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VI th HUNGARIAN PHARMACOPOEIA

AKADÉMIAI KIADÓ, BUDAPEST

VIth HUNGARIAN PHARMACOPOEIA

Edited by B. LÁNG

As we live in a period of international integration of sciences, it is an essential requirement that important national sources of information should be made available for international utilization. Such sources are, in medicine and pharmacy, the National Pharmacopoeias, which permit insight into the pharmaceutical culture of nations.

In the history of the Hungarian Pharmacopoeia, its fifth edition in 1954 was of a paramount importance, since it first took into consideration the points of industrialization of pharmacy, besides pharmaceutical compounding on small-scale. The sixth edition has been fully based on this conception: in addition to simple "rapid informative methods", easy to perform in any pharmacy, ample space has been provided for the most modern instrumental procedures of physical and chemical analysis and biological evaluation.

Thus, this work is indispensable for pharmacologists, pharmaceutical factories and for health authorities exercizing control activity.

AKADÉMIAI KIADÓ
BUDAPEST

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PHARMACOPOEA HUNGARICA VI

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B. LÁNG

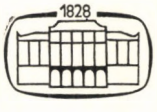
AKADÉMIAI KIADÓ · BUDAPEST 1970

VIth HUNGARIAN PHARMACOPOEIA

VOLUME I

EDITED BY

B. LÁNG



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Foreword

As we live in a period of international integration of sciences it is an essential requirement to render important national sources of information available for international utilization. Such sources are, in medicine and pharmacy, the National Pharmacopoeias, which offer insight into the pharmaceutical culture of nations.

The development of National Pharmacopoeias reflects both the increasing variety of drugs and the advancement in quality requirements and testing methods.

During the past decades the overwhelming part of pharmaceutical compounding has been gradually shifted from pharmacy dispensaries into the large production plants of the pharmaceutical industry. If the Pharmacopoeia is to remain a modern collection of quality norms and test methods the problem arising from this complex development must be taken into consideration.

In this respect the Fifth Hungarian Pharmacopoeia published in 1954, was extremely important. Its editor, Elemér Schulek, a scientist of international reputation in pharmaceutical control, was the first to recognize and to apply the above principle in practice. It was a Pharmacopoeia really to become a general codex of compulsory norms of quality, since its validity extended not only to small-scale compounding but also to large-scale industrial production including even the control activity of the health authorities. Accordingly, besides simple "rapid informative methods", easy to perform in any pharmacy, ample space was provided for the most modern instrumental procedures of physical and chemical analysis, and biological evaluation.

The Sixth Edition of the Hungarian Pharmacopoeia has been wholly based on this principle, only slight adjustments were necessary, especially where new substances and preparations were included.

The appearance of the Sixth Edition in Hungarian coincided with the 100th anniversary of the foundation of the first pharmaceutical factory in the country. Moreover, this decade has brought forth a very rapid development in this branch of industry both in the number of novel products and in the quality and volume of production.

This English version does not only display the present state of Hungarian Pharmacy but also expresses our wish for international cooperation.

Budapest, 6th March, 1969

ANTAL VÉGH
Head of the Editorial Board
of the Hungarian edition

BÉLA LÁNG
Editor of the English edition

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Introduction

Before the First Edition of the Hungarian Pharmacopoeia was published, the Austrian Pharmacopoeia IV and V — *Pharmacopoea Austriaca Ed. IV et V* had been in force in Hungary. The Austrian Pharmacopoeia IV, edited in 1834, was handed over by the Hungarian Governmental Council to the Chief Medical Officer with the decree, dated January 27, 1835, to urge the Medical Faculty of the University of Pest to elaborate the *Hungarian Pharmacopoeia.* This was already ordered at an earlier date, May 20, 1832, at the request of the Hungarian pharmacists.

The case of the Pharmacopoeia advanced slowly, although the Medical Faculty of the University of Pest appointed a committee for the elaboration of a "Dispensatorium" at the faculty meeting held January 8, 1848.

The next step towards the edition of a Hungarian Pharmacopoeia was made in 1848 by the first responsible Hungarian Government. This plan, however, could not be carried out. The responsible Hungarian Government, established at the compromise of 1867, called into existence the National Board of Public Health by the decrees of April 9, and June 14, 1868, respectively. Article 4, paragraphs 1 and 5 of the statutes declared the elaboration of the Hungarian Pharmacopoeia to be an urgent task. The work took 3 years and the first Hungarian Pharmacopoeia (*Pharmacopoea Hungarica*) was published in 1871. It was introduced, on its first page by the decree of the Home Secretary (31.993/1871, December 4, 1871), enacting the provisions of the Pharmacopoeia by March 15, 1872. The "Preface" also contains the list of members of the Editorial Board, elected from the members of the National Board of Public Health, Chairman: Károly Than, Secretary: Lajos Grosz, Members to the Commission: János Wagner, Frigyes Korányi, Kálmán Balogh, Tivadar Margó, Bernát Müller and Gusztáv Jármay.

The Pharmacopoeia was edited bilingually, in Hungarian and Latin on opposite pages. It contained 510 monographs, 46 reagents (2 volumetric solutions) on its XLVI + 581 pages. A 49-page Supplement to the Hungarian Pharmacopoeia — *Additamentum ad Pharmacopoeam Hungaricam* — also in two languages, with 28 pharmaceuticals, 7 antiseptic dressings (*Ligamenta antiseptica*), 4 reagents and 2 test descriptions, completed the material of the Pharmacopoeia.

The Second Edition of the Hungarian Pharmacopoeia — *Pharmacopoea Hungarica Editio Secunda* — was published in April 1888 and its provisions became valid through the decree of the Home Secretary No. 25.000/VII/1888 at October 1, 1888. Chairman of the Pharmacopoeia Commission: Károly Than, Secretary: Lajos Csatáry, Members to the Commission: János Wagner, Frigyes Korányi, Kálmán Balogh, Lajos Tóth, Bernát Müller, Gusztáv Jármay. The Second Edition also appeared bilingually, in Hungarian, and Latin on opposite pages. It contained the description of 516 pharmaceuticals or materials used for the prepara-

tion of medicaments and 79 reagents (9 volumetric solutions) on its XLV + 716 pages. The bilingual Supplement — *Additamentum ad Editionem Secundum Pharmacopoeae Hungaricae* — published in 1898, provided for the storage and preparation of 26 pharmaceuticals, as well as for the packaging and storage of surgical dressings — *Ligamenta chirurgica.*

The Third Edition of the Hungarian Pharmacopoeia — *Pharmacopoea Hungarica Editio Tertia* — was published in 1909 and was enacted January 1, 1910 by the decree of the Home Secretary (No. 100.000/1909).

Members to the Pharmacopoeia Commission were the following: Chairman: Árpád Bókay; Members: Endre Deér, Gusztáv Jármay, Elemér Winkler; Participant Experts: Gyula Kóssa, Béla Lengyel, Sándor Mágócsy-Dietz, Alajos Ströcker, and the late Károly Than. — The Third Edition of the Hungarian Pharmacopoeia contained monographs of 537 pharmaceuticals and materials used for the preparation of medicaments, and 106 reagents (8 volumetric solutions) on its XX + 414 pp. The continuous Hungarian text was followed by the Latin on the same number of pages. Among the equipment the "exact and sensitive balance", the microscope of 300-fold magnifying power and instruments for the preparation of botanical cuttings were listed.

The Fourth Edition of the Hungarian Pharmacopoeia — *Pharmacopoea Hungarica Editio Quarta* — was published in 1933 and was enacted January 1, 1935 by the decree of the Home Secretary (No. 252.091/1934. B.M.). The Fourth Edition was elaborated by a Commission, in collaboration with the National Institute of Public Health, cooperating with Sándor Mágócsy-Dietz, István Bugarszky, and Miklós Matolcsy, Chairman of the Commission: Zoltán Vámossy; Members: Lajos Winkler, Zsigmond Jakabházy, Endre Deér. The Pharmacopoeia was edited in two separate volumes, in Hungarian and Latin. The Latin translation was the work of József Ernyei, Director of the Numismatic Section of the Hungarian National Museum. Hungarian Pharmacopoeia IV contained on its 424 pages (Hungarian Edition) 564 Monographs and 118 reagents (10 volumetric solutions). It was an interesting innovation of the Pharmacopoeia to include also reagents for pathologic, microbiologic and other clinical tests, and their mode of preparation, with the notice that the keeping of these reagents is not compulsory. Each pharmacy was obliged, however, to possess an analytical balance and a microscope. The Hungarian Pharmacopoeia IV also included the literally translated text of the Resolutions of the Second International Brussels Conference (August 20, 1929) which were conditionally adopted by Hungary on October 4, 1930.

The first four editions of the Hungarian Pharmacopoeia reveal the evolution of Hungarian pharmacy. The tests and assays were kept at a level which allowed their application in the laboratories of any pharmacy. Therefore, no methods requiring expensive instrumentation were included. The first four editions agreed on this point.

The development of the industrial production of proprietary preparations ("pharmaceutical specialities") caused a steadily increasing change in the mode of distribution of pharmaceuticals. The supply system of the pharmacies with pharmaceuticals also changed. All these circumstances brought about the necessity to elaborate a pharmacopoeia, which no longer would adapt all its testing methods to the necessarily limited instrumentation of the pharmacy. Its aim could remain no longer the quality control of the medicaments in the detail pharmacies only, but the control had to be extended to the whole country's pharmaceutical supply, using adequate methods. This involves, besides the control of

medicinal substances, also that of compounded preparations. Hungarian Pharmacopoeia V was designed and elaborated in this spirit. Besides the description and the preparation of pharmaceuticals, it also standardized testing methods and so became the norm for the pharmacy, the pharmaceutical industry, as well as for the control institutions. Among others, semi-micro and micro-chemical tests and assays, biological and microbiological methods were also included.

The Fifth Edition of the Hungarian Pharmacopoeia — *Pharmacopoea Hungarica Editio Quinta* — was published in 1954 and became enacted January 1, 1955 by the decree of the Minister of Health No. 1/1954 (XII. 29) Eü.M. It was elaborated by the Pharmacopoeia Commission in collaboration with a Committee of Experts, appointed by the Minister of Welfare. Chairman of the Pharmacopoeia Commission: Elemér Schulek, Secretary: Antal Végh, Scientific collaborator: László Kavlovitz. Members to the Commission: Béla Augusztin, Chairman of the Subcommittee of Pharmacognosy; Béla Issekutz, Chairman of the Subcommittee of Biology; Sándor Mozsonyi, Chairman of the Subcommittee of Galenical Pharmacy; Zoltán Alföldy, Pál Lipták, Tibor Széki, Gábor Vastagh, Members of the Committee of Experts. Lajos Angyal, Antal Babics, Zsigmond Bari, Zoltán Csipke, Lajos Dávid, Sándor Fekete, Imre Fodor, János Halmai, György Kedvessy, Ferenc Kun, Sándor Müller, Géza Petényi, Ödön Rajka, Pál Rom, Sándor Sárkány, István Vitéz.

The Pharmacopoeia Commission worked, in collaboration with the advisors and experts, in four Subcommittees.

The critical evaluation and integration of the work of the Subcommittees was fulfilled by Elemér Schulek with the assistance of Antal Végh, László Karlovitz, György Kedvessy, János Halmai, István Vitéz and László Mittelman.

Hungarian Pharmacopoeia V was published in 3 volumes, on 1627 pages altogether. The large increase of the number of admitted pharmaceuticals and reagents as well as that of tests and assays, the introduction of new physical, chemical, microscopical, bacteriological and biological methods necessitated a new arrangement for the Pharmacopoeia. Volume I, as the general part, included prescriptions of a general character for the preparation and qualification of pharmaceuticals as well as general methods of tests and assays, dose tables, etc. Hungarian Pharmacopoeia V contained 515 reagents and 37 volumetric solutions. Volumes II and III contained 819 monographs in the following arrangement: Volume II contained 334 monographs of chemical substances, 12 lipids (fixed oils, fats, waxes and materials of similar consistency), and 21 essential oils. Volume III contained the monographs of 97 drugs of vegetable and animal origin, 289 pharmaceutical (galenical) preparations, 32 serobiological preparations for human and 26 for veterinary use and finally 7 surgical dressings and sutures. The monographs of the individual groups were listed alphabetically in order of the Latin pharmaceutical titles.

Contrary to all former editions, according to the Fifth Hungarian Pharmacopoeia, the pharmacies were no longer obliged to store all the official articles. Simultaneously, the adopted tests and assays ceased to be limited to the methods practicable only with the necessarily rather limited instrumentation of public pharmacies, as the modern testing of pharmaceuticals frequently requires expensive apparatuses. According to this principle, the articles to be held in stock or prepared by the pharmacies, as well as the implements necessary for the preparation and testing of pharmaceuticals became subject of separate specification by the Ministry of Health. It was regulated further, also by the Ministry, which of

the Informative Tests, and to what extent, must be carried out in the pharmacies.

Following the suggestions of the Pharmacopoeia Commission and the Committee of Experts, the Ministry of Health ensured the Pharmacopoeia's close relation to the pharmaceutical practice and progressed by establishing a permanent Pharmaceutical and Pharmacopoeial Commission within the framework of the Scientific Board of Public Health. For the fulfilment of the experimental work a laboratory directly subordinated to the Ministry of Health was established. This organization ensured a smooth execution of the innovations and alterations connected with the Pharmacopoeia and permitted the preparation of the Addendum to the Fifth Hungarian Pharmacopoeia.

The Addendum to the Fifth Edition of the Hungarian Pharmacopoeia was published in 1958. It was enacted September 1, 1959 by the decree of the Minister of Health [No. 4/1959 (VIII. 25) Eü.M.]. The Ministry entrusted the Pharmaceutical and Pharmacopoeial Commission with the elaboration of the Addendum. The Chairman of the Commission, Elemér Schulek, as Editor in Charge, in cooperation with Vice-Chairman Antal Végh and Secretary István Bayer, reconciled the monographs and performed the critical evaluations. Members to the Commission were: Béla Bölcs, János Halmai, György Kedvessy, Sándor Mozsonyi, Károly Nikolics, Egon Pandula, Pál Rózsa, Gábor Vastagh, István Vitéz.
The construction of the Addendum agrees with that of the Fifth Hungarian Pharmacopoeia. The General Part contains, besides test and assay methods, the fundamentals of statistical calculations indispensable for the evaluation of biological tests. The list of reagents increased by 17 items and the number of newly adopted monographs was enlarged with 21 chemical substances and 37 pharmaceutical (galenical) preparations. The composition or the technique of preparation of 28 pharmaceutical (galenical) preparations underwent substantial alteration, and 2 were completely changed.

The Addendum regulated anew the preparation of medicaments containing incompatible ingredients and assured a greater prevail to the expertise of the pharmacist.

Simultaneously with the publication of the Addendum the Minister of Health ordered (No. 80.687/1959) the elaboration of a new pharmacopoeia and appointed the *Pharmacopoeia Commission* for the Sixth Edition of the Hungarian Pharmacopoeia. Chairman: Elemér Schulek; Vice-Chairman: Antal Végh; Secretary: István Bayer.

Members to the Editorial Committee:

Chemical Subcommittee

Chairman: Elemér Schulek († 1964), Members: István Bayer, Ottó Clauder, István Gyenes, László Kovács, Dénes Kőszegi, József Laszlovszky, Károly Nikolics, Szabolcs Nyiredy, Ernő Pungor, Pál Rózsa, István Simonyi, Gábor Vastagh, Antal Végh, Elemér Vinkler, Flóra Wessel.

Committee of Pharmaceutical Preparations

Chairman: Sándor Mozsonyi. Members: István Éllő, István Floderer, György Kedvessy, Ferenc Kun, Béla Láng, Egon Pandula, János Ragettly.

Committee of Pharmacognosy

Chairman: János Halmai. Members: Othmár Buchmann, Rudolf Giovannini (†1963), Zoltán Kárpáti, István Novák, Pál Rom (†1962), Sándor Sárkány, Kálmán Szász.

Committee of Biology and Pharmacology

Chairman: Béla Issekutz. Members: Zoltán Dirner, György Fekete, Gusztáv Fritz, Miklós Jancsó (†1966), Jenő Kovács, Sándor Simon.

Committee of Microbiology

Chairman: Zoltán Alföldy. Members: László Erdős, Rezső Manninger, Lajos Réthy, József Szathmáry, István Vitéz.

The Pharmacopoeia Commission worked under the chairmanship of Elemér Schulek until his death, October 14, 1964. As the principles of the Hungarian Pharmacopoeia VI have been clearly shaped before that date, these were regarded by the Commission as the professional legacy of Elemér Schulek and the work was continued in conformity with these principles under the direction of the Vice-President, Antal Végh.

In contrast to the Fifth Edition, Hungarian Pharmacopoeia VI omitted the detailed description of some laboratory operations. At the same time the duality, existing between dispensing in the pharmacy and manufacturing on large scale in the factory, has been further developed. The Pharmacopoeia gives but general directives for the preparation of dosage forms, while the detailed instructions for the pharmacies are described by the "Formulae Normales". The active ingredients and the auxiliary materials applied in the "Formulae Normales" are all official in the Pharmacopoeia in which all requirements concerning the various dosage forms are also prescribed.

Although the Pharmacopoeia does not include prescriptions for the large-scale production of pharmaceutical preparations, the principal requirements are laid down for all individual dosage forms and preparations; these requirements are valid also for preparations not official in this Pharmacopoeia.

The Panel of Experts to the Pharmacopoeia Commission

László Auber, Dániel Bagdy, István Bálintffy, Tibor Bánó, Lajos Barcza, Zsigmond Bari, László Biró, Sándor Bordás, Pál Csiki, Zoltán Csipke (†1966), Mrs. Livia Dömök née Kiss Vigh, András Farkas, Mihály Gerendás, László Gyulai, György Habán, Gyula Hidvégi, Győző Hortobágyi, Endre Horváth, István Horváth, István Inczefi, Miklós Jáky, Iván Janovszky, Iréneusz Juvancz, Éva Kálmán, Pál Kertai, József Knoll, Ede Kolos, Endre Kőrös, Dezső Küttel, Antal Lakos, Mária Langfelder, Béla Lányi, György Lenárt, Dezső Mihalik, János Nagy, Sándor Nepper, Ernő Novák, Imre Pál, Mrs. Rozália Péntek née Babel, Mrs. Edit Pozsgay née Kovács, János P. Radó, Endre Siftár, László Szabó, György Szász, Angela Szepesy, István Szórády, Mihály Takács, Géza Takácsi Nagy, Lőrinc Tamáska, Mária Tényi, Pál Tolnay, Tibor Torbágyi, Jenő Trompler, Pál Varga, Erik Zajta.

Participants in experimental work for the Pharmacopoeia Commission
National Institutes

National Institute for Agricultural Quality Control

Mrs. Éva Nechay née Horváth, Mrs. Zsuzsánna Schulek née Lassányi, József Tatár

National Institute of Nutrition

Ernő Dworschák.

National Institute for Pharmacy

Sándor Farkas, Mrs. Alice Fischer née Grünwald, Kálmán Katona, Pál Majlát, Mrs. Éva Zábrák née Göttye, Mrs. Emilia Zoltay née Matolcsy.

National Institute of Public Health

Mária Haraszti, Anna Kovách, Mrs. Ilse Kelemen née Küttel, István Kerényi, Mrs. Emma Szabolcs née Kossányi, Mrs. Erzsébet Vastagh née Varga, Mrs. Ida Vida née Kanizsai Nagy, Mrs. Éva Zöllner, née Iván.

University Institutes

Budapest Medical University, Institute of Pharmaceutical Chemistry

Mrs. Ágnes Bálint née Novotny, Antal Brantner, Mrs. Zsuzsánna Budvári née Bárány, Mrs. Mária Gracza née Lukács, Piroska Kertész, László Khin, Mrs. Vera Lukács née Bányai, Otto Papp, Barnabás Szabó, Mrs. Mária Szász née Zacskó, József Vámos.

Budapest Medical University, Institute of Pharmacognosy

Éva Csapó, Mrs. Edit Kolos née Pethes, Mrs. Gabriella Marczal, József Váradi.

Budapest Medical University, Institute of Pharmacy

Pál Bernát, Mrs. Valéria Fehér née Selmeczi, Mrs. Aranka Gál née Farkas, Péter Keserü, Mrs. Klára Koritsánszky née Ambrus, Béla Kovács, Mrs. Zsuzsánna Pajor née Weszelowszky, István Rácz, Mrs. Ildikó Robogány née Somogyi, Sándor Tarjányi, Barna Tóth, Mrs. Veronika Vajda née Benedek.

Budapest University of Technology, Institute of General Chemistry

László Káplár.

Eötvös Loránd University of Sciences, Institute of Inorganic and Analytical Chemistry

Alexandra Lásztity, Mrs. Zsuzsanna Remport née Horváth.

Szeged Medical University, Institute of Pharmaceutical Technology

Mrs. Mária Dobó née Wayer, Mihály Kata, Adél Mayer, Géza Regdon, Mrs. Éva Regdon née Kiss, Béla Selmeczi.

Szeged Medical University, Institute of Pharmaceutical Chemistry

Mrs. Éva Csapó née Bartos, Mrs. Marianne Gáti née Simon, Ferenc Kliványi, János Lázár, Lajos Simon, Géza Stájer, Mrs. Enikő Stájer née Szabó, János Szabó, István Varga.

Szeged Medical University, Institute of Pharmacognosy

Géza Buzás, András Háznagy, Kálmán Szendrei, László Tóth.

Laboratories of the Pharmaceutical Industry and Other Institutions

Chinoin Chemical and Pharmaceutical Works Ltd.

Sándor Darányi, Jenő Hollós, Mrs. Sára Költő née Binder, Mrs. Dóra Lendvai née Gara, Judit Pálosi.

Chemical Works of Gedeon Richter Ltd.

György Bakó, Csaba Lőrincz, György Németh, Zsuzsanna Réday, László Rigó, István Temesváry, Ferenc Trischler.

United Works of Pharmaceutical and Dietetic Products Ltd.

Mrs. Magdolna Bőjthe née Mátyási, István Krasznai, Zoltán Tóth.

Biological Control Laboratory of the Pharmaceutical Industry

Anikó Zarándi.

Analytical Control Laboratory of the Central Pharmaceutical Wholesale Company

Mrs. Éva Bayer née Gáthy, Paula Bertsch, Magdolna Király, Mrs. Gizella Laszlovszky née Groma, Mrs. Edit Trompler née Király

Pharmacy Centres

Analytical Control Laboratory of the Municipial Pharmacy Centre Budapest

Julia Ambrus, Mrs. Sarolta Bé née Szabó, György Élő, Mrs. Katalin Gyökér née Nagygyőri, László Kálmán, Mrs. Lidia Magyarosy née Emanditi, Mrs. Éva Popp née Petőcz, Mrs. Márta Potsubay née Szentpétery, Zoltán Staudt, Mrs. Ilona Tarján née Szokolay, Karola Vida, Mrs. Margit Vitkóczy née Kranixfeld.

Galenical Laboratories of County Pharmacy Centres

László Gaskó (County of Pest), Mrs. Ágnes Küttel née Thieringer (County of Vas), Mrs. Zsuzsanna Nádler née Geszty (County of Borsod-Abaúj-Zemplén).

Public Pharmacies

Károly Geszti (Budapest), Nándor Göbl (Eger), Dénes Horváth (Sopron), Dezső Huba (Pécs), Pál Kovács (Debrecen), †Lajos Papy (Hódmezővásárhely), Kálmán Szalay (Szombathely).

List of Monographs Official in the Hungarian Pharmacopoeia VI

New admissions to the Hungarian Pharmacopoeia are printed in italics.

"Pharmaceutical Preparations" (Vol. III) modified in composition or in technique of preparation with respect to the Fifth Edition of the Hungarian Pharmacopoeia are marked with an asterisk(*).

1 CHEMICAL SUBSTANCES

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Acetanilidum	Acetanilide	
Acetazolamidum	Acetazolamide	
Acetonum	Acetone	
Acetyldigitoxinum	Acetyldigitoxin	
Acidum aceticum concentratum	Glacial Acetic Acid	
Acidum acetylsalicylicum	Acetylsalicylic Acid	
Acidum arsenicosum anhydricum	Arsenic Trioxide	Arseni Trioxydum
Acidum ascorbicum	Ascorbic Acid	
Acidum benzoicum	Benzoic Acid	
Acidum boricum	Boric Acid	
Acidum citricum	Citric Acid	
Acidum citricum pro infusione	Citric Acid for Infusion	
Acidum dehydrocholicum	Dehydrocholic Acid	
Acidum hydrochloricum concentratissimum	Hydrochloric Acid 38 per cent	
Acidum hydrochloricum concentratum 25%	Hydrochloric Acid 25 per cent	
Acidum lacticum	Lactic Acid	
Acidum nicotinicum	Nicotinic Acid	
Acidum oleinicum	Oleic Acid	
Acidum paraaminosalicylicum	Aminosalicylic Acid	
Acidum phosphoricum concentratum	Concentrated Phosphoric Acid	
Acidum Salicylicum	Salicylic Acid	
Acidum silicicum colloidale	Colloidal Silicium Dioxide	
Acidum tannicum	Tannic Acid	
Acidum tartaricum	Tartaric Acid	
Acidum trichloraceticum	Trichloroacetic Acid	
Aciphenochinolium	Cinchophen	
Adeps lanae	Wool Fat	
Adeps solidus	Solid Fat	
Adrenalinum	Adrenaline	
Aether	Ether	
Aether ad narcosim	Anesthetic Ether	Ether Anesthesicus
Aether chloratus	Ethyl Chloride	Ethylis Chloridum
Aetheroleum anisi	Anise Oil	

Latinized titles of the monograph	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Aetheroleum carvi	Caraway Oil	
Aetheroleum caryophylli	Clove Oil	
Aetheroleum chamomillae	Matricaria Oil	
Aetheroleum cinnamomi	Cinnamon Oil	
Aetheroleum citri	Lemon Oil	
Aetheroleum eucalypti	Eucalyptus Oil	
Aetheroleum foeniculi	Fennel Oil	
Aetheroleum juniperi	Juniper Oil	
Aetheroleum lavandulae	Lavender Oil	
Aetheroleum menthae piperitae	Peppermint Oil	
Aetheroleum pini pumilionis	Mountain Pine Oil	
Aetheroleum pini silvestris	Pine Oil	
Aetheroleum sinapis syntheticum	Synthetic Mustard Oil	
Aetheroleum terebinthinae rectificatum	Rectified Turpentine Oil	
Aethinyloestradiolum	Ethynyl Estradiol	Ethinylestradiolum
Aethionamidum	Ethionamide	
Aethylendiaminum hydratum	Ethylendiamine Hydrate	Ethylenediamini Hydras
Aethylmorphinum hydrochloricum	Ethylmorphine Hydrochloride	
Albumen tannicum	Albumin Tannate	
Alcohol benzylicus	Benzyl Alcohol	
Alcohol cetylstearylicus	Cetostearyl Alcohol	
Alumen	Alum	
Alumen ustum	Dried Alum	
Aluminium chloratum	Aluminium Chloride	
Aluminium hydroxydatum	Aluminium Hydroxide	
Aluminium monostearinicum	Aluminium Monostearate	
Aluminium sulfuricum	Aluminium Sulphate	
Amidazophenum	Aminophenazone	Aminophenazonum
Ammonia soluta concentrata	Strong Ammonia Solution	
Ammonium bitumensulfonicum	Ichthammol	
Ammonium bromatum	Ammonium Bromide	
Ammonium chloratum	Ammonium Chloride	
Amobarbitalum	Amobarbital	
Amphethaminum phosphoricum	Amphetamine Phosphate	
Amylium nitrosum	Amyl Nitrite	Amylis Nitris
Amylum liquefactum	Liquid Glucose	
Apomorphinum hydrochloricum	Apomorphine Hydrochloride	
Aqua demineralisata	Demineralized Water	
Aqua destillata	Distilled Water	
Arecolinum hydrobromicum	Arecoline Hydrobromide	
Argentum aceticum	Silver Acetate	
Argentum colloidale	Colloid Silver	
Argentum nitricum	Silver Nitrate	Argenti Nitras
Argentum proteinicum	Silver Protein	
Atropinum sulfuricum	Atropine Sulphate	Atropini Sulfas
Azophenum	Phenazone	
Barbitalnatrium	Barbital Sodium	Barbitalum Natricum
Barbitalum	Barbital	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Barium chloratum	Barium Chloride	
Barium sulfuricum	Barium Sulphate	Barii Sulfas
Benzaldehydum	Benzaldehyde	
Benzalkonium chloratum	Benzalkonium Chloride	Benzalkonii Chloridum
Benzinum	Light Petroleum	
Benzylium benzoicum	Benzyl Benzoate	
Benzylpenicillin-kalium	Benzylpenicillin Potassium	BenzylpenicillinumKalicum
Benzylpenicillin-natrium	Benzylpenicillin Sodium	Benzylpenicillinum Natricum
Benzylpenicillin-procainum	Benzylpenicillin Procaine	Procaini Benzylpenicillinum
Betainum hydrochloricum	Betaine Hydrochloride	
Bismuthum subgallicum	Bismuth Subgallate	
Bismuthum subnitricum	Bismuth Subnitrate	Bismuthi Subnitras
Bismuthum subsalicylicum	Bismuth Subsalicylate	Bismuthi Subsalicylas
Bolus alba	White Clay	
Bromacetocarbamidum	Carbromal	
Bromvalerocarbamidum	Bromisovalum	
Butobarbitalum	Butobarbital	
Butyrum cacao	Theobroma Oil	
Calcium bromatum	Calcium Bromide	
Calcium carbonicum	Calcium Carbonate	
Calcium chloratum crystallisatum	Crystalline Calcium Chloride	Calcii Chloridum Crystallisatum
Calcium glucinicum	Calcium Gluconate	Calcii Gluconas
Calcium lacticum	Calcium Lactate	Calcii Lactas
Calcium oxydatum	Calcium Oxide	
Calcium phosphoricum	Calcium Phosphate	
Calcium phosphoricum tribasicum	Tribasic Calcium Phosphate	
Calcium sulfuricum ustum	Dried Calcium Sulphate	
Camphora	Camphor	
Carbamidum	Urea	
Carbamidum peroxydatum	Urea Hydrogen Peroxide	
Carbo activatus	Activated Charcoal	
Carboneum dioxydatum	Carbon Dioxide	Carbonei Dioxydum
Carboneum tetrachloratum medicinale	Carbon Tetrachloride	
Carboxymethylcellulosnatrium	Sodium Carboxymethylcellulose	
Caseinum	Casein	
Cellulosum acetylphthalicum	Cellulose Acetate Phthalate	
Cera alba	White Beeswax	
Cetaceum	Spermaceti	
Cetylpyridinium chloratum	Cetylpyridinium Chloride	Cetylpyridinii Chloridum
Chinacrinum	Mepacrine Hydrochloride	Mepacrini Hydrochloridum
Chinidinum sulfuricum	Quinidine Sulphate	Quinidini Sulfas
Chininum hydrochloricum	Quinine Hydrochloride	Quinini Hydrochloridum
Chininum sulfuricum	Quinine Sulphate	Quinini Sulfas
Chloralum hydratum	Chloral Hydrate	Chlorali Hydras
Chloramphenicolum	Chloramphenicol	
Chloramphenicolum palmitinicum	Chloramphenicol Palmitate	
Chlorbutanolum	Chlorobutanol	Chlorobutanolum
Chlorochinum phosphoricum	Chloroquine Diphosphate	Chloroquini Diphosphas
Chloroformium	Chloroform	
Chlorogenium	Chlorogen	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Chloropyraminum hydrochloricum	Chloropyramine Hydrochloride	
Chlorpromazinum hydrochloricum	Chlorpromazine Hydrochloride	Chlorpromazini Hydrochloridum
Cholecalciferolum	Cholecalciferol	
Cholinium chloratum	Choline Chloride	
Cocainum hydrochloricum	Cocaine Hydrochloride	Cocaini Hydrochloridum
Codeinum hydrochloricum	Codeine Hydrochloride	
Codeinum phosphoricum	Codeine Phosphate	Codeini Phosphas
Coffeinum	Caffeine	
Collodium	Collodion	
Cuprum sulfuricum	Copper Sulphate	
Cyanocobalaminum	Cyanocobalamin	
Cyclobarbitalum	Cyclobarbital	
Cycloserinum bitartaricum	Cycloserine Bitartarate	
Desoxycortonum aceticum	Desoxycortone Acetate	Desoxycortoni Acetas
Dienoestrolum	Dienestrol	
Digitoxinum	Digitoxin	
Digoxinum	Digoxin	
Dihydrocodeinum bitartaricum	Dihydrocodeine Bitartarate	
Dimercaprolum	Dimercaprol	
Disulfiramum	Disulfiram	
Ephedrinum hydrochloricum	Ephedrine Hydrochloride	
Ergocalciferolum	Ergocalciferol	
Ergometrinum maleinicum	Ergometrine Maleinate	
Ergotaminum tartaricum	Ergotamine Tartarate	
Ferrum pulveratum	Powdered Iron	
Ferrum reductum	Reduced Iron	
Ferrum sesquichloratum	Ferric Chloride	
Ferrum sulfuricum oxydulatum	Ferrous Sulphate	Ferrosi Sulfas
Formaldehydum solutum	Formaldehyde Solution	Formaldehydi Solutio
Fuchsinum	Magenta	
Gelatina alba	Gelatin	
Glucosum	Glucose	
Glucosum anhydricum	Anhydrous Glucose	
Glucosum pro infusione	Glucose for Parenteral Use	
Glycerinum	Glycerin	Glycerolum
Glycerinum monostearinicum	Glycerin Monostearate	
Gonadotrophinum chorionicum	Chorionic Gonadotrophin	
Heparinnatrium	Heparin	
Hexachlorophenum	Hexachlorophene	
Hexamethylentetraminum	Hexamine	
Hexobarbitalnatrium	Hexobarbital Sodium	
Hexobarbitalum	Hexobarbital	
Histaminum bihydrochloricum	Histamine Bihydrochloride	
Homatropinum hydrobromicum	Homatropine Hydrobromide	Homatropini Hydrobromidum
Hydrargyrum	Mercury	
Hydrargyrum amidochloratum	Ammoniated Mercury	Hydrargyri Aminochloridum
Hydrargyrum bijodatum rubrum	Red Mercuric Iodide	
Hydrargyrum chloratum mite	Mercurous Chloride	
Hydrargyrum oxydatum flavum	Yellow Mercuric Oxide	Hydrargyri Oxydum Flavum

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Hydrargyrum sulfuratum rubrum	Red Mercuric Sulphide	
Hydrochinonum	Hydroquinone	
Hydrochlorothiazidum	Hydrochlorothiazide	
Hydrocodonum Bitartaricum	Hydrocodone Bitartarate	Hydrocodoni Bitratras
Hydrocortisonum	Hydrocortisone	
Hydrocortisonum aceticum	Hydrocortisone Acetate	Hydrocortisoni Acetas
Hydrogenium peroxydatum concentratum 30%	Concentrated Hydrogen Peroxide 30 per cent	
Isoniazidum	Isoniazid	
Isoprenalinum hydrochloricum	Isoprenaline Hydrochloride	Isoprenalini Hydrochloridum
Jodochloroxychinolinum	Iodochlorhydroxyquinoline	
Jodum	Iodine	Iodum
Kalium bromatum	Potassium Bromide	Kalii Bromidum
Kalium carbonicum	Potassium Carbonate	
Kalium chloratum	Potassium Chloride	Kalii Chloridum
Kalium chloratum pro infusione	Potassium Chloride for Parenteral Use	
Kalium citricum	Potassium Citrate	
Kalium hydroxydatum	Potassium Hydroxide	Kalii Hydroxidum
Kalium jodatum	Potassium Iodide	Kalii Iodidum
Kalium natrium tartaricum	Sodium Potassium Tartarate	
Kalium nitricum	Potassium Nitrate	Kalii Nitras
Kalium perchloricum	Potassium Perchlorate	
Kalium permanganicum	Potassium Permanganate	
Kalium rhodanatum	Potassium Thiocyanate	
Kalium sulfuricum	Potassium Sulphate	
Lactosum	Lactose	
Lanalcolum	Wool Alcohols	
Lanatosidum C	Lanatoside C	
Lidocainum	Lidocaine	
Lidocainum hydrochloricum	Lidocaine Hydrochloride	Lidocaini Hydrochloridum
Liothyroninum hydrochloricum	Liothyronine Hydrochloride	
Magnesium carbonicum hydroxydatum	Magnesium Carbonate	
Magnesium oxydatum	Magnesium Oxide	
Magnesium peroxidatum	Magnesium Peroxyde	
Magnesium stearinicum	Magnesium Stearinate	
Magnesium sulfuricum crystallisatum	Magnesium Sulphate	
Magnesium sulfuricum siccatum	Dried Magnesium Sulphate	
Magnesium trisilicicum	Magnesium Trisilicate	
Menachinonum natrium bisulfurosum	Menadione Sodium Bisulphite	
Mentholum	Menthol	
Mercamphamidum	Mercamphamide	
Methadonum hydrochloricum	Methadone Hydrochloride	Methadoni Hydrochloridum
Methandrostenolonum	Methandrostenolone	
Methimazolum	Methimazole	
Methylcellulosum	Methylcellulose	
Methylenum coeruleum	Methylene Blue	
Methylhomatropinium bromatum	Methylhomatropinium Bromide	
Methylium paraoxybenzoicum	Methyl Hydroxybenzoate	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Methylium salicylicum	Methyl Salicylate	
Methylphenydatum hydrochloricum	Methylphenidate Hydrochloride	
Methylrosanilinum chloratum	Methylrosaniline Chloride	
Morphinum hydrochloricum	Morphine Hydrochloride	Morphini Hydrochloridum
Nalorphinum hydrobromicum	Nalorphine Hydrobromide	
Naphthalanum liquidum	Naphthalan Oil	
Naphtholum-β	Betanaphthol	
Natrium aceticum crystallisatum	Sodium Acetate	
Natrium benzoicum	Sodium Benzoate	
Natrium bicarbonicum	Sodium Bicarbonate	
Natrium bicarbonicum pro infusione	Sodium Bicarbonate for Parenteral Use	
Natrium boricum	Borax	Natrii Tetraboras
Natrium bromatum	Sodium Bromide	Natrii Bromidum
Natrium carbonicum crystallisatum	Crystalline Sodium Carbonate	
Natrium carbonicum siccatum	Dried Sodium Carbonate	
Natrium chloratum	Sodium Chloride	Natrii Chloridum
Natrium chloratum pro infusione	Sodium Chloride for Parenteral Use	
Natrium citricum	Sodium Citrate	Natrii Citras
Natrium citricum pro infusione	Sodium Citrate for Parenteral Use	
Natrium citricum acidum	Acid Sodium Citrate	
Natrium citricum acidum pro infusione	Acid Sodium Citrate for Parenteral Use	
Natrium fluoratum	Sodium Fluoride	
Natrium hydroxydatum	Sodium Hydroxide	
Natrium jodatum	Sodium Iodide	
Natrium laurylsulfuricum	Sodium Lauryl Sulphate	
Natrium nitrosum	Sodium Nitrite	
Natrium paraaminosalicylicum	Sodium Para-aminosalicylate	Natrii Para-aminosalicylas
Natrium phosphoricum acidum	Sodium Acid Phosphate	
Natrium phosphoricum crystallisatum	Crystalline Sodium Phosphate	
Natrium pyrosulfurosum	Sodium Pyrosulphite	Natrii Pyrosulfis
Natrium salicylicum	Sodium Salicylate	Natrii Salicylas
Natrium sulfuricum crystallisatum	Sodium Sulphate	
Natrium sulfuricum siccatum	Dried Sodium Sulphate	
Natrium thiosulfuricum	Sodium Thiosulphate	
Neomycinum sulfuricum	Neomycin Sulphate	Neomycini Sulfas
Neostigminium bromatum	Neostigmine Bromide	Neostigmini Bromidum
Neostigminium methylsulfuricum	Neostigmine Methylsulphate	Neostigmini Methylsulfas
Nicetamidum	Nikethamide	
Nicotinamidum	Nicotamide	
Nitrogenium	Nitrogen	
Nitrogenium oxydulatum	Nitrous Oxide	Oxydum Nitrosum
Nitroglycerinum solutum 1%	Glyceryl Trinitrate Solution, 1 per cent	
Noradrenalinum bitartaricum	Noradrenaline Acid Tartarate	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Noracinum	Benzocaine	Ethylis Aminobenzoas
Noscapinum hydrochloricum	Noscapine Hydrochloride	
Novamidazophenum	Noraminophenazone	
Nystatinum	Nystatin	
Oestradiolum benzoicum	Oestradiol Benzoate	Estradioli benzoas
Oestradiolum propionicum	Oestrol Propionate	
Oestronum	Oestrone	
Oleum helianthi	Sunflower-seed Oil	
Oleum jecoris aselli	Cod-liver Oil	
Oleum lini	Linseed Oil	
Oleum ricini	Castor Oil	
Oleylum oleinicum	Oleyl Oleate	
Oxygenium	Oxygen	
Oxytetracyclinum	Oxytetracycline	
Oxytetracyclinum hydrochloricum	Oxytetracycline Hydrochloride	Oxytetracyclii Hydrochloridum
Pancreatinum	Pancreatine	
Papaverinum hydrochloricum	Papaverine Hydrochloride	Papaverini Hydrochloridum
Paraffinum liquidum	Liquid Paraffin	
Paraffinum solidum	Solid Paraffin	
Paraldehydum	Paraldehyde	
Pentetrazolum	Pentetrazol	
Pepsinum	Pepsin	
Pethidinum hydrochloricum	Pethidine Hydrochloride	Pethidini Hydrochloridum
Phenacetinum	Phenacetin	
Phenlaxinum	Phenlaxin	
Phenobarbitalnatrium	Phenobarbital Sodium	Phenobarbitalum Natricum
Phenobarbitalum	Phenobarbital	
Phenolphthaleinum	Phenolphthalein	
Phenolum	Phenol	
Phenomerborum	Phenylmercuric Borate	
Phenylbutazonum	Phenylbutazone	
Phenytoinum	Phenytoin	
Pholedrinum	Pholedrine	
Physostigminum salicylicum	Physostigmine Salicylate	Physostigmini Salicylas
Pilocarpinum hydrochloricum	Pilocarpine Hydrochloride	
Pix juniperi	Cade Oil	
Plumbum oxydatum	Lead Monoxide	
Polyoxaethenum-400	Macrogol 400	
Polyoxaethenum-1540	Macrogol 1540	
Polyoxaethenum-4000	Macrogol 4000	
Polyoxaethenum stearinicum	Macrogol Stearate	
Polyvidonum	Polyvidone	
Prednisolonum	Prednisolone	
Procainum hydrochloricum	Procaine Hydrochloride	Procaini Hydrochloridum
Progesteronum	Progesterone	
Promethazinum hydrochloricum	Promethazine Hydrochloride	Promethazini Hydrochloridum
Propylenglycolum	Propylene Glycol	
Propylium paraoxybenzoicum	Propyl Hydroxybenzoate	
Pyridoxinum hydrochloricum	Pyridoxine Hydrochloride	Pyridoxini Hydrochloridum
Reserpinum	Reserpine	
Resorcinum	Resorcinol	
Riboflavinum	Riboflavine	
Rubrum Congo	Congo Red	
Rutinum	Rutin	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Saccharimidum	Saccharin	
Saccharosum	Sucrose	
Saccharosum pro infusione	Sucrose for Parenteral Use	
Salicylamidum	Salicylamide	
Scopolaminum hydrobromicum	Scopolamine Hydrobromide	Hyoscini Hydrobromidum
Sorbitolum	Sorbitol	
Sorboxaethenum laurinicum	Sorbimacrogol Laurate	
Sorboxaethenum oleinicum	Sorbimacrogol Oleate	
Sorboxaethenum stearinicum	Sorbimacrogol Stearate	
Spiritus concentratissimus	Alcohol 96 per cent	Ethanolum
Spiritus concentratus	Alcohol 90 per cent	
Spiritus dilutus	Diluted Alcohol 70 per cent	Ethanolum Dilutum
Stearinum	Stearic Acid	
Stibium sulfuratum aurantiacum	Antimony Pentasulphide	
Streptomycinum sulfuricum	Streptomycin Sulphate	Streptomycini Sulfas
Strychninum nitricum	Strychnine Nitrate	
Sulfacetamidnatrium	Sulphacetamide Sodium	Sulfacetamidum Natricum
Sulfadimidinum	Sulphadimidine	
Sulfaguanidinum	Sulphaguanidine	
Sulfamethoxypyridazinum	Sulphamethoxypyridazine	
Sulfur praecipitatum	Precipitated Sulphur	
Sulfur pulveratum lotum	Purified Sulphur Powder	
Sympaethaminum	Phenylephrine Hydrochloride	
Talcum	Talc	
Terpinum hydratum	Terpin Hydrate	
Testosteronum propionicum	Testosterone Propionate	Testosteroni Propionas
Tetracainum hydrochloricum	Tetracaine Hydrochloride	Tetracaini Hydrochloridum
Tetracyclinum hydrochloricum	Tetracycline Hydrochloride	Tetracyclini Hydrochloridum
Theobrominum	Theobromine	
Theobrominum natrium salicylicum	Theobromine Sodium and Sodium Salicylate	Theobrominum Natricum et Natrii Salicylas
Theophyllinum	Theophylline	
Thiaminum hydrochloricum	Thiamine Hydrochloride	Thiamini Hydrochloridum
Thiomersalum	Thiomersal	
Thymolum	Thymol	
Thyreoideum siccum	Thyroid	Thyroidea
Tocopherolum aceticum	Tocopheryl Acetate	
Tolazolinum hydrochloricum	Tolazoline Hydrochloride	Tolazolini Hydrochloridum
Triaethanolaminum	Triethanolamine	
Trichloraethylenum	Trichloroethylene	Trichloroethylenum
Tricresolum	Cresol	Cresolum
Trimetozinum	Trimetozine	
Urethanum	Urethane	
Vanillinum	Vanillin	
Vaselinum album	White Soft Paraffin	
Vaselinum album ophthalmicum	White Soft Ophthalmic Paraffin	
Vaselinum flavum	Yellow Soft Paraffin	
Viride nitens	Brilliant Green	
Xanthacridinum	Euflavine	
Zincum chloratum	Zinc Chloride	
Zincum oxydatum	Zinc Oxide	Zinci Oxydum
Zincum sulfuricum	Zinc Sulphate	Zinci Sulfas

II VEGETABLE AND ANIMAL DRUGS

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Absinthii herba	Wormwood	
Agar-agar	Agar	
Aloë	Aloes	
Althaeae folium	Marshmallow Leaf	
Althaeae radix	Marshmallow Root	
Amylum solani	Potato Starch	
Amylum tritici	Wheat Starch	
Anisi vulgaris fructus	Anise	
Aurantii pericarpium	Orange Peel	
Balsamum peruvianum	Peru Balsam	
Belladonnae folium	Belladonna Leaf	
Belladonnae radix	Belladonna Root	
Benzoë	Benzoin	
Calami rhizona	Sweet Flag Root	
Cantharis	Spanish Fly	
Capsici fructus	Paprika	
Cardui benedicti herba	Holy Thistle	
Carvi fructus	Caraway	
Caryophylli flos	Clove	
Centaurii herba	Centaury	
Chamomillae flos	Matricaria	
Chinae succirubrae cortex	Cinchona Bark	
Cinnamomi cassiae cortex	Cinnamon	
Coriandri fructus	Coriander	
Cynosbati fructus	Rose Fruit	
Digitalis purpurae folium	Digitalis Leaf	
Equiseti herba	Horsetail	
Foeniculi fructus	Fennel	
Frangulae cortex	Frangula Bark	
Gentianae radix	Gentian Root	
Gummi arabicum	Acacia	
Herniariae herba	Herniary	
Hirudo	Leech	
Hyoscyami folium	Hyoscyamus Leaf	
Ipecacuanhae radix	Ipecacuanha Root	
Juniperi fructus	Juniper Fruit	
Lavandulae flos	Lavender Flower	
Leonuri lanati herba	Horehound	
Lini placentae farina	Linseed Meal	
Lini semen	Linseed	
Liquiritiae radix	Liquorice	
Malvae flos	Wild Mellow Flower	
Malvae folium	Wild Mellow Leaf	
Marrubii herba	White Horehound	
Menthae crispae folium	Spearmint Leaf	
Menthae piperitae folium	Peppermint Leaf	
Millefolii herba	Milfoil	
Ononidis radix	Restharrow Root	
Opium nativum	Opium	
Plantaginis lanceolatae folium	Plantain Leaf	
Primulae radix	Primrose Root	
Quercus cortex	Oak Bark	
Ratanhiae radix	Ratanhia Root	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Rhei rhizoma	Rhubarb Rhizome	
Salviae folium	Salvia Leaf	
Sambuci flos	Elder Flower	
Saponariae albae radix	Soap-Root	
Scilla siccata	Squill	
Sennae folium	Senna Leaf	
Sennae fructus	Senna Fruit	
Serpylli herba	Wild Thyme	
Stramonii folium	Thorn-Apple Leaf	
Strychni semen	Nux Vomica	
Taraxaci radix	Dandellion Root	
Thymi vulgaris herba	Thyme	
Tiliae flos	Lime Blossom	
Tragacantha	Tragacanth	
Trifolii fibrini folium	Buckbean Leaf	
Uvae ursi folium	Bearberry Leaf	
Valerianae rhizoma et radix	Valeriana Root	
Veratri rhizoma	White Veratrum Rhizome	
Verbasci flos	Verbascum Flower	

III PHARMACEUTICAL PREPARATIONS

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Acidum aceticum dilutum 20%	Dilute Acetic Acid, 20 per cent	
Acidum hydrochloricum dilutum 10%	Dilute Hydrochloric Acid, 10 per cent	
Acidum phosphoricum dilutum 10%	Dilute phosphoric Acid, 10 per cent	
Acidum trichloraceticum solutum 50%	Trichloroacetic Acid Solution, 50 per cent	
Aluminium aceticum tartaricum solutum	Aluminium Acetate Tartarate Solution	
Ammonia soluta 10%	Dilute Ammonia Solution, 10 per cent	
Aqua calcis	Calcium Hydroxide Solution	
Aqua destillata pro injectione	Water for injection	Aqua pro Injectione
Axerophtholum solutum	Axerophthol Solution	
Azophenum coffeinum citricum	Phenazone with Caffeine and Citric Acid	
Bacillus radioyttrii (^{90}Y) oxydati	Radioyttrium Oxide Rod (^{90}Y)	
Barium sulfuricum conditum	Flavoured Barium Sulphate	
Benzalkonium chloratum solutum 10%	Benzalkonium chloride Solution, 10 per cent	Benzalkonii Chloridi Solutio
Calcium bromatum solutum 33.3%	Calcium Bromide Solution, 33.3 per cent	
Calcium chloratum solutum 50%	Calcium Chloride Solution, 50 per cent	
Coffeinum citricum	Caffeine and Citric Acid	
Coffeinum natrium benzoicum	Caffeine and Sodium Benzoate	Coffeinum et Natrii Benzoas

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Collemplastrum adhaesivum	Adhesive Plaster	
Collemplastrum salicylatum	Adhesive Plaster with Salicylic Acid	
Collodium flexile	Flexible Collodion	
Diluendum benzaldehydi	Concentrated Benzaldehyde Solution	
Diluendum foeniculi	Concentrated Fennel Oil Solution	
Diluendum menthae	Concentrated Peppermint Oil Solution	
Elixirium thymi compositum*	Compound Thyme Elixir	
Elixirium tonisans	Tonic Elixir	
Emplastrum diachylon*	Diachylon Plaster	
Ergocalciferolum solutum	Ergocaliferol Solution	
Extractum aloës siccum	Aloes Dry Extract	
Extractum belladonnae siccum	Belladonna Leaf Dry Extract	
Extractum liquiritiae fluidum	Liquorice Fluid Extract	
Extractum liquiritiae venale	Crude Liquorice Dry Extract	
Extractum strychni siccum	Nux Vomica Dry Extract	
Ferrum sesquichloratum solutum 50%	Iron(III) Chloride Solution, 50 per cent	
Granulatum calcii phosphorici	Calcium Phosphate Granulate	
Granulatum carbonis activati	Activated Charcoal Granulate	
Granulatum magnesii trisilicici	Magnesium trisilicate Granulate	
Hydrogelum methylcellulosi	Methylcellulose Hydrogel	
Hydrogenium peroxydatum dilutum 3%	Hydrogen Peroxide Solution, 3 per cent	
Infusio glucosi	Dextrose Infusion	
Infusio glucosi cum kalio	Dextrose Infusion with Potassium	
Infusio glucosi salina	Saline Dextrose Infusion	
Infusio invertosi 10%	Invertose Infusion, 10 per cent	
Infusio natrii bicarbonici	Sodium Bicarbonate Infusion	
Infusio natrii chlorati	Sodium Chloride Infusion	Natrii Chloridi Injectio
Infusio natrii lactici	Sodium Lactate Infusion	
Infusio natrii lactici cum kalio	Sodium Lactate Infusion with Potassium	
Infusio natrii lactici salina	Saline Sodium Lactate Infusion	
Infusio salina	Compound Saline Infusion	
Injectio acetyldigitoxini	Acetyldigitoxin Injection	
Injectio acidi ascorbici 10%	Ascorbic Acid Injection, 10 per cent	
Injectio acidi nicotinici	Nicotinic Acid Injection	
Injectio adrenalini 0.1%	Adrenaline Injection, 0.1 per cent	Adrenalini Injectio
Injectio aminophyllini	Aminophylline Injection	
Injectio amphethamini phosphorici	Amphetamine Phosphate Injection	
Injectio atropini sulfurici 0.1%	Atropine Sulphate Injection, 0.1 per cent	
Injectio axerophtholi oleosa	Axeropthol Oily Injection	
Injectio benzylpenicillinkalii	Benzylpenicillin-Potassium Injection	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from he titles of the Hungarian Pharmacopoeia VI
Injectio benzylpenicillinprocaini	Benzylpenicillin-Procaine Injection	
Injectio calcii gluconici	Calcium Gluconate Injection	Calcii Gluconatis Injectio
Injectio chlorochini phosphorici	Chloroquine Phosphate Injection	
Injectio chloropyramini hydrochlorici	Chloropyramine Hydrochloride Injection	
Injectio chlorpromazini hydrochlorici	Chloropromazine Hydrochloride Injection	Injectio Chlorpromazini Hydrochlorici
Injectio cholecalciferoli oleosa	Cholecalciferol Oily Injection	
Injectio coffeini natrii benzoici 20%	Caffeine and Sodium Benzoate Injection	Coffeini et Natrii Benzoatis Injectio
Injectio corticotrophini	Corticotrophin Injection	
Injectio cyanocobalamini	Cyanocobalamin Injection	
Injectio desoxycortoni acetici oleosa	Desoxycortone Acetate Injection	Desoxycortoni Acetatis Injectio
Injectio digoxini	Digoxin Injection	Digoxini Injectio
Injectio ephedrini hydrochlorici	Ephedrine Hydrochloride Injection	
Injectio ergocalciferoli oleosa	Calciferol Injection	
Injectio ergometrini maleinici	Ergometrine Injection	Ergometrini Maleatis Injectio
Injectio ergotamini tartarici	Ergotamine Injection	Ergotamini Tartaratis Injectio
Injectio glucosi 20%	Dextrose Injection, 20 per cent	
Injectio glucosi 40%	Dextrose Injection, 40 per cent	
Injectio gonadotrophini chorionici	Chorionic Gonadotrophin Injection	
Injectio heparini	Heparin Injection	
Injectio hexobarbital natrii	Sodium Hexobarbital Injection	
Injectio hydrocortisoni acetici	Hydrocortisone Acetate Injection	
Injectio insulini	Insulin Injection	Insulini Injectio
Injectio lidocaini hydrochlorici	Lidocaine Hydrochloride Injection	Lidocaini Hydrochloridi Injectio
Injectio menachinoni	Menadione Sodium Bisulphite Injection	
Injectio mercamphamidi	Mercamphamide Injection	
Injectio methadoni hydrochlorici	Methadone Hydrochloride Injection	
Injectio morphini hydrochlorici 1%	Morphine Hydrochloride Injection, 1 per cent	
Injectio morphini hydrochlorici 2%	Morphine Hydrochloride Injection, 2 per cent	
Injectio morphini hydrochlorici 3%	Morphine Hydrochloride Injection, 3 per cent	
Injectio morphini hydrochlorici 2% cum atropino sulfurico 0.05%	Morphine Hydrochloride 2 per cent, and Atropine Sulphate 0.05 per cent Injection	
Injectio nalorphini hydrobromici	Nalorphine Hydrobromide Injection	
Injectio natrii chlorati 10%	Sodium Chloride Injection, 10 per cent	
Injectio natrii citrici, 3.3%	Sodium Citrate Injection, 3.3 per cent	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Injectio natrii nitrosi, 4%	Sodium Nitrite Injection, 4 per cent	
Injectio natrii nitrosi, 10%	Sodium Nitrite Injection, 10 per cent	
Injectio natrii radiochromici (^{51}Cr)	Sodium Radiochromate (^{51}Cr) Injection	
Injectio neostigminii methylsulfurici	Neostigmine Injection	Neostigmini Methylsulfatis Injectio
Injectio noradrenalini bitartarici	Noradrenaline Bitartarate Injection	
Injectio oxytocini	Oxitocin Injection	Oxytocini Injectio
Injectio papaverini hydrochlorici 4%	Papaverine Hydrochloride Injection, 4 per cent	
Injectio pentetrazoli	Pentetrazol Injection	Pentetrazoli Injectio
Injectio pethidini hydrochlorici	Pethidine Hydrochloride Injection	Pethidini Hydrochloridi Injectio
Injectio pholedrini	Pholedrine Injection	
Injection procaini hydrochlorici 2%	Procaine Hydrochloride Injection, 2 per cent	
Injectio procaini hydrochlorici 4%	Procaine Hydrochloride Injection, 4 per cent	
Injectio procaini hydrochlorici 2% cum adrenalino 0.005%	Procaine Hydrochloride, 2 per cent, and Adrenaline, 0.005 per cent, Injection	
Injectio procaini hydrochlorici 4% cum adrenalino 0.005%	Procaine Hydrochloride, 4 per cent, and Adrenaline, 0.005 per cent, Injection	
Injectio progresteroni oleosa	Progesterone Injection	Progesteroni Injectio
Injectio promethazini hydrochlorici	Promethazine Hydrochloride Injection	
Injectio pyridoxini hydrochlorici	Pyridoxine Hydrochloride Injection	
Injectio radioauri (^{198}Au) colloidalis	Colloidal Radiogold (^{198}Au) Injection	
Injectio reserpini	Reserpine Injection	
Injectio riboflavini	Riboflavine Injection	Riboflavini Injectio
Injectio rubri Congo 1% A. U. V.	Congo Red Injection for Veterinary Use, 1 per cent	
Injectio streptomycini sulfurici	Streptomycin Sulphate Injection	
Injectio strychnini nitrici 0.1%	Strychnine Nitrate Injection, 0.1 per cent	
Injectio sympaethamini	Sympaethamine Injection	
Injectio thiamini	Thiamine Injection	
Injectio tocopheroli acetici oleosa	Tocopherol Acetate Injection	
Injectio tolazolini hydrochlorici	Tolazoline Hydrochloride Injection	
Injectio zinc-insulini protaminati	Protamine Zinc Insulin Injection	Insulini Zinci Protaminati Injectio
Linimentum saponatum camphoratum	Camphorated Soap Jelly	
Liquor-formaldehydi saponatus	Formaldehyde Solution with Soap	
Massa polyoxaetheni	Polyoxethene Mass	

Latinized tlties of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Mixtura chloralobromata*	Chloral and Bromide Mixture	
Mixtura pectoralis	Expectorant Mixture	
Mucilago gummi arabici*	Acacia Miculage	
Mucilago methylcellulosi	Methylcellulose Mucilage	
Natrium bromatum solutum 33.3%	Sodium Bromide Solution, 33.3 per cent	
Natrium lacticum solutum 20% pro infusione	Sodium Lactate Solution for Infusion, 20 per cent	
Oculentum hydrosum*	Hydrous Ophthalmic Ointment	
Oculentum simplex*	Simple Ophthalmic Ointment	
Oleum pro injectione	Oil for Injection	
Pasta zincini oleosa	Oily Zinc Oxide Paste	
Pasta zinci oxydati	Zinc Oxide Paste	
Pasta zinci oxydati salicylata	Zinc Oxide Paste with Salicylic Acid	
Phenolum liquefactum	Liquefied Phenol	
Phenomerborum solution 0.1%	Phenomerbor Solution, 0.1 per cent	
Pulvis Caroli	Alkaline Laxative Salt	
Pulvis opii	Powdered Opium	
Pulvis opii et ipecacuanhae	Opium and Ipecacuanha Powder	
Pulvis sennae compositus	Compound Senna Powder	
Sapo kalinus	Potash Soap	
Sapo stearini	Stearic Soap	
Sirupus aurantii	Orange Peel Syrup	
Sirupus cacao	Cocoa Syrup	
Sirupus chloramphenicoli palmitinici	Chloramphenicol Palmitate Syrup	
Sirupus ferri chlorati oxydulati	Ferrous Chloride Syrup	
Sirupus laxans	Laxative Syrup	
Sirupus liquiritiae*	Liquorice Syrup	
Sirupus simplex*	Simple Syrup	
Solutio acidi borici 3%	Boric Acid Solution, 3 per cent	
Solutio adrenalini, 0.1%	Epinephrine Solution	
Solutio anticoagulans	Anticoagulant Solution	
Solutio arsenicalis*	Arsenic Solution	
Solutio calcii sulfurati	Calcium Polysulphide Solution	
Solutio cholinii chlorati	Choline Chloride Solution	
Solutio conservans*	Preservative Solution	
Solutio jodi spirituosa	Alcoholic Iodine Solution	Iodi Solutio Spirituose
Solutio lanatosidi C	Lanatoside C Solution	
Solutio natrii radiojodati ^{131}J	Sodium Radio-Iodide ^{131}I Solution	Natrii Radio-iodidi ^{131}I Solutio
Solutio ophthalmica cum benzalkonio	Ophthalmic Solution with Benzalkonium	
Solutio ophthalmica cum phenomerboro*	Ophthalmic Solution with Phenomerbor	
Solutio pholedrini	Pholedrine Solution	
Solutio sympaethamini	Sympaethamine Solution	
Solvens pro oculoguttis cum benzalkonio	Solvent for Ophthalmic Solutions with Benzalkonium	
Solvens pro oculoguttis cum phenomerboro*	Solvent for Ophthalmic Solutions with Phenomerbor	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Solvens viscosa	Viscous Solvent	
Sparsorium infantum*	Children's Dusting Powder	
Species althaeae		
Species antiasthmatica ad-fumigationem	Antiasthmatic SmokingPowder	
Species cholagoga	Cholagoge Tea	
Species diuretica*	Diuretic Tea	
Species laxans	Laxative Tea	
Spiritus anisatus	Anise Spirit	
Spiritus camphoratus	Camphor Spirit	
Spiritus salicylatus	Salicylic Acid Spirit	
Spiritus saponatus	Soap Spirit	
Suppositorium glycerini*	Glycerin Suppositories	
Suppositorium haemorrhoidale*	Haemorrhoidal Suppositories	
Suspensio siccans	Drying Suspension	
Tabletta acetazolamidi	Acetazolamide Tablets	
Tabletta acetyldigitoxini	Acetyldigitoxin Tablets	
Tabletta acidi acetylsalycilici g 0.5	Acetylsalicylic Acid Tablets	Acidi Acetylsalicylici Compressi
Tabletta acidii ascorbici	Ascorbic Acid Tablets	Acidi Ascorbici Compressi
Tabletta aethionamidi	Ethionamide Tablets	
Tabletta amidazopheni g 0.1	Aminophenazone Tablets, 0.1 g	
Tabletta amidazopheni g 0.3	Aminophenazone Tablets, 0.3 g	
Tabletta ammonii chlorati intestinsolvens g 0.5	Enteric-Coated Ammonium Chloride Tablets, 0.5 g	
Tabletta amobarbitali	Amobarbital Tablets	Amobarbitali Compressi
Tabletta amphetamini phosphorici	Amphetamine Phosphate Tablets	
Tabletta atropini sulfurici mg 1/3	Atropine Sulphate Tablets, 1/3 mg	Atropini Sulfatis Compressi
Tabletta azopheni coffeini citrici g 0.5	Caffeine, Phenazone and Citric Acid Tablets, 0.5 g	
Tabletta barbitali g 0.5	Barbital Tablets, 0.5 g	
Tabletta carbamidi peroxydati g 1	Peroxide-Urea Tablets, 1 g	
Tabletta carbonis activati g 0.25	Charcoal Tablets, 0.25 g	
Tabletta chinini hydrochlorici	Quinine Hydrochloride Tablets	Quinini Hydrochloridi Compressi
Tabletta chloramphenicoli	Chloramphenicol Tablets	
Tabletta chlorochini phosphorici	Chloroquine Phosphate Tablets	Chloroquini Diphosphatis Compressi
Tabletta chlorogenii g 1	Chlorogene Tablets, 1 g	
Tabletta chloropyramini hydrochlorici	Chloropyramine Hydrochloride Tablets	
Tabletta chlorpromazini hydrochlorici	Chloropromazine Hydrochloride Tablets	Chlorpromazini Hydrochloridi Compressi
Tabletta codeini hydrochlorici g 0.01	Codeine Hydrochloride Tablets, 0.01 g	
Tabletta codeini hydrochlorici g 0.02	Codeine Hydrochloride Tablets, 0.02 g	
Tabletta cyclobarbitali	Cyclobarbital Tablets	
Tabletta cycloserini bitartarici	Cycloserine Hydrogen Tartarate Tablets	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Tabletta dienoestroli	Dienestrol Tablets	Dienestroli Compressi
Tabletta digitoxini	Digitoxin Tablets	Digitoxini Compressi
Tabletta digoxini	Digoxin Tablets	Digoxini Compressi
Tabletta disulfirami	Disulphiram Tablets	
Tabletta ephedrini hydrochlorici	Ephedrine Hydrochloride Tablets	Ephedrini Hydrochloridi Compressi
Tabletta hexamethylentetramini g 0.5	Hexamine Tablets, 0.5 g	
Tabletta hydrochlorothiazidi	Hydrochlorothiazide Tablets	Hydrochlorothiazidi Compressi
Tabletta jodchloroxychinolini	Iodochlorhydroxyquin Tablets	
Tabletta lanatosidi C	Lanatoside C Tablets	Lanatosidi C Compressi
Tabletta liothyronini hydrochlorici	Lyothyronine Hydrochloride Tablets	
Tabletta methadoni hydrochlorici	Methadone Hydrochloride Tablets	
Tabletta methandrostenoloni	Methandienone Tablets	
Tabletta methimazoli	Methimazole Tablets	
Tabletta methylphenidati hydrochlorici	Methylphenidate Hydrochloride Tablets	
Tabletta natrii paraaminosalicylici	Sodium Aminosalicylate Tablets	Natrii Para-aminosalicylatis Compressi
Tabletta natrii salicylici intestinosolvens g 0.5	Enteric-Coated Sodium Salicylate Tablets, 0.5 g	
Tabletta neostigminii bromati	Neostigmine Bromide Tablets	Neostigmini Bromidi Compressi
Tabletta nicotinamidi	Nicotinamide Tablets	Nicotinamidi Compressi
Tabletta nitroglycerini mg 0.5	Glyceryl Trinitrate Tablets, 0.5 mg	
Tabletta papaverini hydrochlorici	Papaverine Hydrochloride Tablets	
Tabletta pethidini hydrochlorici	Pethidine Hydrochloride Tablets	Pethidini Hydrochloridi Compressi
Tabletta phenobarbitali g 0.015	Phenobarbital Tablets, 0.015 g	
Tabletta phenobarbitali g 0.1	Phenobarbital Tablets, 0.1 g	
Tabletta phenolphthaleini g 0.2	Phenolphthalein Tablets, 0.2 g	
Tabletta phenolphthaleini g 0.5	Phenolphthalein Tablets, 0.5 g	
Tabletta prednisoloni	Prednisolone Tablets	Prednisoloni Compressi
Tabletta promethazini hydrochlorici	Promethazine Hydrochloride Tablets	Promethazini Hydrochloridi Compressi
Tabletta pyridoxini hydrochlorici	Pyridoxine Hydrochloride Tablets	
Tabletta reserpini	Reserpine Tablets	
Tabletta saccharimidi	Saccharin Tablets	
Tabletta sulfadimidini g 0.5	Sulphadimidine Tablets, 0.5 g	Sulfadimidini Compressi
Tabletta sulfaguanidini g 0.5	Sulphaguanidine Tablets, 0.5 g	Sulfaguanidini Compressi
Tabletta sulfamethoxypyridazini g 0.5	Sulphamethoxypyridazine Tablets, 0.5 g	Sulfamethoxypiridazini Compressi
Tabletta thiamini	Thyamine Tablets	
Tabletta thyreoidei	Thyroide Tablets	
Tabletta tocopheroli acetici	Tocopherol Acetate Tablets	
Tabletta tolazolini hydrochlorici	Tolazoline Hydrochloride Tablets	

Latinized titles of the monographs	English titles of the monographs	Titles of the International Pharmacopoeia differing from the titles of the Hungarian Pharmacopoeia VI
Tabletta trimetozini	Trimetozine Tablets	
Tinctura amara	Bitter Tincture	
Tinctura aromatica	Aromatic Tincture	
Tinctura aurantii	Orange Peel Tincture	
Tinctura aurantii pro sirupo	Orange Peel Tincture for Syrup	
Tinctura belladonnae	Belladonna Tincture	
Tinctura benzoes	Benzoin Tincture	
Tinctura cantharidis	Cantharides Tincture	
Tinctura capsici	Capsicum Tincture	
Tinctura chamomillae	Chamomille Tincture	
Tinctura chinae composita*	Compound Cinchona-Bark Tincture	
Tinctura ipecacuanhae	Ipecacuanha Tincture	Ipecacuanhae Tinctura
Tinctura opii	Opium Tincture	Opii Tinctura
Tinctura ratanhiae	Krameria Tincture	
Tinctura saponariae	Saponaria Tincture	
Tinctura strychni	Nux Vomica Tincture	
Tinctura thymi*	Thyme Tincture	
Tinctura valerianae aetherea	Ethereal Valeriana Tincture	
Tinctura valerianae spirituosa	Alcoholic Valeriana Tincture	
Tinctura veratri	White Veratrum Tincture	
Unguentum aluminii acetici tartarici*	Aluminium Acetate Tartrate Ointment	
Unguentum argenti nitrici	Silver Nitrate Ointment	
Unguentum diachylon*	Diachylon Ointment	
Unguentum emolliens*	Emollient Ointment	
Unguentum emulsificans anionicum*	Anionic Emulsifying Ointment	
Unguentum emulsificans non-ionicum*	Non-Ionic Emulsifying Ointment	
Unguentum glycerini*	Glycerin Ointment	
Unguentum hexachloropheni 1%	Hexachlorophene Ointment, 1 per cent	
Unguentum hydrargyri	Mercury Ointment	
Unguentum hydrophilicum anionicum*	Anionic Hydrophilic Ointment	
Unguentum hydrophilicum non-ionicum*	Non-Ionic Hydrophylic Ointment	
Unguentum hydrosum*	Hydrous Ointment	
Unguentum naphthalani	Naphthalan Ointment	
Unguentum oleosum*	Oily Ointment	
Unguentum polyoxaetheni	Polyoxethene Ointment	
Unguentum simplex*	Simple Ointment	
Unguentum stearini*	Stearine Ointment	
Unguentum zinci oxydati*	Zinc Oxide Ointment	
Vaselinum acidi borici*	Boric Acid Ointment	
Zincum chloratum solutum 50%	Zinc Chloride Solution, 50 per cent	
Zincum gelatinosum	Zinc Gelatin	

IV SEROBIOLOGICAL PREPARATIONS FOR HUMAN USE

Sera humana pro haemagglutinatione
Sera humana pro haemagglutinatione cryodehydrata
Serum antianthracicum equinum
Serum antidiphthericum
Serum antigangraneosum equinum tetravalens
Serum antigangraenosum equinum tetravalens cryodehydratum
Serum antirhusiopathicum equinum
Serum antirhusiopathicum suillum
Serum antitetanicum
Serum bovinum normale
Serum equinum normale
Serum humanum anti-D (anti-Rh_0) pro diagnosi
Serum ovinum normale
Toxinum diphthericum pro diagnosi
Toxinum scarlatinosum assorptum
Toxinum scarlatinosum pro diagnosi
Toxoidum diphthericum
Toxoidum diphtherico-tetanicum assorptum
Toxoidum tetanicum assorptum
Tuberculinum pristinum sine albumoso paratum
Tuberculinum purificatum cryodehydratum
Vaccinum BCG cryodehydratum
Vaccinum cholericum
Vaccinum contra rabiem
Vaccinum diphtherico-pertussico-tetanicum assorptum
Vaccinum febris exanthematicae cryodehydratum
Vaccinum paratyphosum-A cryodehydratum
Vaccinum paratyphosum-B (S. Schottmülleri) cryodehydratum
Vaccinum pertussicum
Vaccinum pertussicum assorptum
Vaccinum typhoso-tetanicum assorptum
Vaccinum typhosum-assorptum
Vaccinum typhosum purificatum cryodehydratum
Vaccinum variolae

V SEROBIOLOGICAL PREPARATIONS FOR VETERINARY USE

Anatoxinum contra tetanum aluminio hydroxydato assorptum A.U.V.
Malleinum A.U.V.
Serum contra anthracem A.U.V.
Serum contra aphthas epizooticas ex animalibus hyperimmunisatione paratum A.U.V.
Serum contra enteritidem necrositantem infectiosam porcellorum A.U.V.
Serum contra febrem canum catarrhalem A.U.V.
Serum contra febrem canum catarrhalem et hepatitidem infectiosam canum A.U.V.
Serum contra hepatitidem infectiosam canum A.U.V.
Serum contra morbum Aujeszkyi A.U.V.
Serum contra pasteurellosem A.U.V.
Serum contra pestem suillam A.U.V.
Serum contra pestem suillam et partim (30%) contra rhusiopathiam A.U.V.
Serum contra rhusiopathiam A.U.V.
Tuberculinum A.U.V.
Vaccinum contra abortum infectiosum A.U.V.
Vaccinum contra anthracem assorptum A.U.V.
Vaccinum contra anthracem saponino paratum A.U.V.
Vaccinum contra aphthas epizooticas A.U.V.
Vaccinum contra choleram avium assorptum A.U.V.

Vaccinum contra enteritidem necrotisantem infectiosam porcellorum A.U.V.
Vaccinum contra enterotoxaemiam ovium sorptum A.U.V.
Vaccinum contra febrem canum catarrhalem A.U.V.
Vaccinum contra gangraenam emphysematosam A.U.V.
Vaccinum contra hepatitidem infectiosam canum A.U.V.
Vaccinum contra morbum Aujeszkyi A.U.V.
Vaccinum contra paratyphum columbarum A.U.V.
Vaccinum contra pestem avium A.U.V.
Vaccinum contra pestem suillam viro alpinisato paratum A.U.V.
Vaccinum contra rabiem A.U.V.
Vaccinum contra rhusiopathiam assorptum A.U.V.
Vaccinum contra stomatitidem pustulosam contagiosam ovium A.U.V.
Vaccinum contra variolam avium A.U.V.
Vaccinum contra variolam ovinam A.U.V.

VI BLOOD PREPARATIONS

Holosanguis humanus conservatus
Massa erythrocytae
Massa erythrocytae lota
Plasma humanum individuale liquidum
Plasma humanum individuale congelatum
Plasma humanum individuale cryosiccatum
Plasma humanum mixtum liquidum
Plasma humanum mixtum congelatum
Plasma humanum mixtum cryosiccatum
Sanguis granulocytis thrombocytisque spoliatus
Suspensio thrombocytaea

VII SURGICAL DRESSINGS AND SUTURES

Chorda siccata
Chorda sterilisata
Fascia cum calcio sulfurico usto
Fascia e tela parata
Fascia separatim texta
Filum linteum
Filum sericeum
Filum sericeum sterilisatum
Lana gossypii
Lana ligni
Lana ophthalmica
Tela

List of monographs included in the Hungarian Pharmacopoeia V but not Admitted to the Hungarian Pharmacopoeia VI

I CHEMICAL SUBSTANCES

Acetum pyrolignosum rectificatum
Acetylcholinum bromatum
Acidum hydrargyro-salicylicum
Acidum nitricum concentratum
Acidum nitricum dilutum 10%
Acidum nitricum fumans
Acidum sulfuricum concentratum
Acidum sulfuricum dilutum 10%
Aether aceticus
Aether petrolei
Alcohol absolutus
Alcohol cetylicus
Allylbarbituralum
Ammonium bicarbonicum
Ammonium phosphoricum
Aqua chlorata

Bismuthum oxyjodogallicum
Bismuthum tribromphenylicum

Calcaria chlorata
Calcium hypophosphorosum
Carbamylcholinum chloratum
Chininum bihydrochloricum
Chininum bisulfuricum
Chloroformium ad narcosim
Chlorphenanum
Cholesterinum

Diacetylmorphinum hydrochloricum
Dicumarolum
Diphentoinnatrium

l-Ephedrinum hydrochloricum
Eucalyptolum

Ferrum albuminatum
Ferrum oxalicum oxydulatum

Gametocidum
Gonadotropinum e sero

Hexacitraminum
Hexylresorcinum
L-Histidinum hydrochloricum
Hydrargyrum bichloratum corrosivum
Hydrargyrum oxycyanatum
Hypophyseos partis posterioris extractum

Jodoformium
Jodophthaleinnatrium
Jodoxysulfochinolinum

Kalium bicarbonicum
Kalium bitartaricum
Kalium chloricum
Kalium guajacolsulfonicum
Kresolum crudum

Manganum chloratum

Natrium anilarsonicum
Natrium arsenicicum
Natrium methylarsonicum
Novarsenolum

Phenarsoxydum hydrochloricum
Phenisatinum
Phenylium salicylicum
Physostigminium sulfuricum
Pix fagi
Plumbum aceticum
Plumbum carbonicum hydroxydatum
Proguanilum hydrochloricum
Pyrogallolum

Santoninum
Stibium kalium tartaricum
G-Strophanthinum
Sulfamethylpyrimidinum
Sulfamethylthiazolum
Sulfanilamidum
Sulfathiazolum

Tetrachloraethylenum

FATS, WAXES AND MATERIALS OF SIMILAR CONSISTENCY

Adeps suillus
Cera flava
Oleum sesami
Sebum

ESSENTIAL OILS

Oleum aurantii
Oleum bergamottae
Oleum chenopodii
Oleum geranii
Oleum rosmarini
Oleum terebinthinae
Oleum thymi

II VEGETABLE AND ANIMAL DRUGS

Amygdala dulcis
Amylum maydis

Cardamomi fructus
Colocynthidis fructus
Colophonium

Digitalis lanatae folium

Filicis maris rhizoma

Galla
Graminis rhizoma

Jalapae resina
Jalapae tuber

Kamala depuratum

Lacca in tabulis
Lichen islandicus

Manna
Mastix
Mel

Podophyllinum

Salep tuber
Scillae bulbus
Secale cornutum
Senegae radix
Sinapis nigrae semen
Strophanthi semen

Terebinthina communis
Terebinthina laricina
Tormentillae rhizoma

III PHARMACEUTICAL PREPARATIONS

Adeps lanae hydrosus
Aqua benzaldehydi
Aqua cinnamomi spirituosa
Aqua foeniculi
Aqua Goulardi
Aqua menthae piperitae
Aqua plumbica

Charta sinapisata
Chininum tannicum Rozsnyai
Coffeinum natrium salicylicum
Collemplastrum hydrargyri
Collemplastrum saponatum salicylatum
Collodium cum acido salicylico

Decoctum saponariae

Emplastrum hydrargyri
Emulsio benzylii benzoici
Emulsio oleosa
Emulsio olei jecoris
Emulsio olei ricini
Emulsio paraffini cum phenolphthaleino
Extractum frangulae fluidum
Extractum frangulae siccum
Extractum gentianae spissum
Extractum liquiritiae spissum
Extractum opii siccum
Extractum rhei siccum
Extractum secalis cornuti fluidum
Extractum stramonii siccum

Faex compressa
Faex praeparata siccata
Faex praeparata spissa

Infusum frangulae compositum
Infusum ipecacuanhae
Infusum sennae compositum
Injectio arsenicalis 1% cum procaino hydrochlorico
Injectio atropini sulfurici 0.05%
Injectio benzpropamini phosphorici 1%
Injectio bismuthi subsalicylici oleosa 10%
Injectio calcii bromati 10%
Injectio calcii chlorati 10%
Injectio camphorae aetherea 20%
Injectio camphorae oleosa 20%
Injectio chinini hydrochlorici 15% et urethani 15%
Injectio coffeini natrii salicylici 20%

Injectio glucosi 10%
Injectio hypophyseos partis posterioris
Injectio isotonica Ringeri
Injectio morphini hydrochlorici 1% cum atropino sulfurico 0.05%
Injectio natrii bromati 10%
Injectio natrii chlorati isotonica
Injectio natrii jodati 10%
Injectio natrii nitrosi 8%
Injectio natrii salicylici 10%
Injectio natrii thiosulfurici 10%
Injectio opii concentrati 2%
Injectio opii concentrati 4%
Injectio g-penicillin-kalii oleosa
Injectio g-penicillin-natrii
Injectio g-penicillin-natrii oleosa
Injectio g-penicillin-procaini oleosa
Injectio riboflavini 0.5%
Injectio sulfamethylpyrimidini 20%
Injectio sulfamethylthiazoli
Injectio sulfathiazoli 20%
Injectio testosteronoli propionici

Kalium sulfuratum crudum

Linimentum ammoniatum
Linimentum calcis
Liquor ferri albuminati saccharatus
Liquor kresoli saponatus

Mucilago ad catheterem
Mucilago gummi arabici
Mucilago salep
Mucilago tragacanthae

Novarsenolum pro injectione in ampulla

Oculentum album 1%
Oculentum flavum 1%
Oculogutta argenti
Oculogutta atropini
Oculogutta physostigmini fortior
Oculogutta physostigmini mitior
Oculogutta pilocarpini
Oculogutta resorcini
Oculogutta zinci
Oculogutta zinci cum adrenalino
Opium concentratum

Phenarsoxydum hydrochloricum pro injectione in ampulla
Pilula ferri sulfurici oxydulati
Pilula laxans
Plumbum aceticum basicum solutum
Pulvis dentrificius albus
Pulvis frangulae compositus

Sapo kalinus venalis
Sebum salicylatum
Sirupus hypophosphorosus compositus
Sirupus kalii guajacolsulfonici
Sirupus rubi idaei
Solutio colophonii
Solutio fuchsini cum resorcino
Solutio jodi acida
Solutio natrii chlorati isotonica
Solutio strophanthini 0.5%
Sparsorium sulfaboricum
Spiritus aethereus
Spiritus ammoniatus anisatus
Spiritus dentifricius
Suppositorium mercamphamidi

Tabletta hydrargyri bichlorati corrosivi g 1
Tabletta hydrargyri oxycyanati g 1
Tabletta natrii bromati effervescens g 1
Tabletta natrii chlorati g 0.9
Tabletta opii concentrati g 0.01
Tabletta paraformaldehydi g 1
Tabletta phenacetini g 0.5
Tabletta phenamidi g 0.5
Tabletta proguanili hydrochlorici
Tabletta santonini g 0.025
Tabletta sulfamethylpyrimidini g 0.5
Tabletta sulfanilamidi g 0.5
Tabletta sulfathiazoli g 0.5
Tabletta theobromini jodati
Tinctura digitalis
Tinctura gallae
Tinctura gentianae
Tinctura pomi ferrata
Tinctura strophanthi
Tinctura tormentillae

Unguentum cantharidatum veterinarium
Unguentum hydrargyri 30%
Unguentum infantum
Unguentum plumbi tannici
Unguentum sulfuratum flavum
Unguentum sulfuratum fuscum

Vaselinum cholesterinatum
Vinum album
Vinum rhei

IV SURGICAL DRESSING

Tela sec. Billroth

Classification of the Pharmacopoeia

The first volume of the Pharmacopoeia summarizes the general rules for the preparation and qualification of pharmaceuticals and the generally used test and assay methods. "Tables of the Hungarian Pharmacopoeia VI" (henceforth Vol. IV) is essentially an appendix of Volume I.

Volume I consists of four sections: Principles, Tests and Assays, Substances, Preparations and Reagents.

The section "*Principles*" includes, besides the principles of Hungarian Pharmacopoeia VI, general notices.

The section "*Tests and Assays*" includes methods for Physical Tests and Determinations (I), Physicochemical Tests and Determinations (II), Chemical Tests and Assays (III), Biological and Microbiological Tests and Assays (IV) and Tests and Assays of Containers (V).

The section "*Substances and Preparations*" classifies the general notices concerning the storage and the special test and assay methods for Chemical Substances (I), Vegetable and Animal Drugs (II), Pharmaceutical Preparations (III), Serobiological Preparations, Diagnostics and Blood Preparations (IV) and Surgical Dressings and Sutures (V).

The section "*Reagents*" deals with the composition and preparation of reagents test and volumetric solutions, etc., required for the tests and assays of the Pharmacopoeia. Volumes II and III include monographs in the following classification:

I Chemical Substances
II Vegetable and Animal Drugs
III Pharmaceutical Preparations
IV Serobiological Preparations for Human Use
V Serobiological Preparations for Veterinary Use
VI Blood Preparations
VII Surgical Dressings and Sutures

The Essential Oils (*aetherolea*), Fats, Waxes and Materials of Similar Consistency are classified among the Chemical Substances.

The monographs of the individual sections are listed alphabetically according to the order of their Latinized titles.

The structure of the individual monographs is presented in the following.

I CHEMICAL SUBSTANCES

1 *Title and formula* — Designation of strength of action (+), main title in Latin and its abbreviation, English title, synonyms (Latin synonyms), registered trade names ®, structural formula, chemical name, empirical formula, molecular weight.

2 *Required Content of Active Ingredient in per cent or in Units.*

3 *Properties* — Description (consistency, taste, colour, odour, etc.), solubility, reaction, melting-range, etc. (In the case of pharmaceuticals of vegetable origin, the scientific name of the plant.)

4 *Physical Indexes* — Specific gravity, melting-point, boiling-point, optical rotation, refractive index, light absorption, viscosity, etc.

5 *Identification.*

6 *Qualitative Tests* — Insoluble and colouring matter, acidity, alkalinity, tests for contaminant kations and anions, special tests, test for readily carbonizable substances.

7 *Quantitative Tests* — Loss on drying, residue on ignition, etc., chemical indexes (acid value, iodine value, etc.), chemical and biological assay,s respectively.

8 *Informative Tests.*

9 *Notices referring to tests and assays.*

10 *Storage, Expiration.*

11 *Incompatibilities.*

12 *Dose* (Maximal and usual doses).

13 *Eventual notices, references to statutes.*

II VEGETABLE AND ANIMAL DRUGS

1 *Title* — Designation of strength of action (✠), Latinized name of the drug as main title and its abbreviation; English title, Latin synonym. In the Latinized title the name of the plant precedes the denomination of the part of the plant used.

2 *Scientific* (and in some cases also the English) *name* of the plant, family and occurrence.

3 *Required Content of Active Ingredient in per cent.*

4 *Description* — odour, taste, macroscopical description.

5 *Microscopical Description.*

6 *Identification.*

7 *Qualitative Tests*—Test for other parts of the plant, tests for foreign matter.

8 *Quantitative Tests and Assays*—Determination of water, ash, sand, aqueous (alcoholic) extractives, assay of active ingredients.

9 *Storage, Expiration.*

10 *Dose.*

11 *Notices.*

III PHARMACEUTICAL PREPARATIONS

The structure of the monographs on pharmaceutical preparations is identical with that of group I, except that the description of the mode of preparation precedes that of the properties. The latter is followed by the physical indexes and the subsequent tests and assays.

IV SEROBIOLOGICAL PREPARATIONS FOR HUMAN USE

1 *Title* — Designation of strength of action (✠), main title in Latin and its abbreviation; English title.

2 *Description* — In individual cases the scientific name of the microorganisms. Potency, preservation, packaging.

3 *Tests and Assays* — Sterility tests, safety tests, total microbial count, control of antigen content, protein content, potency assay.

4 *Directions for Expiration and Storage.*

V SEROBIOLOGICAL PREPARATIONS FOR VETERINARY USE

The structure of the monographs is identical with that of group IV.

VI BLOOD PREPARATIONS

1 *Title* — Main title in Latin and its abbreviation; English title.
2 *Description.*
3 *Tests and Assays.*
4 *Directions for Expiration and Storage.*

VII SURGICAL DRESSINGS AND SUTURES

1 *Title* — Main title in Latin and its abbreviation; English title.

2 *Description* — Size, respectively weight and package, directions for sterilization.

3 *Identification.*
4 *Qualitative tests.*
5 *Quantitative Tests and Assays.*
6 *Sterility Tests.*
7 *Storage.*

NOTE: Volumes I, II and III have indexes each at the end listing the most important entries of the individual volumes. The cumulative index at the end of Volume IV (Appendix) contains the most important entries of all four volumes.

Principles

Any material used to influence the living organism with *therapeutic* aim is falling under the term *drug*. Prevention of disease is also considered as a therapeutic intention. The Pharmacopoeia contains the description of the most important and current drugs, pharmaceutical forms, some auxiliary materials used for the preparation of such, as well as surgical dressings, sutures, appliances and diagnostics. It describes methods for the quality control of substances, preparations, etc. and contains directions for the preparation and storage of pharmaceuticals. *The Pharmacopoeia is the official standard for the control and qualification of drugs.*

In quotations of Hungarian Pharmacopoeia VI the term "Pharmacopoeia" is always referring to the Sixth Edition.

For articles official in one of the former Hungarian Pharmacopoeias, but not admitted to the present Edition, those requirements included in the last Hungarian Pharmacopoeia in which the article was official remain valid. For articles not admitted in Hungarian Pharmacopoeia VI, requirements of foreign pharmacopoeias are valid or those prescribed by the National Institute, authorized by the Ministry of Health.

NOMENCLATURE

The Pharmacopoeia generally uses nonproprietary names adopted by the World Health Organisation. According to deep-rooted tradition, but in contrast to the international usage, the latinized titles are kept in attributive construction. For the titles of monographs of vegetable and animal drugs, and for the titles of some of the pharmaceutical preparations, however, the genitive form is used, corresponding to the international denomination. In both cases, the name of the substance and the preparation is in the singular. The international names of items for which different titles have been adopted, the differing names of the International Pharmacopoeia are enumerated in the list of official monographs (Vol. I, p. 27).

The formerly adopted latinized titles have been changed by the Pharmacopoeia only in cases of necessity. From the point of view of pharmaceutical practice, short latinized names are used in many cases, which, even in their abbreviated form, cannot be mistaken and thus are suitable for general usage. For the same purpose, the shortest abbreviation of the full title that may be used by the physician, is given in parentheses under the main title of the monograph. Instead of the long latinized scientific name of some of the organic compounds, the Pharmacopoeia applies an abbreviated latinized name which differs from the registered trade names of the respective compound.

The titles of monographs of Hungarian Pharmacopoeia V changed in the Sixth Edition are included in the respective monographs as synonyms.

ORTHOGRAPHY

The latinized names follow the orthography of the Latin language. In current text this circumstance is indicated by a distinctive type setting, either by the use of italics or by putting the name between quotation marks.

ATOMIC AND MOLECULAR WEIGHTS

The atomic weights used in the Pharmacopoeia are those adopted internationally in 1962 (C = 12.000) (Vol. IV, Table XX).

The molecular weights are the sum of atomic weights rounded off to two decimals. Equivalent weights are calculated from the rounded off molecular weights.

SYMBOLS AND ABBREVIATIONS

Vol. I	Volume I of the Pharmacopoeia
Vol. II	Volume II of the Pharmacopoeia
Vol. III	Volume III of the Pharmacopoeia
Vol. IV	Supplementary volume: "Tables of the Hungarian Pharmacopoeia VI"
AGR	analytical grade reagent
At.Wt.	atomic weight
A.U.V.	*ad usum veterinarium* (drugs for veterinary use)
C.I.	Colour Index, 1956
F	factor
g/g%	per cent weight in weight (g/100 g)
g/v%	per cent weight in volume (g/100 ml)
H.I.	hemolytic index
i.art.	intra-arterial(ly)
i.glut.	intragluteal(ly)
im.	intramuscular(ly)
iv.	intravenous(ly)
I.U.	International Unit(s)
Im. U.	Immunisation Unit(s)
lg	logarithm to the base 10
M	molar
Mol.Wt.	molecular weight
mval	millivalency
N	normal
pH	hydrogen exponent
Ph.Hg.V.	Hungarian Pharmacopoeia V
p.	part (weight); and page
per rect.	per rectum

pro inhal.	for inhalation
pv.	part, volume
qu.s.	quantum satis (a proper amount)
®	Registered trade name (used as an upper index)
sc.	subcutaneous(ly)
torr	mm. mercury column
v/v%	per cent volume in volume

Abbreviations of metric units are given in the chapter *Physical Tests and Determinations* (Vol. I, p. 60).

GENERALLY USED EXPRESSIONS

Where *water* is called for in the text of the Pharmacopoeia, unless otherwise specified, *distilled water* of room temperature is to be used. The term solution denotes *aqueous solution.*

"Boiled and cooled water" is distilled water that has been boiled vigorously for 5 minutes and cooled.

Where *alcohol* is called for in the text of the Pharmacopoeia, unless otherwise specified, *Alcohol* (*90 per cent*) (*spiritus concentratus*) should be used.

The term *per cent,* used without qualifications means, for drugs per cent weight in weight, for reagent solutions usually per cent weight in volume. All statements of percentage of alcohol — whether used as ingredient of a drug or as reagent — refer to per cent volume in volume, at 15°.

All temperatures are expressed in centigrades (Celsius) (°). For the denomination of approximative temperatures the following terms are used:

cold	2 to 5°
cool	5 to 15°
room temperature	15 to 25°
warm (water)	60 to 70°
hot (water)	85 to 90°
water- or steam-bath about	100°

The term "*gentle heating*" means heating at approximately 50°.

The term "*on a water-bath*" means heating on a bath of boiling water; "*in a water bath*" means heating immersed in a bath of boiling water.

The concentration of solutions is designated by the Pharmacopoeia by 1 + 9; 1 + 19 etc., meaning that 1 part by weight of the substance is to be dissolved in 9 parts by weight of the solvent, etc.

The approximative solubility of the pharmacopeial substances is indicated by the descriptive terms enumerated in Table 1.

TABLE 1

Descriptive term of solubility	Parts of Solvent Required for 1 Part of Solute (at room temperature)
Very soluble	< 1
Freely soluble	1–10
Moderately soluble	10–100
Slightly soluble	100–1000
Very slightly soluble	1000–10 000
Practically insoluble	< 10 000

TABLE 2

Reaction	pH	Indicator	Acidic ⟶ Alkaline Colour		
Strongly acidic	2.0	Thymol Blue	red	→	orange
		Thymol Blue	orange	→	yellow
Acidic	2.0–5.0	Methyl Red	red	→	onion-red
		Methyl Red	onion-red	→	yellow
Weekly acidic	5.0–6.5	Bromothymol Blue	yellow	→	greenish-yellow
Neutral	6.5–7.5	Bromothymol Blue	greenish-yellow	→	greenish-blue
		Phenol Red	yellow	→	pink
Weekly alkaline	7.5–9.0	Phenol Red	pink	→	red
		Thymol Blue	yellow		greenish-grey
Alkaline	9.0–12.0	Thymol Blue	greenish-grey		blue
		Alizarin Yellow	yellow	→	onion-red
Strongly alkaline	< 12.0	Alizarin Yellow	onion-red	→	red

Acidity and alkalinity (reaction) are characterized approximatively by the terms listed in Table 2.

The letter R-(reagent) before the name of the reagent means a solution of exact concentration or a reagent of special quality (Vol. I, p. 265).

The letter I-(indicator) before the name of a solution or reagent designates an indicator solution or indicator, respectively.

SYMBOL OF PHARMACEUTICALS IN RELATION TO POTENCY

Potent drugs, designated by the symbol ✠ and hypnotics by ✠ respectively, placed before the main title, must be stored separated from other drugs. Drugs for which storage in the poison-cabinet is compulsory are designated by the symbol ✠✠ and narcotic drugs by the symbol ✠✠, respectively.

Prescription and dispensing of drugs designated by the symbols ✠, ✠, ✠✠, ✠✠ are subject to special regulations.

SYMBOL OF VETERINARY PHARMACEUTICALS

Drugs used exclusively for veterinary medicinal purposes are distinguished by the letters A.U.V. (*ad usum veterinarium*).

STORAGE

The pharmacopoeial regulations affect mainly storage conditions in the pharmacy. For storage of drugs in bulk and wholesale quantities the sense of the pharmacopoeial regulations should be adapted accordingly. Drugs must be stored as a rule in dry rooms in which the relative humidity does not or only transitionally exceed 60 per cent and where, unless otherwise specified, the temperature should be between 15° to 25°.

Drugs should be preserved according to their properties, in covered or closed containers the material of which does not interact with the article placed in it so as to alter strength, quality or purity of the drug.

The Pharmacopoeia prescribes for the storage of drugs covered, closed, well-closed, hermetic containers and containers or ampoules insuring sterility.

Covered containers protect the contents from mechanical contamination. For this purpose wood or porcelain containers or lined drawers are used.

Closed containers protect the contents even from fine particulate contamination and insect damage (for example: metal containers, jars with bakelite or plastic closures).

Well-closed containers protect the contents from humidity, prevent eventual evaporation and loss of humidity (water of crystallization) for example: bottles with ground stoppers, from which, when shaken in inverted position water does not leak out.

A *hermetic container* insures perfect sealing and excludes the exchange of air. Hermetic sealing can be attained by fusion of glass containers, by the use of rubber closures, by dipping a bottle stoppered with well-fitting corks into melted paraffin, or closures with tight gaskets.

For the preservation and storage of drugs the Pharmacopoeia uses the following special expressions.

Protected from light means that the drugs must be preserved in light-resistant cabinets or containers, and may be stored in standardized amber-coloured glass containers in the dispensing or prescription department of the pharmacy.

In a light place means that the articles must be kept in colourless glass containers in scattered light, protected from direct sunshine.

In a cool place means that the article has to be preserved at temperatures between 5° to 15°.

In a cold place means that the article must be preserved at temperatures between 2° to 5°.

Strong smelling (malodorous) drugs must be stored in a *separate cabinet,* possibly in a cool place.

Drugs designated by the symbols ✠✠ or ✠✠ must be *stored in the poison-cabinet.*

Further pharmacopoeial regulations concerning the storage of drugs may be found in the general chapter of Vol. I; requirements relating to the individual articles are included in the respective monographs.

With the storage of drugs labour-safety measures, anti-inflammatory precautions and special measures required for radioactive materials must also be taken into consideration.

In the prescription department of the pharmacy, generally only the necessary quantity of drugs should be stored, especially of those for which storage in a cool place is prescribed.

EXPIRATION

Drugs may be stored only as long as they meet the requirements described in the general part and the monographs of the Pharmacopoeia, respectively.

Individual drugs may undergo transformation even under proper storage conditions, therefore such must only be kept in store for a limited time period (*expiration date*). With expiration of this time limit any stock of such drugs must be renewed.

Drugs that are not subject to expiration date may in general be preserved for 5 years.

Chemical substances, unless otherwise specified, must be checked by complete analysis after 5 years. Depending on the analytical result, the stock must be renewed or the storage period may be extended for 5 more years, on one occasion.

Unless otherwise specified, the stock of pharmaceutical preparations, fixed and essential oils must be renewed after 5 years.

Regulations concerning the storage of vegetable drugs and powders of such, microbiological and serobacteriological preparations, as well as surgical dressings are included in the respective monographs.

For the storage of pharmaceuticals prepared in the pharmacy, authoritative requirements are included in the individual monographs of the Pharmacopoeia or of the "Formulae Normales".

DOSE

Statements relating to the maximal single and daily dose and the usual dose range for adults are given at the end of monographs (Vols I and II).

Maximum single and daily doses and the usual dose range for infants and children, respectively, are contained in Table II of Vol. IV.

Since the magnitude of the dose also depends on the mode of administration, the Pharmacopoeia indicates before the doses the route of administration in the individual monographs, as well as in Tables I and II of Vol. IV, where the route of administration is denominated by abbreviations according to Vol. I, p. 53.

If the route of administration is not indicated, the dose always refers to the oral route.

The physician may prescribe potent drugs in even higher doses than the maximum doses of the Pharmacopoeia, provided that the legal provisions concerning the transgression of maximal doses are observed.

The usual dose range is informative.

The usual dose range of drugs prescribed for veterinary purposes is summarized in Table III of Vol. IV.

The statement of dosage is given by the Pharmacopoeia in *gram (eventually milligram), millilitre, number of drops, number of pieces* and *international units*, respectively.

QUALITATIVE REQUIREMENTS

Any article of *pharmacopoeial quality* must comply with all requirements of the Pharmacopoeia.

The requisites of the Pharmacopoeia represent compulsory standards for materials used directly as medicaments, but do not affect the requirements prescribed for the same materials by the National Standards of the Hungarian People's Republic when used for any other purpose than for pharmaceutical ones.

Medicaments chemically or in composition identical with any of the official articles of the Pharmacopoeia, but available under any other name, must comply with all requirements of the Pharmacopoeia.

TESTS AND ASSAYS

The aim of tests and assays is to establish the *identity* and the *quality* of the material by suitable methods.

The suitable, generally used physical, physicochemical, chemical, biological and microbiological methods are described by the chapter "Tests and Assays" in Volume I.

Special test and assay methods for chemical substances (gases, volatile oils, fats, radioactive isotopes), vegetable drugs, individual dosage forms, blood-preparations, serobiological preparations, surgical dressings and sutures are described by the chapter "*Substances and Preparations*" in Volume I. The monographs complete these methods with those necessary for the tests and assays of the individual substances or preparations.

From the point of view of qualification of the articles, above all the description given in the single monograps under the title *Properties* is to be taken into consideration. (Data under this title referring to solubility, reaction and in some cases to melting range, are merely of informative character.)

Indexes, that is properties measurable by physical and physicochemical methods, as characteristics of identity and quality, are generally included into the monograph under the subtitle *Physical indexes*. The procedures of these determinations are described in the chapters *Physical Tests and Determinations* and *Physicochemical Tests and Determinations*, respectively. With instrumental analyses, the directions for the use of the instrument must be taken into consideration besides the directions of the Pharmacopoeia.

General methods important for the establishment of identity and quality are described by the chapter *Chemical Tests and Assays*.

The *Qualitative Tests* are very important for the qualification of drugs by establishing the presence of foreign matter and impurities.

Some of the impurities may influence the therapeutic value, the presence of others indicates careless preparation, or that impurities endangering curative action became admixed to the drug. Impurities may also affect adversely the stability of drugs and preparations and the manufacture of those.

The Pharmacopoeia contains in each monograph individual tests for the prospective contaminating substances. This does not mean, however, that the sample may contain other, not specified foreign impurities dangerous to the health, or refer to improper handling or manufacture even in case the Pharmacopoeia does not prescribe the concerning test.

For the establishment of some of the qualitative requirements, including the active ingredient content, quantitative methods are prescribed under the subtitle *Quantitative tests.*

Pharmacopoeial quality is determined jointly by the data included into the description, preparation, the physical indexes and in the requirements, respectively, represented by the test and assay methods.

The values of weight, volume and temperature indicated by the physical, physicochemical tests and determinations and by the chemical tests and assays may deviate by ± 3 units in the last cipher.

This does not affect limit numbers, data of limit values (physical indexes, qualitative requirements) and such cases in which the expression *accurately weighed* is used (*2.15*, Vol. I, p. 62) and the prescribed quantity of the material to be weighed is given in the monograph only approximatively.

If a pharmacopoeial method cannot be applied for certain reasons (small quantity of available sample, etc.) other methods may be used. In such cases the method must be described in the test record. The pharmacopoeial method is decisive, however, in any case of disagreement.

Drugs that have been submitted to complete pharmacopoeial analysis may be checked in the pharmacy only by the *Informative Tests* (see D, Vol. I, p. 132).

Tests and assays

I PHYSICAL TESTS AND DETERMINATIONS

1 Length

The unit of length is the metre (m). In this Pharmacopoeia the one-hundredth part of the metre, the *centimetre* (cm) is used as unit.

1 Metre (m)	= 100	cm	$= 10^{2}$ cm
1 Decimetre (dm)	= 10	cm	= 10 cm
1 Millimetre (mm)	= 0.1	cm	$= 10^{-1}$ cm
1 Micrometre (μm)	= 0.0001	cm	$= 10^{-4}$ cm
former name: Micron (μ)			
1 Nanometre (nm)	= 0.0000001	cm	$= 10^{-7}$ cm
former name: Millimicron (mμ)			

2 Mass and Weight

The unit of mass is the kilogram (kg). In this Pharmacopoeia the one-thousandth part of the kilogram, the *gram*, is used as unit.

1 Kilogram (kg)	= 1000	g	$= 10^{3}$ g
1 Centigram (cg)	= 0.01	g	$= 10^{-2}$ g
1 Milligram (mg)	= 0.001	g	$= 10^{-3}$ g
1 Microgram (μg)	= 0.000001	g	$= 10^{-6}$ g
former name: gamma (γ).			

The mass units are commonly referred to as weights.

2.1 Measurement of Weight (Mass)

2.11 Balances and Scales

For pharmacopoeial tests standardized sets of analytical weights, and a freely swinging or air-damped analytical balance of 0.1 mg sensitivity, and constructed for a maximum load of 200 g are used.

The balance must be placed on a stand free of vibrations provided with rubber damping sheets, in a place protected from radiant heat, from water vapours and from acid fumes. The balance must be adjusted properly (adjustment of sensitivity, levelling, etc.). It is convenient to protect the pans by thin glass discs.

2.12 Check of Analytical Balances

2.121 Check of stability of the equilibrium position

The equilibrium position of the balance is to be checked several times (five- or six-times), in the case of a freely swinging balance by successive swings, in the case of air-damped balance by direct reading. After each check the balance must be arrested. The equilibrium position of the balance must be determined when it is loaded with 20 g and 200 g, respectively. The deviation of the equilibrium position expressed in divisions found in two successive determinations performed with equal loads must be not more than one division.

2.122 Check of sensitivity

The measure of sensitivity of a balance is the difference expressed in divisions, by which the equilibrium position is shifted when 1 mg is placed in one of the pans.

In the case of freely swinging balances the equilibrium position of the unloaded balance is determined thrice and the average value is taken. Then one of the pans is loaded with 1 mg, the equilibrium position is determined thrice and the average value is taken. The difference between the two equilibrium positions expressed in divisions is the sensitivity of the balance; it must be not less than 4 to 5 divisions.

In the case of an air-damped balance or of a balance fitted with optical scale, a 10 mg standardized weight is placed on the left pan of the balance, and the equilibrium position is read on a scale fixed to the pointer of the balance.

In a similar manner, the sensitivity is determined with the following weights placed successively on the pan: 1, 5, 20, 50, 100 and 200 g.

The ring-shaped weights are checked by standardized weights, placed on the left pan.

2.13 Use of Analytical Balances

Before weighing, open the box for a short while to allow equalization of temperature. During swinging, however, the box must be closed.

When a freely swinging balance is used, the first two-three swings are generally disregarded. The pointer must not swing too far (to the buffer).

In pharmacopoeial weighings a simple weighing method is used in most of the cases, provided that all weighings are carried out on the same balance in a similar manner. (For this reason the balance used for weighing the samples must be used — in principle — also for the standardization of the volumetric solutions.) An object of approximately known weight is placed into the left pan, and it is counterbalanced approximatively by adding weights in decreasing order (down to 10 mg), and the final counter-balance is done by the proper adjustment of the rider. Milligrams and tenths of a milligram are read on the scale of the rider. The weighing is performed in similar manner when air-damped or optical scale balances are used, the only difference is that the milligrams and the tenths of a milligram are read on an optical scale.

When small quantities of a substance (e.g. in the case of a residue on ignition a few milligrams or tenths of a milligram) must be weighed in a large vessel, and a longer period elapses between the two weighings, atmospheric pressure and the temperature may alter to such an extent that the change in buoyancy may

cause an error of a few milligrams. This error can be eliminated by taring the empty vessel with another vessel of similar quality and weight.

Generally the weight must not be reduced to vacuum (zero pressure), if the densities of the samples are nearly the same. If the reduction of the weight to vacuum appears to be necessary, all other weighings connected with the test are to be reduced to vacuum.

For precise weighings use a calibrated set of weights, and apply the "simple Borda method" (see *Calibration of volumetric flask, 3.121,* Vol. I, p. 64).

TABLE 3

Weight	Tolerance ± mg	
	Quality I weights	Quality II weights
100 g	0.5	0.3
50 g	0.5	0.3
20 g	0.3	0.2
10 g	0.3	0.2
5 g	0.2	0.1
3 g	0.2	0.1
2 g	0.2	0.1
1 g	0.2	0.1
500 mg	0.15	0.08
300 mg	0.15	0.06
200 mg	0.15	0.06
100 mg	0.10	0.05
50 mg	0.10	0.04
30 mg	0.10	0.03
20 mg	0.08	0.03
10 mg	0.08	0.03

2.14 Analytical Weights

Analytical weights are standardized by the National Bureau of Standards.

Standardization must not be labelled on the individual weights. The standardized analytical weights may deviate from their denominations as presented in Table 3.

2.15 Accuracy of Weighing

A sample is "accurately weighed" if the uncertainty in the reading is not more than ±0.2 mg, using the simple weighing method.

The result of weighing is given to four digits. If an approximative weighing is sufficient, a hand-balance can be used. In such cases, the quantity of the sample to be weighed is given in grams or milligrams omitting the decimals.

3 Volume (Capacity)

The unit of the volume is the *cubic metre* (m^3). A cubic metre is the volume of a cube of 1 m edge.

In chemistry, a *litre* (1 = 1 dm^3) is used as the unit.

In this Pharmacopoeia, one-thousandth part of the litre, the millilitre is used as unit.

1 Hectolitre (hl)	=	100 l	$= 10^2$ l
1 Decilitre (dl)	=	0.1 l	$= 10^{-1}$ l
1 Centilitre (cl)	=	0.01 l	$= 10^{-2}$ l
1 Millilitre (ml)	=	0.001 l	$= 10^{-3}$ l
1 Microlitre (μl)	=	0.000001 l	$= 10^{-6}$ l

3.1 Measurement of Volume (Capacity)

3.11 Volumetric Glasswares

Use glasswares, scrupulously clean, calibrated at 20° with distilled water for the measurement of volume. The lowest point of the meniscus must be adjusted to the thin line etched around the neck, and the volume must be read so that the nearer and farther side of the ring be tangential to the lower edge of the meniscus.*

3.111 Volumetric flasks

Use volumetric flasks the inner diameter of which at the etched ring does not exceed the following values

Volume of flask (ml)	20	50	100	250	500	1000
Greatest acceptable inner diameter of the neck (mm)	7	9	11	13	16	19

3.112 Pipet

Use pipets the inner diameters of which at their narrow part holding the etched ring are 2.5 to 3.5 mm for 1 to 10 ml, 3.5 to 5.5 mm for 10 to 100 ml capacities. The outflow tip of the pipets must be 15 to 25 mm.

Pipets are available with single or with double marks. For accurate measurements use pipets with double marks. The upper engraved ring of the pipets must be 20 mm above the widening part, the lower engraved ring of the double mark pipets must be 30 to 40 mm from the orifice of the pipet. The narrow tube of the pipets above the upper engraved ring must be not less than 110 mm. Pipets with defective tip must not be used.

Using a single mark pipet allow the liquid to flow out with a 15 sec post flow; the tip of the pipet must touch the wall of the receiver. Using a double mark pipet adjust the meniscus of the liquid to the lower engraved ring after a 15 sec post flow. The orifice of the pipets must be made of such size that the free outflow time must range as given below.

Capacity of the pipet	1 to 10	20 to 50	100 ml
Outflow time	15 to 20	22 to 30	32 to 40 sec

3.113 Burets

For pharmacopoeial tests 12 ml burets, graduated to 0.05 ml provided with glass stopcock are most frequently employed. The inner diameter is 6 mm, the length of the graduated part about 420 mm (about 35 mm/ml). The tip of the glass stopcock must be made of a size that 40 drops of water must make a volume of 0.9 to 1 ml. The outflow of burets must be about 10 ml per one minute. The volume of the outflown liquid must be read after three minutes.

* Glasswares coated inside with silicone resins may be used too. In this case the meniscus is flat. The inner walls of such glasswares are not wetted by aqueous standard solutions. If the calibrated glasswares are coated with silicone resin after calibration, they must be calibrated again.

The glass stopcock of a buret must be slightly lubricated with soft paraffin or with a mixture of soft paraffin and bees wax (9 + 1); the glass stopcock — if properly lubricated — must be transparent.

3.12 Check of volumetric vessels

Allow to stand overnight in the room in which the calibration is to be made, sufficient amounts of freshly boiled and cooled water in a flask, covered with a beaker. Place the clean and dry glasswares to be calibrated or used for the calibration at least 1 hour prior to calibration, near the balance; open the door of the balance box in order to egalize the temperature. Before the measurements, the temperatures of the water and the air must be measured, and they must not differ by more than $\pm 0.5°$.

TABLE 4

Calibration Table*

t° (water)	R_t	lg R_t
15°	1.00208	.00090
16°	1.00221	.00096
17°	235	.00102
18°	250	.00108
19°	266	.00115
20°	283	.00123
21°	1.00301	.00131
22°	321	.00139
23°	341	.00148
24°	363	.00157
25°	385	.00167
26°	1.00409	.00177
27°	433	.00188
28°	458	.00199
29°	485	.00210
30°	512	.00222

* If the temperature of the water does not correspond to integer R_t and lg R_t values, respectively, calculate the exact values by interpolation. Determine the weight of water up to 100 g on an analytical balance. Express the calculated volume with 0.01 ml accuracy. This accuracy is sufficient for pharmacopoeial tests.

3.121 Calibration of volumetric flasks

Place the flask on the right pan of the balance together with calibrated weights (m') numerically equal to the nominal volume of the flask. Tare the flask and the weights by placing other weights (not calibrated) on the left pan. Measure the temperature of the water used for calibration with 0.1° accuracy. Litt the flask off the balance and fill with water up to the mark. Perform the accurate adjustment of the meniscus using a glass tube with capillary tip. Remove any drops adhering to the neck above the meniscus, with a roll of filter paper. Place the flask again on the right pan of the balance, and remove the weights. Finally re-adjust the equilibrium position of the balance by placing calibrated weights (m'') beside the flask.

The weight of the water in the flask at $t°$ is thus $(m' - m'') = m$ gram. Multiply this weight with value R_t (see Table 4) corresponding to the temperature of water, to obtain the volume of the flask at 20°. Record adequately the calculated volume (capacity) on the flask

The volume of volumetric flasks may deviate from the nominal volume by not more than the values given below:

Volume (ml)	50	100	250	500	1000	2000
Tolerated maximum deviation (ml)	+0.04	+0.06	+0.10	+0.15	+0.25	+0.40

If the deviations found on checking do not exceed the values given above, no corrections must be applied.

3.122 Calibration of pipets

Determine the tare weight of a glass-stoppered conical flask of an adequate volume together with calibrated weights numerically equal to the nominal volume of the pipet according to *3.121*. Then fill the pipet with water of known temperature, and measure water into the tared flask, as described in *3.112*. Proceed further as directed in *3.121*. Repeat the operation checking again the temperature of the water. The deviation between the two measurements must not exceed 10 mg. Record the true capacity of the pipet.

The volume of the pipets may deviate from the nominal volume by not more than the values given below:

Volume (ml)	2	5	10	20	25	50	100
Tolerated maximum deviation (ml)	+0.006	+0.01	+0.015	+0.02	+0.025	+0.035	+0.05

If the deviations found on checking do not exceed the values given above, no corrections are necessary.

3.123 Calibration of burets

Check 12 ml burets at 1 ml intervals, 50 ml burets at 5 ml intervals. Fill the buret to the zero mark with water of known temperature (determined with 0.1° accuracy), and allow to flow out 0–1, 0–2, 0–3.0, etc. volumes. It is sufficient to wait for one minute after filling, before adjusting the level to the zero mark. Wipe the tip off to remove adhering water. The outflow time is 6 seconds per millilitre. Adjust the meniscus to the required mark with a 0.01 ml accuracy three minutes after the outflow. Further proceed as directed under *3.121* and *3.122*, respectively. Repeat the operation checking again the temperature of water. The deviation between the two measurements must not exceed 10 mg. Compile the nominal and corrected values in a table or present graphically.

The volume of individual intervals of the buret may deviate from the nominal volume with a value not exceeding the deviation obtained for a pipet of corresponding volume.

If the deviations found on checking the individual intervals of the buret do not exceed the tolerated deviations of pipets of corresponding volumes, no corrections are necessary.

3.13 Use of volumetric vessels at temperatures other than 20°

If standard solutions (up to 0.1 **N**) are used at temperatures other than 20° (t'), corrections must be applied, according to Table 5, to obtain volumes corresponding to 20°. (In the case of glacial acetic acid solutions see Vol. I, p. 133.)

TABLE 5

Consumed ml	10°	12°	14°	16°	18°	20°°	22°	24°	26°	28°	30°
10	+0.01	+0.01	+0.01	+0.01	0.00	0.00	0.00	−0.01	−0.01	−0.02	−0.02
20	+0.03	+0.02	+0.02	+0.01	+0.01	0.00	−0.01	−0.02	−0.03	−0.03	−0.03
25	+0.03	+0.03	+0.02	+0.02	+0.01	0.00	−0.01	−0.02	−0.03	−0.04	−0.05
30	+0.04	+0.03	+0.03	+0.02	+0.01	0.00	−0.01	−0.02	−0.04	−0.05	−0.07
40	+0.05	+0.04	0.04	+0.03	+0.01	0.00	−0.02	−0.03	−0.05	−0.07	−0.09
50	+0.06	+0.06	+0.05	+0.03	+0.02	0.00	−0.02	−0.04	−0.06	−0.09	−0.12

3.14 Accuracy of Volume Measurements

Use for accurate volume measurements calibrated volumetric flasks, pipets and burets. When the use of a volumetric flask, pipet or buret is necessary, the volume of the liquid is given in this Pharmacopoeia to two decimal digits, e.g. 25.00 ml. The deviation between the prescribed and real volumes must not exceed ±0.01 ml.

When an approximate measurement of the given volume is sufficient, also graduated volumetric cylinders can be applied. In such cases the volume of the liquid is given only by integers (e.g. 25 ml) or to one decimal digit (e.g. 2.5 ml).

4 Temperature

In this Pharmacopoeia, the centigrade (*Celsius*) thermometric scale is used for expressing temperatures. Zero degree *Celsius* is equal to 273.16 degree *Kelvin* (°K).

4.1 Thermometers and Measurement of Temperature

For the measurement of temperature, use *standardized mercury thermometers*, for highly accurate measurement thermometers consider also the correction table belonging to the thermometer.

The length of the thermometers ranges generally 300 to 400 mm, with an external diameter of 6 to 7 mm. The diameter of the mercury reservoir varies from 5 to 6 mm, and its length from 10 to 15 mm. The upper end of the capillary tube is widened to a small safety reservoir.

If not otherwise directed, for the pharmacopoeial tests the following thermometers are used:

SCALE RANGE

from 0° to + 50° graduated into tenth-degrees (for calibrations, density and viscosity measurements)
from 0° to +110° graduated into integer degrees
from 0° to +250° graduated into integer degrees
from 0° to +360° graduated into integer degrees

Immerse the thermometer into the liquid or into the vapour phase at least until the beginning of the scale. When a thermometer calibrated for immersion is used, the liquid should reach the particular mark. Read the thermometer after thermal equilibrium has been attained between the thermometer and the liquid and vapour, respectively. Prior to reading gently tap the thermometer with a wooden rod. Read the thermometer held in vertical position.

When accurate readings are needed, the use of a magnifier is advisable. The tenth and hundredth of degrees should be estimated. Unless immersion points are marked, thermometers are calibrated for total immersion. For this reason for the actual reading apply a correction for the length of the mercury thread which is not exposed to the temperature to be measured. A wider (about 25 mm in diameter) and longer (about 30 cm long) glass tube is pulled on the thermometer. By the aid of a one-bore cork, place an auxiliary thermometer into it so as the mercury reservoir of the latter should be at half-height of the mercury thread not being immersed into the liquid.

Read the correction from the nomogram (Fig. 1) where

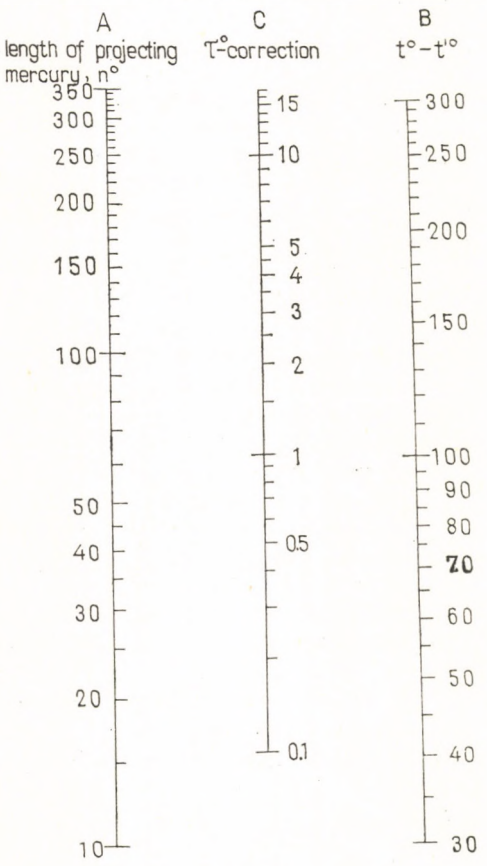

FIG. 1

$n°$ = the length (measured in scale degrees) of mercury thread not being immersed into the liquid

$t°$ = observed temperature

$t'°$ = temperature read on the auxiliary thermometer

$\tau°$ = correction in degrees

4.11 Check of Thermometers

Check thermometers by comparing them with a calibrated thermometer. Fix both thermometers to each other with a rubber ring or with a thread so that their mercury reservoirs should be at the same height. Immerse them into a gradually heated bath. The mercury threads of the thermometers should be immersed completely into the liquids. Stir the liquid bath and read the thermometers at about every 10 degrees and record the values or plot on a graph.

The standard points of the thermometer can be checked directly by immersing the thermometer into melting ice (melting point = 0.0°), into boiling water [boiling point = $100.0° + 0.037(b_0 - 760.0)$, where b_0 is the barometric pressure reduc-

ed to 0°*] and into boiling naphthalene [boiling point = 218.0° + 0.058 $(b_0 - 760.0)$].

4.2 Determination of Melting Point

Perform the determination of melting point in an apparatus shown on Fig. 2. Place into the apparatus a thermometer, by the aid of a ground joint, so that the bissector of the mercury reservoir should be about 2.5 to 3 cm below the top orifice of the triangle-shaped part. (Capillary tubes placed into the 55 mm long side arms, sealed in a 30° angle, should touch the bissector of the mercury reservoir of the thermometer.)

Apply two thermometers: one with a scale range from 0° to 250°, graduated into half-degrees, and another with a scale range from 0 to 350°, graduated into integer degrees.

The length of the thermometers from the bottom of the ground joint to the bottom of the mercury reservoir should be 380 mm. An opening of 2 mm diameter, on the mantle about 1 cm under the ground joint of the apparatus serves for pressure egalization.

Fix the apparatus on a Bunsen-stand. Use liquid paraffin or silicone oil as a liquid bath. Prepare capillary tubes of about 1 mm diameter and 8 to 10 cm length from resistent, alkali-free glass tubes, of 4 to 5 mm inner diameter and 0.2 to 0.3 mm wall thickness. Use tubes scrupulously cleaned, and rinsed with distilled water prior to preparation of capillaries.

Preserve capillary tubes protected from dust. Finely powder the substance to be tested and unless otherwise directed in the monograph, spread out in a thin layer and dry in a dessiccator over concentrated sulphuric acid for 24 hours. Fill the capillary tubes with the sample to 2 mm height by the aid of an about 1 m long "dropping tube". (After each use clean and dry the dropping tube.) Protect the apparatus from air currents. Heat the triangle-shaped part of the apparatus with a microburner, equipped with a funnel.

Place the packed capillary tube into the apparatus at a temperature 15 to 20° lower than that of the expected melting point. The capillary tube should touch the bissector of the mercury reservoir. Control heating so that the temperature of the liquid bath should rise by 1 to 2 degrees per minute.

If the substance decomposes near the melting point, apply „rapid heating" method. In this case the rate of heating is about 4 to 5 degrees per minute.

Observe two temperatures at the measurement of the melting point: first when the substance settles or just starts to liquefy, then the total quantity of the substance melts to a clear liquid. Use a magnifier for the observation for more accurate reading. Correct the

TABLE 6

Compound	Melting-point, °C
Azobenzene	68.0
Vanillin	81.5
Norcaine	90.5
Acetanilide	114.5
Phenacetin	135.0
Salicylic acid	159.0
Sulphanilamide	165.5
Sulphaguanidine[1]	190.0
Caffeine[1]	236.0

[1] Free of crystal water.

* The reduction of barometric pressure is performed as described in the paragraph "*Measurement of pressure*", Vol. I, p. 73.

mean value of the two observed temperatures by a value read on the calibration curve of the apparatus. This temperature is the corrected melting point of the substance. The difference in the two values should be not greater than the difference in the limits given in the single monographs. Perform at least two parallel measurements.

For calibration, use the substances listed in Table 6.

The differences between the observed data and the values given are to be plotted. (Corrections for outstanding mercury thread height should not be performed in this case, of course.)

The melting point of two samples is regarded as *identical* according to the Pharmacopoeia if the melting points of mixtures prepared from equal quantities of the sample of the tested substance and of a reference substance of known melting point have the same melting point as the reference substance of known melting point.

Eutectic temperature is the temperature at which the mixture settles, separates from the walls of the capillary tube and a molten drop appears beside the solid phase. To measure the eutectic temperature, weigh equal quantities of the substance to be tested and of the eutectic forming substance into a porcelain mortar and triturate thoroughly.

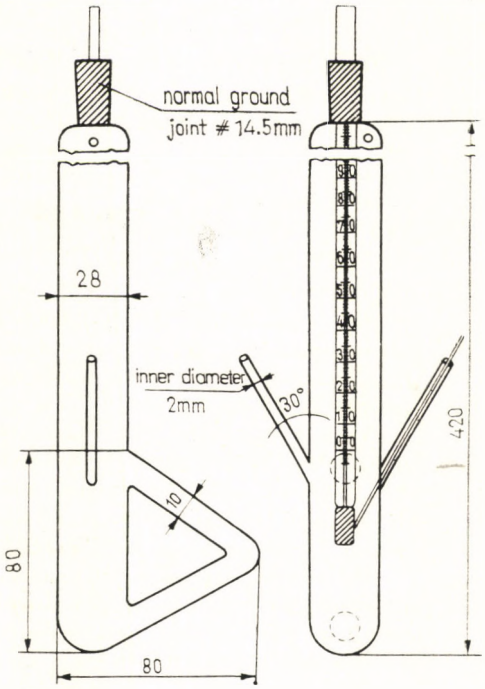

FIG. 2

4.3 Determination of Solidifying Point

4.31 Determination of Solidifying Point Using the Zhukov Apparatus

For the determination, use a double-walled Zhukov flask (outer diameter 40 mm, inner diameter 24 mm, outer height 85 mm, inner height 24 mm) (Fig. 3), the space between the two walls of which is evacuated. Insert through a one-bore cork a thermometer (ranging from 20 to 80° and graduated into 0.2 degrees) into the vessel.

Heat the substance, under stirring, to a temperature higher by 15 to 20° than the expected solidyfying point, and fill the flask to its 3/4 volume with the melted sample. Stopper the flask by means of the cork so that the mercury reservoir of the thermometer should reach right into the middle of the melt.

When the temperature of the melt is only 3 to 4 degrees higher than that of the expected solidifying point start shaking the vessel constantly. When the melt becomes turbid, read the temperature every minute, with 0.1° accuracy.

When the temperature decrease stops for a while or the rate of decrease is less than 0.1° per minute, the *solidifying* point is attained.

In particular cases, the temperature sinks under the solidifying point, and a sudden temperature increase is observed at a certain point. The highest value in the temporary temperature increase is in this case the solidifying point.

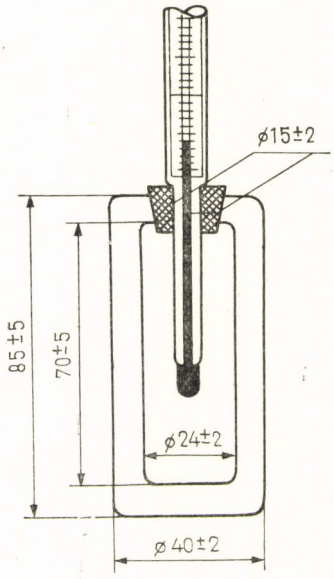

FIG. 3

4.32 Determination of Solidifying Point, Using a Rotating Thermometer

For the determination of this type use a cylindrical thermometer 0 to 100°, graduated into 0.2 degrees. The length of the graduated part should be 250 mm; the mercury reservoir should be of olive form, length: 10 to 12 mm, width 5 to 7 mm.

Insert through a one-bore cork the thermometer into a 100 ml wide-necked conical flask, which serves as an air-bath. Heat the sample on the water-bath under stirring, to a temperature higher by 8 to 10 degrees than the expected solidification point. Place the air-bath equipped with the thermometer in the same water-bath. Then lift off the air-bath the thermometer, together with the cork, and immerse the mercury reservoir of the thermometer into the melted sample so that 1 drop should hang on its tip. Transfer the thermometer into the air-bath and fix the conical flask horizontally. Rotate the horizontally oriented thermometer around its longitudinal axis with a speed of 2 sec per turnover. The temperature when the drop on the mercury reservoir makes the first turnover together with the thermometer is the solidification point of the sample.

4.4 Determination of Drop Point

Drop point is the temperature at which a solid becomes dropable under given conditions.

For the determination, use Ubbelohde thermometer (Fig. 4) graduated into integer degrees in at least 1 mm distances. Its mercury reservoir is 6 mm long and 3.5 mm wide. To the bottom part of the thermometer, a cylindrical metal-socket is fixed and on the latter, another metal socket can be screwed so that its bottom rim should be at the same height as the mercury reservoir. On the second metal socket a small opening serves for pressure egalization, inside there are three blocking hooks 7 mm from the bottom rim. The bottom rim consists of two jaws holding a metal socket (test vessel) cylindrical at the top and funnel-like at the bottom. The metal socket is 12.0 to 12.5 mm long with a wall-thickness of 1.2 to 1.4 mm. This reduces to a 2 mm high rim with a bottom orifice of 3.0 to 3.3 mm inner diameter. The blocking hooks serve for controlling the position of the test vessel in that the walls of test vessel remain at equal distances from the mercury reservoir of the thermometer. A test tube of 180 mm length and 35 mm inner diameter is used as an air-bath, into which a thermometer is fixed with the aid of a one-bore, sidelong incised cork. The test tube is immersed up to its 3/4 length into a 1-litre beaker filled with water.

Stand the test vessel (thimble), with its bottom orifice downwards, on a glass plate, and maintain a temperature by 20 to 30° below the expected drop point. Transfer the sample, bubble-free, into the test vessel. If the sample swells, smooth down with a knife. Slip the filled test vessel into the thermometer socket. Place the thermometer equipped with the metal socket into a test tube and immerse

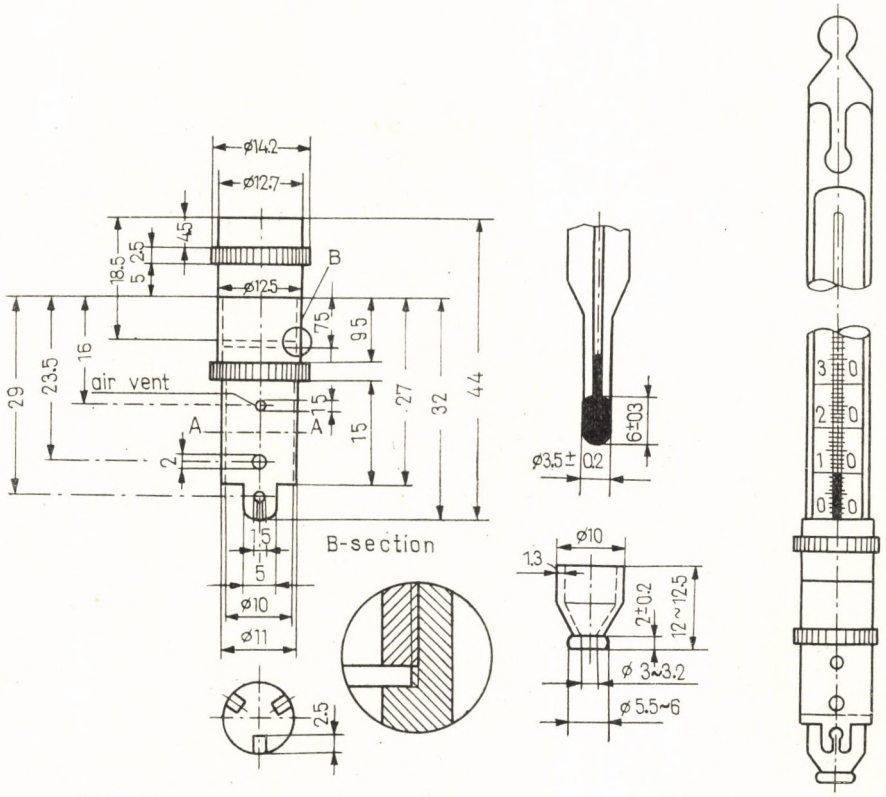

FIG. 4

the whole apparatus into the water bath. Heat from 10 degrees below the expected drop point, with the rate of about 1 degree per minute. Record the temperature at which the first drop separates. Perform at least two measurements, and regard the mean value as the drop point of the sample.

When a solid is tested, heat it to 10 degrees above the expected dropping point and transfer the liquid bubble-free into the test vessel. Allow the assembled thermometer to stand at 20° for 9 hours and perform the determination thereafter as directed above.

4.5 Determination of Boiling Point

In the course of the determination, that temperature or temperature range is recorded, where the sample or a certain portion of the sample distils at 760 torr pressure.

Use the apparatus shown in Fig. 5 for the determination. Place the vessel of the apparatus on an asbestos plate (12×12 cm, and 2 to 3 mm thick) so that its bottom should close the hole, 40 mm in diameter, on the centre of the asbestos plate. Unless otherwise directed in the monograph, transfer 50 ml of liquid into the vessel and add about 0.2 g of pumice to prevent retarded boiling. Insert a

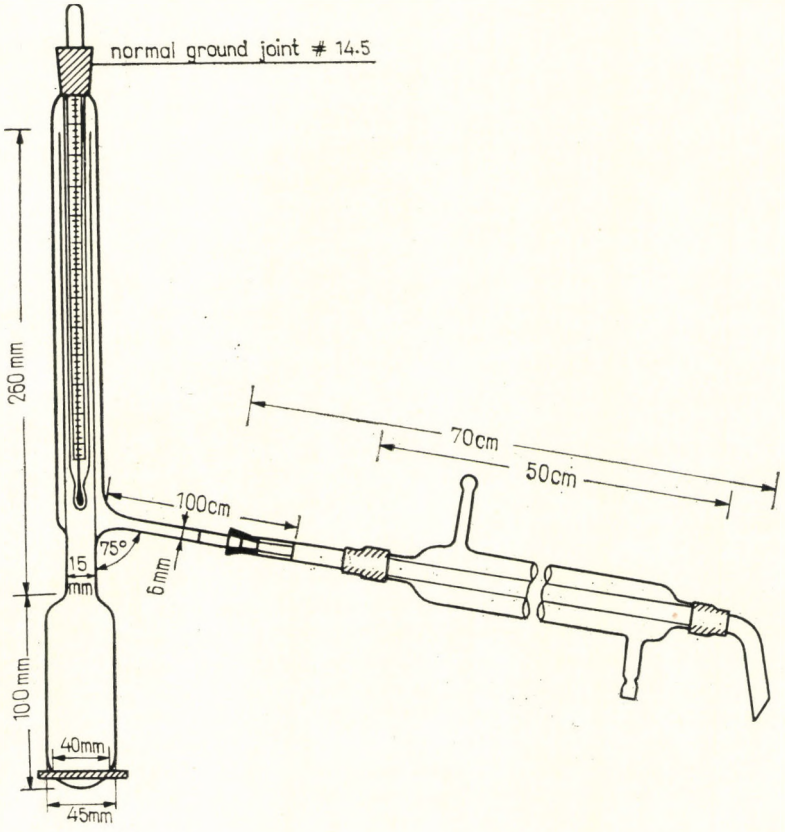

FIG. 5

ground thermometer into the apparatus so that the mercury reservoir of the thermometer should be at the height of the side arm. Heat the liquid over a small flame at a rate of distilling 3 to 5 ml per minute. Record the boiling point at first after distilling 1 ml of the liquid. The recorded temperature or temperature range should be the same as those given in the respective monograph. If the expected boiling point of a liquid is above 100°, proceed with distillation until the volume of the liquid in the vessel is reduced to 1 ml.

If liquids of low boiling point (about 50°) are tested, cover the funnel of the burner with a wire-gauze, or place the vessel in a water-bath, and heat cautiously.

When liquids of boiling points above 150° are tested, water cooling can be omitted, a single, jacketless cooling tube of adequate diameter may be used.

Use the same apparatus also for the determination of the boiling point of liquids the boiling point of which rises during distillation. In such cases, the cor-

responding monographs give directions concerning the boiling range of the different fractions.

Reduce boiling points to 760 torr, with a sufficient accuracy, as follows: determine the boiling point of water in the described apparatus and as a correction value add to or substract from the boiling point of the sample, the deviation from 100°, respectively.

5 Pressure

The unit of pressure in this Pharmacopoeia is one *torr*, which is one seven hundred and sixtieth part of the normal atmospheric pressure:

760 torr = 1 atm.

In laboratories, the commonly accepted measure of pressure is the height in millimetres of a mercury column. One torr is equal to the hydrostatic pressure of a mercury column of 1 mm height. In practice, *technical atmospheric pressure* (at) and *atmospheric overpressure* (ato) are also used.

The following relation exists:

at — 1 = ato, or ato + 1 = at.

5.1 Measurement of Pressure

Perform the measurement of gas pressure by a *mercury manometer* in which the mercury level in the reservoir is adjustable and both the upper and the lower meniscus can be read separately. Perform the measurement always with an adjusted manometer. The result is expressed in torr (mmHg).

Reduce the recorded value (b) to 0° in general. This value is obtained by the formulae:

in the case of a glass scale $b_0 = b - 0.000174\, b.t.$
in the case of a brass scale $b_0 = b - 0.000163\, b.t.$

where b = the recorded value of barometer indication
t = temperature of air and mercury, respectively
b_0 = the reduced value of pressure.

(The correction at a pressure near 760 torr is about $\frac{t}{8}$ mm.)

Other corrections (e.g. due to capillary depression, vapour pressure of mercury and graviational acceleration) are neglected in practical measurements. The change in atmospheric pressure due to height, however, is to be considered. Every 11 m difference in height is equal to a pressure difference of 1 torr. The barometer should be located at the same storey where pressure measurements are performed or the height difference should be considered. Perform measurements of low pressure by a *U-shaped manometer*. The pressure is indicated by the difference in the heights of mercury columns of the legs.

6 Specific Gravity, Density

The density (ϱ_t) of a homogeneous substance at temperature $t°$ is, in absolute terms, the mass in grams of one volume unit (ml) of the material. This is equal to the mass in volume unit, reduced to vacuum. The density of a substance on a fixed temperature and pressure is a constant characteristic of the substance.

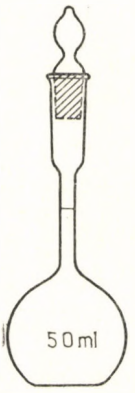

FIG. 6

Unless otherwise indicated, in this Pharmacopoeia densities measured at 20° are used (ϱ_{20}). The density of water at 20° is 0.99823.

Comparison of densities at the same temperature leads to the specific gravity, $d_t/_t$; the ratio of the masses of equal volumes of the material and water at $t°$.

Unless otherwise indicated, in this Pharmacopoeia specific gravities measured at 20° are used ($d_{20}/_{20}$). The specific gravity of water at 20° is 1.00000.

6.1 Measurement of Specific Gravity

The specific gravity measurement of a liquid is performed by determining the weight of an exactly known volume contained in a pyknometer of 50 ml volume (Fig. 6). The narrow part of the neck of the pyknometer is of 4 mm inner diameter. Weigh the empty and dry pyknometer accurately, then fill with water of about 20° temperature. Adjust to exact volume after the content of pyknometer has attained exactly 20° in a thermostat. Dry the neck of the pyknometer, if necessary, with a small strip of filter paper. Then wipe the pyknometer and place it into the box of a balance for 15 minutes, and weigh again accurately. The weight difference gives the weight of the liquid in the pyknometer.

Then clean the vessel and determine the weight of water at 20° in the same manner.

Perform the measurement of the specific gravity of a viscous liquid (oil, syrup, etc.) as described above. Replace, however, the pyknometer with a 50 ml volumetric flask.

The ratio of the weights of the liquid (μ) and water (μ_w), μ/μ_w, is the specific gravity of the liquid ($d_{20}/_{20}$).

6.11 When specific gravities to only three decimal digits are to be measured, also a Westphal-balance can be used. By using a Westphal-balance, directly the specific gravity values are obtained, if it is balanced in air, unloaded, and with a "float" immersed in water at 20° at 1.0000 load.

The volume of the "float" is, to every gram of the greater rider, 1.002821 ml. The adjusted balance can be used between any temperatures of 10 to 30° to obtain values accurate to 3 decimal digits.

6.2 Calculation of Density from Specific Gravity

Use the following formula to calculate the density (ϱ_{20}) from the specific gravity:

$$\varrho_{20} = \frac{\mu}{\mu_w} \times 0.99703^* + 0.0012$$

* lg 0.99703 = .99871.

These calculations can be simplified by calculating only the μ/μ_w ratios (specific gravity), and subtracting corrections (k)* from these values. The corrections are listed in Table 7. The density values are given to three decimal digits.

Specific gravity measured by means of a Westphal balance at 20° can be converted into density also with the aid of Table 7.

TABLE 7

Correction to the calculation of density

$\frac{\mu}{\mu_w} = fs_{20/20}$	k	$\frac{\mu}{\mu_w} = fs_{20/20}$	k
0.4209–0.4545	0.0001	1.2627–1.2962	0.0026
0.4546–0.4882	2	1.2963–1.3299	27
0.4883–0.5218	3	1.3300–1.3636	28
0.5219–0.5555	4	1.3637–1.3973	29
0.5556–0.5892	5	1.3974–1.4309	30
0.5893–0.6228	6	1.4310–1.4646	0.0031
0.6229–0.6565	7	1.5757–1.4983	32
0.6566–0.6902	8	1.4984–1.5319	33
0.6903–0.7239	9	1.5320–1.5656	34
0.7240–0.7575	10	1.5657–1.5993	35
0.7576–0.7912	0.0011	1.5994–1.6329	36
0.7913–0.8249	12	1.6330–1.6666	37
0.8250–0.8585	13	1.6667–1.7003	38
0.8586–0.8922	14	1.7004–1.7340	39
0.8923–0.9259	15	1.7341–1.7676	40
0.9260–0.9596	16	1.7677–1.8013	0.0041
0.9596–0.9932	17	1.8014–1.8350	42
0.9933–1.0269	18	1.8351–1.8686	43
1.0270–1.0606	19	1.8687–1.9023	44
1.0607–1.0942	20	1.9024–1.9360	45
1.0943–1.1279	0.0021	1.9361–1.9696	46
1.1280–1.1616	22	1.9697–2.0033	47
1.1617–1.1952	23	2.0034–2.0370	48
1.1953–1.2289	24	2.0371–2.0707	49
1.2290–1.2626	25	2.0708–2.1043	50

Density values calculated from the formula may differ in one unit in the fourth decima from the values obtained using corrections values.

* $k = \frac{\mu}{\mu_w} \times 0.00297—0.0012.$

Note. In informative tests, the density can also be measured with an areometer. Areometer should be read at the lower tangential plane of the meniscus, with the exception of alcoholometers, which should be read at the upper margin of the meniscus.

7 Viscosity

Unless otherwise indicated, viscosity in this Pharmacopoeia is the absolute *dynamic viscosity* ($\eta_{20°}$) measured at 20° and expressed in *poise* (*P*) or *centipoise* (*cP*).

The absolute *kinematic viscosity* ($\nu_{t°}$) of a material is the ratio of the absolute dynamic viscosity of the material and its density ($\varrho_{t°}$) measured at the same temperature. The unit is one *stokes* (*St*) or one *centistokes* (*cSt*).

The following relations exists:

$$\eta_{t°} = \nu_{t°} \times \varrho_{t°}$$

(*Relative viscosity* is the ratio of viscosities of two materials at the same temperature. It is a dimensionless value.)

7.1 Determination of Viscosity

Perform the determination of viscosity of a liquid by using a tube viscometer, and by measuring the flow time of a certain volume of the liquid through a capillary of given length and diameter. (For viscosity measurement of liquids of a viscosity higher than 2000 *cP* is the use of a falling-ball viscometer more suitable.)

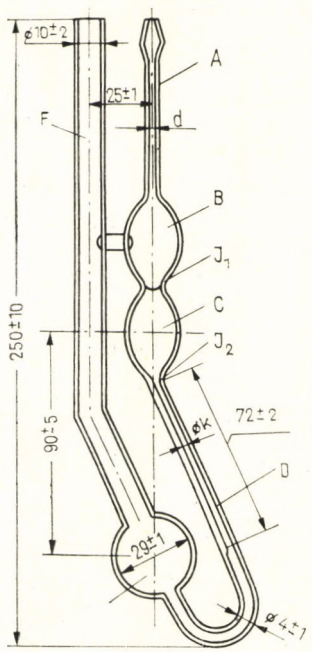

FIG. 7

Measure the flow time in a calibrated, standard tube viscometer (Fig. 7). The data of a standard capillary series being used in the measurements with a tube viscometer are shown in Table 8.

Unless otherwise directed in the monographs, determine viscosity or flow time at 20.00°. Immerse the viscometer into a thermostat (with a capacity of 2.5 litres per viscometer, but not less than 5 lit) and with a thermostatic control of ±0.05°. The level of the bath liquid should be above bulb *B*, and the bottom of the viscometer at least 40 mm apart from the bottom of the thermostat. Care must be taken that the meniscus of the liquid should be readable free from parallactic error. Up to +70°, use water as bath liquid.

Control the temperature using a thermometer with a 15 degree range, graduated into tenth degrees, calibrated for immersion. At the measurement of the viscosity, the middle of the mercury reservoir of the thermostat should be at a height equal to that of the middle of the capillary tube, *K*.

Data for the capillary series:

TABLE 8

Code number of capillary	k Inner diameter of the capillary (mm)	d Inner diameter of tube (mm)	Viscosity range (cSt)	Flow time to be secured (sec)
4	0.40–0.45		1.1– 5	400–1800
6	0.60–0.65		4 – 15	300–1000
10	0.97–1.03	2.5±0.5	12 – 100	120–1000
12	1.20–1.30		30 – 250	120–1000
18	1.80–1.90		150 –1200	120–1000
22	2.20–2.30	3.5±0.5	340 –2700	120–1000
28	2.70–2.90		1000 –8000	120–1000

Determine flow time with a stopper watch measuring 1/5 seconds.

Measurements of flow time. Scrupulously degrease, rinse and dry the viscometer. Fit a rubber hose on tube F, turn the viscometer over and draw the liquid (filtered if necessary!) through tube A up to mark J_2. Then invert the viscometer, wipe the top part of tube A and remove the rubber hose. Immerse the viscometer into a thermostat so that the two legs (F and A) should stand vertically. (The vertical position is secured by a supporting cover.) After 10 to 15 minutes, when the temperature equilibrium has been reached, draw the liquid through tube A to the mark at the top of bulb C. Measure the time required for the meniscus to fall from J_1 to J_2. This is the flow time (τ). Repeat the measurement at least five times. Consider only those values of flow time which do not deviate by more than ±0.5 per cent.

The absolute dynamic viscosity of the liquid is given by the formula

$$\eta_{20°} = K \times \tau \times \varrho_{20°}$$

where $\eta_{20°}$ = the absolute dynamic viscosity in cP at 20.00°
K = the calibration constant of the viscometer at 20.00° (according to calibration certificate)
τ = the mean value of flow times, in seconds
$\varrho_{20°}$ = the density of the liquid at 20.00° (see Section 6, Vol. I, p. 74).

If the measurement is performed at a temperature other than 20°, the calibration constant should be corrected. The correction factors are given below:

°C	20	37.8	50	100
$f_{t°}$	1.000	0.999	0.998	0.995

The mean values of parallel measurements should not deviate by more than 1 per cent of the minimum value.

II PHYSICOCHEMICAL TESTS AND DETERMINATIONS

1 Refractive Index

Refractive index is the ratio of the velocities of light in vacuo and in the medium (or in a solution of given concentration), and the ratio of the sine of the angle of incidence and sine of the angle of refraction, respectively. This is called *absolute refractive index:* n_λ^t, where t = the temperature and λ = the wavelength of light in nm.

Refractive index is a characteristic constant for the particular medium at a given wavelength, temperature and pressure, and when solutions are tested, at a given concentration. Refractive indexes for the D line of sodium (Na_D = = 589.3 nm) measured at 20° are given throughout in this Pharmacopoeia. Its symbol is: n_D^{20}.

(*Relative refractive index* is the ratio of absolute refractive indexes of two media. The refractive index of air is 1.00027 and thus the index referred to air differs by only 0.08 per cent from the absolute refractive index, which difference may be neglected in practical work.)

1.1 Determination of Refractive Index

The determination of refractive index is performed using an *Abbé type* refractometer, which measures the limit angle of the total reflection, and is suitable to test substances of a refractive index between 1.3 and 1.7. White light may be used, to prevent a coloured, indistinct boundary between the light and dark fields due to the differences in refractive indexes for light of different wavelengths, a system of two direct-vision prisms is placed in front of the objective of the telescope. The scale where the refractive index can be directly read, should be adjusted at intervals to a glass plate of known refractive index.

Each of the pair of detachable double prisms, which serve for the measurement of the refractive index can be kept at a constant temperature by means of a built-in thermostatic cell. *Clear liquids* should be placed between the detachable prisms, and their refractive index measured by transillumination. When reading the refractive index, the compensator should be thus adjusted that the light and dark portions of the field should form a distinct border without any coloured edge, and the border should coincide with the hair-cross. The value of the refractive index (n_D^{20}) related to the sodium light (Na_D = 589.3 nm) at the prescribed temperature (20°) may be read directly on the scale of the refractometer provided with a nonius.

Greasy substances are smeared on the top prism and tested by transillumination if possible.

The measurement of greater amounts of liquids is performed by using an *immersion refractometer*, which is also provided with a dispersion compensator and thus, white light can be used as well. The refractive indexes corresponding to the values read on the arbitrary scale can be found in tables.

2 Optical Rotation

Optical rotation or optical activity is the property of substances or their solutions, prepared with optically inactive solvents, that they laevorotate (—) or dextrorotate (+) the plane of polarized light. The measure of rotation is the angle of rotation (α) expressed in degrees.

The angle of rotation is depending on the wavelength of the polarized light (λ), the temperature (t), the layer thickness and the specific rotation, and in the case of solutions also on the solvent and the concentration.

Specific rotation is the angle of rotation which is observed when the optically active substance or its solution of a concentration of 1 gram per ml rotates at a temperature of $t°$, the plane of polarized light of λ wavelength. Under given conditions specific rotation is a characteristic constant of the matter. Its symbol is: $[\alpha]_\lambda^t$.

2.1 Determination of Optical Rotation

The measurement of optical rotation is performed by using a half-shade polarimeter with a scale readable to 0.1 degrees. Optical rotation values in this Pharmacopoeia are given mostly for the D-line of sodium ($Na_D^\lambda = 589.3$ nm). Unless otherwise directed in the monograph, measurement must be performed in a 20.0 cm tube at 20° ($\pm 1°$).

Transfer the solution or the liquid bubble-free into a 20.0 cm tube, then stopper and place in the apparatus. The glass plates should not be pressed strongly to the tube since, due to pressure, the glass is becoming double-refractive which may cause some error in the reading.

Adjust the analyzer to *darker half-shade* and read the angle. Repeate the adjustment and read thrice at every measurement, and record the mean value. Adjust the analyzer alternately from the one half of the field and from the other half. Then fill the tube with water or with the solvent applied, and place it into the apparatus and establish the "zero-point" as directed above. The difference in angles is the angle of rotation (α).

The preparation is dextrorotatory if the analyzer has to be rotated clockwise and laevorotatory if it has to be rotated anti-clockwise. The angle of dextrorotation is denoted by + and that of laevorotation by —.

2.2 Calculation of Specific Rotation

The equation for the calculation of the specific rotation of liquids from the measured angles of rotation is as follows:

$$[\alpha]_\lambda^t = \frac{\alpha}{l \times \varrho}$$

where $[\alpha]_\lambda^t$ = the specific rotation
α = the observed rotation in degrees
ϱ = the density of the liquid
l = the layer thickness in decimeters.

The specific rotation of solutions can be calculated from the measured angles of rotation by the formula:

$$[\alpha]_\lambda^t = \frac{100 \times \alpha}{l \times c}$$

where $[\alpha]_\lambda^t$ = the specific rotation
α = the observed rotation in degrees
l = the layer thickness in decimetres
c = the concentration of solute in grams per 100 ml of solution.

When, instead of specific rotation values, optical rotation data are given in this Pharmacopoeia, these are valid only for the specified concentration and solvent, and experimental condition.

3 Light Absorption

Transmittance (τ) is the ratio of intensities of the transmitted (I) and the incident light (I_0).

Light absorption (A) is the ratio of intensities of the absorbed ($I_0 - I$) and the incident light (I_0).

Extinction (E) or *optical density* is the logarithm of the ratio of intensities of the incident (I_0) and the transmitted light (I).

Relative transmittance (τ_r) is the ratio of intensities of light transmitted through the solution and the solvent.

Relative light absorption (A_r) is the ratio of intensities of light absorbed by the solution and transmitted through the solvent.

Relative extinction (E_r) is the difference in the extinctions of solution and solvent under identical experimental conditions:

$$\lg \frac{I_o}{I} - \lg \frac{I_o}{I'} = \lg \frac{I'}{I} = E_r$$

where I' = the intensity of light transmitted through the solvent.

Specific extinction ($E_{1\,cm}^{1\%}$) is the extinction of a solution in 1 cm layer thickness, containing 1 g of solute per 100 ml of solution

$$E_{1\,cm}^{1\%} = \frac{E_r}{c \times d},$$

where E_r = the relative extinction
c = the concentration of solute in grams per 100 ml of solution
d = the layer thickness in centimetres.

Molar extinction coefficient (ε) is the extinction of a solution, of 1 cm layer thickness, containing 1 g mole of solute per 1 litre:

$$\varepsilon = \frac{E_{1cm}^{1\%}}{10} \times \text{Mol. Wt.}$$

3.1 Determination of Light Absorption

3.11 Spectrophotometry

Light absorption is measured by means of a spectrophotometer applicable in a particular energy range: in the visible, ultraviolet and infrared regions. Spectrophotometers may be provided either with prisms or with gratings. The selection of energy regions is continuous. Beside the nominal wavelength value the energy width allowed to pass through the system to be measured should also be known. Namely, this latter determines how close is the extinction value measurable at the nominal wavelength to the extinction given for the nominal wavelength. The result of a measurement is the arithmetic mean of the extinctions within the band. Cells used in spectrophotometers should be selected according to the energy region. The light absorption of cells in the region under investigation must be negligible.

Energy is expressed in wavelength, wavenumber or frequency. In Table 9, the conversion of the three energy terms are given.

TABLE 9

Wavelength (*nm*)	Frequency (*f*)	Wave number (cm^{-1})	Wavelength (*nm*)	Frequency (*f*)	Wavenumber (cm^{-1})
1000	300.0	10 000	430	697.7	23 256
975	307.7	10 256	420	714.3	23 810
950	315.9	10 526	410	731.7	24 390
925	324.3	10 811	400	750.0	25 000
900	333.3	11 111	390	769.2	25 641
875	342.9	11 429	380	789.5	26 316
850	352.9	11 765	370	810.8	27 027
825	363.6	12 121	360	833.3	27 778
800	375.0	12 500	350	857.1	28 571
775	387.1	12 903	340	882.4	29 412
750	400.0	13 333	330	909.1	30 303
725	413.8	13 793	320	937.5	31 250
700	428.6	14 286	310	867.7	32 258
675	444.4	14 815	300	1000.0	33 333
650	461.5	15 385	290	1034.5	34 483
625	480.0	16 000	280	1071.4	35 714
600	500.0	16 667	270	1111.1	37 037
575	521.7	17 391	260	1513.8	38 462
550	545.5	18 182	250	1200.0	40 000
525	571.4	19 048	240	1250.0	41 667
500	600.0	20 000	230	1304.3	43 478
475	631.6	21 053	220	1363.6	45 455
450	666.7	22 222	210	1438.5	47 619
440	681.8	22 727	200	1500.0	50 000

Wavelength is given in *nm*, wavenumber in cm^{-1} and frequency in *f* (Fresnel). Equations for the conversion are the following:

$$\frac{1}{\lambda} = n, \quad \lambda = \frac{c}{\nu}, \quad f = 10^{-12}\frac{c}{\lambda},$$

where λ = the wavelength in *nm*
n = the wavenumber in cm^{-1}
ν = the frequency in sec^{-1}
c = the velocity of light (3.10^{10} cm. sec^{-1}).

In the infrared region, wavelengths or wavenumbers are used (see Table 10).

3.12 Procedure

3.121 Calibration of spectrophotometers

The calibration of a spectrophotometer is performed by checking the scale reading of the spectrophotometer at the two limits of the measuring region. For calibration, the lines of a mercury lamp at 253.7; 313.16; 334.15; 365.48; 404.66

TABLE 10

λ (μ)	$\frac{1}{\lambda}$ (cm^{-1})
10.5	953
11.0	919
11.5	869
12.0	834
12.5	801
13.0	770
13.5	742
14.0	714
14.5	689
15.0	677
15.5	646
16.0	626
16.5	606
17.0	588
17.5	572
18.0	556
18.5	541
19.0	527
19.5	513
20.0	500

TABLE 11

Group	Measuring range	
	Wavelength (μ)	Wavenumber (cm^{-1})
OH	2.66–2.98	3355–3760
NH	2.94–3.00	3335–3400
≡CH	2.95–3.04	3290–3390
$=CH_2$	3.06–3.60	2780–3270
CH_3	3.15–3.69	2710–3175
≡CD	3.71–4.13	2420–2695
$=CD_2$	4.26–4.86	2060–2350
CD_3	4.30–4.92	2030–2325
C≡N	4.31–5.25	1905–2320
N≡N	4.67	2141
C≡C	4.51–5.68	1760–2215
C=N	5.94	1684
C=O	5.47–6.25	1600–1830
C=C	5.48–6.60	1515–1825
N=N	6.35	1575
CH_3	6.72–7.66	1305–1488
CH_2	6.63–7.85	1274–1508
SH	7.76	1289
CH	7.22–22.3	450–1385
CF	8.3	1205
OF	9.01	1110
CCO	9.12–11.33	882–1096
C=S	6.57–15.22	657–1522
OF	12.05	830
N=N	16.68	600
CC≡N	19.7	508
CC≡C	29.8	335

and 435.83 nm or those of a hydrogen discharge tube at 486.13 and 656.28 nm are used.

In the infrared region, calibration is performed using polystyrene films, water vapour or ammonia.

3.122 Identification and qualitative tests

For these tests in the visible and ultraviolet region the logarithmic values of extinctions or of extinction coefficients are plotted against energy values.

When corresponding monograph in this Pharmacopoeia prescribes that the spectrum of the substance under investigation must coincide with the spectrum of the "Standard", the spectrum of the test substance must be recorded under conditions similar to that of the latter.

In infrared measurements the substance is identified by the absorption band of the characteristic groups (see Table 11).

In particular cases, the position of the absorption maxima (possibly minima) of the absorption curve are to be established; this is the case with identification tests, when measurements may be performed without standards.

Specific extinction values are used for qualitative and quantitative purposes. The qualitative nature of the tests is indicated by the limit values given in the monographs. The specific extinction values may serve as bases for estimating the active ingredient content of the tested sample.

The ratios of extinctions measured at two or more wavelengths, further various relations existing between extinctions may indicate the purity (or degree of decomposition) of the preparations as well.

3.123 Quantitative tests

For quantitative determinations, the measurement of extinction is to be performed at a particular wavelength. In the case of a multicomponent system, this procedure is applicable only if one of the components is to be measured and the other components do not show any absorption at that particular wavelength.

In the monographs of this Pharmacopoeia, the wavelength specified for the measurement is always given. Further, in some cases the deviation allowed from the given wavelength in a measurement performed at a given wavelength, with respect to conditions influencing the absorption maximum, is indicated in nm. In similar cases, within the wavelength region given in brackets after the exact wavelength value, the position of the actual absorption maximum should be established and the measurement should be performed at this wavelength.

In individual monographs of this Pharmacopoeia, the layer thickness and the concentration of the solution are given. If the measurement is performed under different (layer-thickness, concentration) circumstances, care must be taken to obtain neither low (below 0.2) nor high (above 0.7) relative extinction values; the solvent and the pH of the solution should be the same as directed in the monograph.

Perform the measurement by filling two cells of the same layer thickness with the solvent, and determine the relative extinction. Use this value as the correction factor of the cells. Fill the test solution into one of the cells and measure again the relative extinction.

Express the result of an assay mostly in percentage of the standard. For this reason perform the measurement of the extinction of the standard similarly, and regard the result as 100 per cent.

The most accurate procedure is to plot a calibration curve (plotting extinction against concentration) under similar conditions both for the standard substance and for the tested sample.

3.13 Photometry

It is an absorption measurement method where colour filters are used to select a particular energy range, and the extinction is measured either visually or by means of a photocell. Photometers may be used generally in the visible region.

Instruments with colour filters, where wide energy range can only be selected are not suitable for pharmacopoeial identification tests, for the measurement of physical indexes, and for qualitative and purity tests.

Due to the great difference between the nominal energy range and the actual band width and to the fact, that wavelengths between single colour filters can not be set, the spectrophotometric values prescribed in the monographs (extinction, specific extinction, and concentration) are not reproducible when measured by photometry.

However, a spectrophotometer can be replaced by a photometer for concentration measurements, when the energy range of one of the colour filters involves the wavelength specified by the particular monograph. Establish the extinction of the preparation also by the calibration curve plotted for the standard, and determine the concentration of the sample graphically.

4 Hydrogen Exponent (pH Value)

The hydrogen ion concentration is a value characteristic of aqueous solutions, A convenient scale for expressing hydrogen ion concentrations is the pH scale. this latter being the negative logarithm of the hydrogen ion concentration: pcH.

The pcH value of a neutral aqueous solution is 7, acidic solutions have pcH values below 7 and alkaline solutions above 7.

Since the potentiometric measurement of the hydrogen ion concentration can be performed only by measuring the hydrogen ion activity, for the characterization of the hydrogen ion content of solutions the negative logarithm of the hydrogen ion activity is also suitable. This is denominated by paH. If the activity coefficient is close to 1 as it is true in dilute solutions, then the values of both expressions of hydrogen ion concentration are nearly identical, hence in this Pharmacopoeia pH values are used instead of both pcH and paH values.

4.1 Determination of pH Value

According to specifications of this Pharmacopoeia, the pH value of solutions is to be measured by potentiometry. Colorimetric pH measurements can be performed only in those cases when special tests have proved that the dye applied to the measurement does not interact with any of the components of the solution in a manner by which the dissociation of the dye is altered.

Perform the colorimetric measurement by transferring the preparation into an alkali-resistent glass vessel and dissolving it in freshly boiled and cooled water. To a 10 ml portion of the solution, add the specified amount of an indicator solu-

tion, and compare the colour of the solution with the colour of a buffer series (Table 35, Vol. I, p. 360) containing the same indicator.

4.11 Electrometric Determination of pH

Perform the measurement in a cell consisting of a glass electrode and a reference electrode. Since the liquid-liquid potential at the interface of the two liquid phases makes the measurement uncertain, thus a standard solution with a nearly identical pH value must be used for comparison. In dilute solutions where the liquid-liquid potential is practically unchanged, the pH of the unknown solution can be interpreted by the equation:

$$\mathrm{pH}(x) - \mathrm{pH}(s) = \frac{(E_x - E_s)\,F}{2.3026\,RT},$$

where $\mathrm{pH}(x)$ = the pH value of the unknown solution
$\mathrm{pH}(s)$ = the pH value of the standard solution
$E_x - E_s$ = the potential difference measured by the cell
R = the gas constant
T = the absolute temperature
F = the Faraday number

and

$$2.3026\,\frac{RT}{F} = 59.16 \text{ mV /pH at } 25°.$$

The buffer solutions listed in Table 12 are official in this Pharmacopoeia. The temperature dependence of these solutions are shown in Table 13.

TABLE 12

Solution	pH at 25°	Buffer capacity mole/pH	Dilution value,* $\Delta\,\mathrm{pH}_{1/2}$
Potassium hydrogen tartrate, saturated at 25°	3.56	0.027	+0.05
Potassium hydrogen phthalate, 0.05 M	4.01	0.016	+0.05
0.025 M KH_2PO_4 + 0.025 M $Na_2HPO_4 \cdot 2H_2O$	6.86	0.029	+0.08
0.01 M $Na_2B_4O_7 \cdot 10H_2O$	9.18	0.020	+0.01

In strongly acid solutions, 0.05 M potassium tetraoxalate solution is used which has a pH value of 1.68 at 25° and its dilution value, $\Delta\,\mathrm{pH}_{1/2}$ is +0.19. In strongly alkaline solutions, a calcium hydroxide solution, saturated at 25° is used. Its pH value is 12.45 and dilution value is —0.28.

* Dilution value, designated by $\Delta\,\mathrm{pH}^1/_2$ is defined as the change of pH that results from diluting the buffer solution with an equal volume of pure water.

TABLE 13

Temperature °C	Potassium hydrogen tartrate, saturated at 25°	Potassium hydrogen phthalate 0.01 M	0.25 M KH_2PO_4 + + 0.025 M $Na_2HPO_4 \cdot 2\ H_2O$	0.01 M $Na_2B_4O_7 \cdot 10\ H_2O$
	pH			
0	—	4.01	6.98	9.46
10	—	4.00	6.92	9.33
20	—	4.00	6.88	9.22
25	3.56	4.01	6.86	9.18
30	3.55	4.01	6.85	9.14
40	3.54	4.03	6.84	9.07
50	3.55	4.06	6.83	9.01
60	3.56	4.09	6.84	8.96
70	3.58	4.12	6.85	8.93
80	3.61	4.16	6.86	8.89
90	3.65	4.20	6.88	8.85

Note. The error of pH measurement using ordinary instruments is between 0.02 and 0.05 pH, mostly due to the liquid–liquid interface potential. Using a pH-meter provided with an oxcillating condenser, the error of the measurement remains the same, the reproducibility, however, increases significantly (between 0.001 and 0.005 pH).

5 Chromatographic Analysis

In this Pharmacopoeia, paper chromatographic and thin-layer chromatographic procedures are official for some identification and purity tests.

5.1 Paper Chromatography

5.11 Chromatographic Chamber

In the paper chromatographic tests, glass vessels (reservoirs, tanks) are used in which the chromatographic papers (see *5.12*) can be placed conveniently. The paper strip should touch neither the walls, nor the bottom or the cover of the chamber. The chamber should be saturated with solvent vapours, prior to each run. Most convenient is to use a 20×30 cm chamber, which is 60 cm high. The chamber must be equipped with an airtight cover and a device serving to fix the paper strip.

5.12 Chromatographic Paper

Apply, in pharmacopoeial tests, chromatographic papers stored in a dustfree place and protected from humidity and vapours of chemicals. The type of the paper is specified in the monographs. The paper should be of suitable texture and thickness. Cut the chromatographic paper, along the fibre, into 6×35 cm strips. Establish fibre position by placing a drop onto the paper. (The position of the fibre is indicated by the direction of the principal axis of the elliptical spot.)

5.13 Ascending Chromatography

Place a particular portion of the solution of the sample, as directed by the monographs, by means of a micro-pipet onto the start line of an air-dry chromatographic paper. The start line should be 5 cm apart from the bottom margin of the strip. (When more than one spot are on the paper strip, they should be 2 cm apart from each other and from the margin of the paper.) When more than one drop are placed onto a certain start point, allow the solvent to evaporate after each drop. The spot should be not more than 1 cm in diameter.

Fill the solvent mixture into the tank. The solvent mixture should form a 2 to 3 cm high liquid layer. The saturation period is prescribed in the monographs. It is the most convenient to allow the solvent mixture to saturate the chamber overnight. Place the chromatographic paper into a chamber saturated with solvent vapours.

Perform the chromatographic test at 20 $(\pm 3)^\circ$ for a period specified by the monographs. Remove after the run, the chromatogram, quickly mark the location of the solvent front, and dry the sheet at room temperature, unless otherwise directed.

5.14 Development of Chromatograms

Spray the dried chromatogram with a developer solution specified by the monographs.

5.15 Evaluation of Chromatograms

The chromatograms can be evaluated either without or after development. Unless otherwise directed by the monographs, test the developed chromatogram immediately after drying. In some cases the use of an analytical ultraviolet lamp is indicated in the test and the observation is to be performed either at 254 nm or at 366 nm; the wavelength is specified by the monographs.

Calculate the R_f value of the spots observed in the chromatogram by the formula:

$$R_f = \frac{a}{b}$$

where a = the distance between the centre of the spot and the start point
b = the distance between the solvent front and the start point.

The R_f-value (retention factor) is characteristic of a particular substance under prescribed experimental conditions (type of chromatographic paper, solvent mixture, temperature, etc.).

In some cases it is necessary to calculate the relative retention value (R_x) of several spots observed on the chromatogram. This can be performed by the formula:

$$R_x = \frac{a}{c}$$

where a = the distance between the centre of the spot and the start point
c = the distance between the centre of the spot of substance x and the start point
x = the substance to which the reference is made.

5.2 Thin-Layer Chromatography

5.21 Chromatographic Chamber

Perform thin-layer chromatographic tests in an air-tightly closed small chamber, where a flat glass plate can be placed without damage to the layer. (Apply a chamber of 20×10 cm base area and 21 cm height for 20×20 cm glass plates.)

5.22 Preparation of the Adsorbent Layer

Scrupulously degrease and clean selected and uniform 20×20 or 8×20 cm glass plates. Triturate 25 g of an adsorbent prepared for purposes of thin-layer chromatography and containing gypsum with 50 ml of water or suspend by shaking. Pour the suspension immediately into the spreader and spread the suspension by moving the spreader over the glass plate to form a uniform adsorbent layer of desired thickness (about 200–250 μm) over the entire surface of the plate within 4 minutes after trituration being completed. (The given quantity is sufficient to prepare five 20×20 cm plates.)

Dry the adsorbent layer at room temperature for 10 to 15 minutes in a place protected from dust and vapours of chemicals, and then activate them in a drying cabinet for a period specified in the monographs. Preserve the glass plates over silica gel in a desiccator or in an air-tightly closed cabinet.

5.23 Procedure

Apply a portion of the solution of the preparation at a start line about 1.5 cm apart from the edge of the plate, with the aid of a micropipet graduated into microlitres. Care should be taken not to damage the layer. When several spots are applied, the spots must be 1 cm apart from each other and about 1 cm from the edge of the plate.

Line the chamber with filter papers saturated with a solvent mixture specified in the monographs and pour the same solvent mixture into the chamber. The quantity of the solvent mixture should be chosen with respect to the dimensions of the chamber, to form a 0.5 to 1 cm layer at the bottom of the chamber, the air space of which should become saturated with the vapours of the solvent mixture within 1 hour. After the lapse of 1 hour place the plate in the chamber so that it should not touch neither walls, or the bottom of the chamber, nor the filter paper lining. Perform the chromatographic test at 20° (±3°) until the solvent ascends to a point 13.5 cm above the start line. Remove the plate, dry it in a place protected from dust and vapours of chemicals at room temperature unless otherwise directed.

5.24 Development of Chromatograms

Proceed according to *5.14.*

5.25 Evaluation of Chromatograms

Proceed as described in *5.15.*

5.3 Identification Tests

When in the individual monograph reference is made to a standard preparation, test the standard simultaneously with the sample. When no concentration of the standard preparation is indicated, the standard solution should be of the same concentration as the sample. When the reference substance is not designated as "standard", then use a substance of pharmacopoeial grade.

5.4 Purity Tests

The aim of the chromatographic tests is to detect contaminants. These tests are semiquantitative under prescribed conditions.

The chromatographic purity tests render the detection and identification of limit-bound related compounds and decomposition products possible.

For tests of contaminants, the use of reference substances is generally not prescribed; orientative R_f-values of the substance under investigation are given in the individual monographs.

III CHEMICAL TESTS AND ASSAYS

1 Sampling

The control of pharmaceuticals starts with the sampling. As the circumstances of sampling are of decisive importance to the analytical result, the mode of sampling and the quantity of the sample must be in accordance with the quantity and material properties of the batch size to be sampled and the aims of the tests and assays. The sample (samples) must perfectly characterize the material.

Prior to sampling, the material must be inspected and the observations recorded.

Samples may only be drawn from appropriately labelled, intact, undamaged packages (containers). Damaged containers must be treated separately and the contents of such must not be used for pharmaceutical purposes. In exceptional cases, motivated by high price or difficulty of replacement, even such material may be utilized, provided it is not objectionable macroscopically and responds, after thorough homogenization, to the requirements of all tests and assays.

Sampling may be performed either according to *series* or according to *individual packages*.

From the point of view of sampling, a *series* is the pharmaceutical product of one delivery, carrying identical production or control number, independently of the total quantity and number of packages, and the individual quantity contained in each single unit. Usually large quantities, consignments and stocks are sampled *serially*. From series, consisting of not more than 20 packaging units, samples are drawn from 2 units; in case of series containing more than 20 units, sampling is performed from 10 per cent, but not more than 10 units. If the individual units are packed in collective packages, sampling is based on the number of the individual units. In such cases care should be taken to select the units proportionally from the collective packages to ensure that the samples characterize the whole series.

Sampling must be performed individually in all cases when, by serial sampling, the contents of the units within one series differ macroscopically in colour, odour, particle size or prove to be inhomogeneous during the tests.

The sample may be a *partial sample*, *average sample*, or *counter sample*.

A *partial sample* is a sample drawn from definite layers of individual packaging units and is characteristic of these.

An *average sample* is the sample obtained by homogenization of partial samples drawn from individual packages. Average samples may only be prepared from

macroscopically homogeneous partial samples. Average samples may also be taken directly from macroscopically uniform packages, following thorough homogenization.

A *counter sample* is a sample taken simultaneously in the same way and quantity as the partial sample or average sample, and which is handed over in a sealed container to the party providing the sampled material.

The quantity of the individual partial and average samples must not be less than the double of that required for the tests and assays.

Sampling must be performed with clean and dry *utensils* (spoon, pipet, poison-syphon, tube or drill probe). If necessary, the sampling utensil must be rinsed with the sampled liquid and the material used for rinsing must be rejected. For drawing each partial sample and average sample for each of the packaging units a separately cleaned sampler must be used.

Depending on the physical state of the material, the samples are taken from the selected packages as follows:

For the sampling of *powdered and crystalline materials* homogenize the macroscopically uniform partial samples drawn from different layers (i.e. by thorough mixing on a clean sheet of paper with cards). Should the partial samples macroscopically not be uniform or contain foreign matter, treat and test the samples individually. Transfer the sample to a clean and dry glass container and close it with a tightly fitting closure. Seal containers of hygroscopic or weathering materials hermetically. Sample large bulk quantities of *vegetable(animal) drugs* according to the respective standards.

Sample *unctuous materials and oils* according to the standards prescribed for sampling of vegetable and animal oils, fats, mineral oils and paraffins. In case of materials filled in collapsible tubes sample whole tubes.

Note. Samples of unctuous medicaments must be filled into containers that are impervious to the material, provided with a closure which does not react chemically with the ointments or the material dispersed in it.

Sample *liquids* from previously thoroughly agitated containers to ensure that any sediment is evenly distributed. Transfer the required quantity to the sampling container by means of pouring, pipeting, etc. If the contents of very large containers cannot be homogenized by agitation, take partial samples from different layers of the material, according to the standards for sampling solvents, and test these separately.

Draw liquid samples into clean and dry bottles provided with tightly fitting closures, made of materials that do not react chemically with the sample.

Note. Alkali-sensitive liquids must be filled into bottles of hydrolytic class II quality glass. The sampling of *gases* is described by the *Tests and Assays of Gases*, under *1*, Vol. I, p. 183.

Serobacteriological preparations, surgical dressings and pharmaceutical preparations are sampled according to the prescriptions of the valid standards and instructions.

The sampling for *sterility tests* is described by the *Sterility Tests*, under *4.11*, Vol. I, p. 170.

Draw average samples individually from bulk materials, stored in the pharmacy, according to the rules described above, provided that they are macroscopically homogeneous. Homogenize vegetable (and animal) drugs, teas, etc. thoroughly and sample unsifted drugs including the powdered parts. Agitate all liquids thoroughly before sampling.

In cases when separate sampling of separated parts seems to be appropriate, draw partial samples and test these individually.

The quantity of samples drawn in pharmacies must also be in accordance with the planned tests and assays.

Draw samples of medicaments compounded on medical order with special attention. Take care that the sample be not only characteristic of the preparation itself but should also reflect the accuracy of compounding.

The sampling of dosage forms prepared in the pharmacy is regulated in detail by special decree.

Seal the containers of samples and counter samples in such a manner that these cannot be opened without breaking the seal.

Store the samples in accordance with the properties of the material (storage prescription of the Pharmacopoeia).

2 Preparation of Material for Measurement

Prior to the test, disintegrate, powder and homogenize the carefully stored sample according to the directions. From the prepared sample, measure an amount exceeding the required quantity by about 20 per cent — in the case of powders, drugs or ointments — into a clear, dry, glass-stoppered bottle and in the case of liquids into a glass-stoppered bottle or glass-stoppered conical flask. Preserve the residual sample in a carefully closed state.

Unless other methods of preparation (ignition or drying) are specified in the monographs, apply *airdry* samples in the tests. Spread the powdered sample in a thin layer on a large watch-glass (or on a strip of filter paper) kept in a dust-free, dry place until equilibrium with air humidity is attained. When the drying of the sample is specified, use a desiccator containing an appropriate desiccant. Also desiccators are used when previously ignited or dried samples are allowed to cool. As desiccants, employ concentrated sulphuric acid, anhydrous calcium chloride, calcium oxide, phosphorus pentoxide or silica gel coloured blue with a cobalt(II) salt. (The blue silica gel turns pink, when it absorbs a certain amount of water. It can be regenerated by drying at 130° to 150°. Overheating should be avoided since at higher temperature silica gel looses its activity.) When substances with water of crystallization are dried in a way that the water of crystallization should not be removed, apply as desiccant a substance with approximately the same or only slightly lower vapour tension as that of the sample.

Dry organic substances sensitive to heat at room temperature in a *vacuum-desiccator* or in a *vacuum drying pistol*. Using the latter one, drying can be performed at different temperatures. Apply phosphorus pentoxide as a desiccant. Substances not sensitive to heat are to be dried in an *electric oven* at 105° to 110° and then allowed to cool in a desiccator. The electric drying oven should be provided with an automatic thermal control between 40° and 250° making possible to adjust the selected temperature within $\pm 2°$.

For *qualitative tests* it is sufficient to weigh the samples on a hand-scale.

Samples both for *the measurement of physical constants* and for *quantitative assay* weighed previously on a hand-scale are transferred into a tared weighing vessel, placed in the balance cabinet and weighed after temperature equilibration (5 to 10 minutes). (Hygroscopic substances and those absorbing carbon dioxide should be transferred into glass-stoppered vessels.) When a compound unstable

in air is to be weighed use a glass vessel with ground-glass stopper and perform weighing as in the preceding case. When substances which undergo changes in air are to be weighed in crucibles, place the crucible into a glass-stoppered weighing vessel, and weigh them together. Dry glass or porcelain vessels when wiped, accumulate electric charges, and for this reason, prior to weighing, they should be kept in thebalance cabinet for 15 minutes.

Weighing of samples on an analytical balance is described under "*Measurement of Weight*" (*2.1*, Vol. I, p. 60).

Substances of soft consistence (ointments, greases) are weighed in glass-stoppered weighing vessels or in aluminium foil, cerate paper or cellophane.

The measurement of liquids is described under *Measurement of Volume* (*3.1*, Vol. I, p. 63).

The sampling of gases is described under *Tests and Assays of Gases* (*1.1*, Vol. I, p. 183).

The weighing of substances to be digested is described under *Preparation of Organic Material prior to Digestion*.

3 Preparation of Organic Material prior to Digestion

3.1 Digestion of Small Quantities

Small quantities (0.1–0.5 g) of *solids*, if this is not opposed by the test and by the nature of the substance, are weighed in paper capsules prepared from pure cellulose. These capsules must not contain weighable quantities of the component to be determined (e.g. nitrogen, arsenic, metals, sulphate, phosphate, etc.). Mostly cigarette tube paper cut off the mouth piece and folded at one end by the aid of pincers is suitable (its weight is approximately 20 mg). Cigarette tube capsules due to their hygroscopic nature should not be touched with the fingers. The capsule is weighed accurately in a glass-stoppered weighing vessel, then the sample, previously weighed on a hand balance with 10 mg accuracy, is transferred into the capsule by means of a small funnel fitted cautiously onto the capsule by the aid of the pincers and re-weighed accurately.

Small quantities of *liquids*, oils, greases and greasy substances, etc. are weighed in thin-walled vessels prepared from a glass tube of 5 mm diameter. The rim of the vessels must be carefully smoothed. Ointments can be weighed also on cellophane sheets.

For digestion, apply generally a 100 ml digestion flask (Kjeldahl-flask). Mount the flask in 45° angle under a hood so that the developing vapours should pass from the flask directly into the vent of the air exhaustor. To prevent retarded boiling, add 1 to 2 glass beads into the flask. Transfer the sample or the capsule containing the sample into the digestion flask, add 2 to 5 ml of concentrated R-sulphuric acid and 2 to 5 ml of concentrated hydrogen peroxide solution. (When the sample is digested together with the capsule, apply 8 to 10 ml of *concentrated R-sulphuric acid*.) After ceasing of the vigorous reaction between sample and digestion mixture, cautiously heat the nitric acid with a small flame until sulphuric acid fumes appear. (The flame should not reach the sides of the flask above the level of the liquid !) If the liquid turns brown, again add 1 to 2 ml of *concentrated hydrogen peroxide* with the aid of a bent-tipped pipet. Remove tha flame during this operation. Then proceed with boiling until sulphuric acid fumes appear. If

the liquid turns brown, repeat the whole operation until a clear, colourless liquid is obtained.

For the digestion of *carbohydrates, fats and proteins* (preparations containing protein) transfer the sample, accurately weighed, in a small paper capsule, into the digestion flask, and add 5 ml of *50 per cent nitric acid*. Then reduce the volume of the liquid by boiling to about 1 ml. Repeat this procedure four- to five-times. Then add 5 ml of water and 2 ml of *concentrated R-sulphuric acid*, and heat the flask over a small flame until sulphuric acid fumes appear. If the liquid turns brown, add with a bent tipped pipet 1 to 2 ml *concentrated hydrogen peroxide solution* to the hot mixture, and proceed with digestion according to the previous procedure ("If the liquid turns brown, again add . . ."). (The addition of concentrated nitric acid is advisable also in other procedures when digestion with a mixture of concentrated R-sulphuric acid and concentrated hydrogen peroxide proceeds too slow.)

3.2 Digestion of Larger Quantities

Weigh larger quantities (0.5 g or more) of *solids* in small beakers (50–100 ml) and dissolve in water or in dilute sulphuric acid. Treat substances insoluble in water or in dilute sulphuric acid with a few millilitres of concentrated R-sulphuric acid, and then dilute to about 10 ml with 50 per cent sulphuric acid. Suspend substances insoluble even in concentrated sulphuric acid, in water or in dilute sulphuric acid.

Measure larger volumes of *solutions* in beakers of adequate size.

Dissolve greases in chloroform.

For digestion, mix in a 100 ml (or 250 ml) digestion flask 5 to 10 ml of concentrated R-sulphuric acid with 5 to 10 ml of concentrated hydrogen peroxide solution. To this mixture add a 0.5 to 2 ml portion of the solution (or suspension) of the sample by means of a bent-tipped pipet. After subsiding of the vigorous reaction, heat the flask over a small flame until sulphuric acid fumes appear. If the liquid turns brown, proceed as described under *3.1*. Perform the digestion of the sample in 0.5 to 2 ml portions. In quantitative tests, rinse the beaker containing the sample and the pipet thrice with 2 to 3 ml portions of water and digest also the washing liquid.

For the digestion of larger quantities of *carbohydrates, fats and proteins* (preparations containing proteins) transfer the sample in a 200 to 500 ml flask, add 50 to 200 ml of 30 per cent nitric acid and heat the mixture on a water-bath until liquified.

Measure larger volumes of *solutions* containing carbohydrates or proteins into a conical flask of adequate size, and add an equal volume of concentrated nitric acid. Heat the mixture on a water-bath until the foaming ceases.

Digest 1 to 5 ml portions of the prepared sample in a 250 ml digestion flask with a mixture of 10 ml of concentrated R-sulphuric and 20 ml of 50 per cent nitric acid. Care should be taken to avoid the separation of carbon particles. To prevent this, apply, if necessary, 30 per cent nitric acid and concentrated hydrogen peroxide solution, alternately.

Note. After digesting small or larger quantities of substances, reduce the volume of the obtained concentrated sulphuric acid solution to 0.5 to 1 ml by heating vigorously while passing air, filtered through a cotton wool, over the liquid. Then dilute the contents of the flask with a certain quantity of water, specified in the

monographs, and perform the test as directed. If the diluted sulphuric acid solution contains solid particles, they must be dissolved by boiling.

Caution! Preparations containing silver should be digested with at least 10 ml of 50 per cent nitric acid. A mixture containing only nitric acid must not be concentrated, owing to explosion hazards; the evaporated portions of nitric acid must be replaced at due intervals.

(A) IDENTIFICATION TESTS

Identification of a sample is generally performed in test tubes, by simple and specific reactions. Several reactions are prescribed only in such cases, when it seems to be necessary to make a distinction between similar substances.

(B) QUALITATIVE TESTS

The test is used to detect those impurities which affect the quality.

For the qualitative tests it is sufficient to weigh the substance on pharmaceutical hand scales.

1 Tests for Insoluble and Colouring Matter

For the testing of solids, dissolve the substance in a suitable solvent; in the case of liquids inspect a portion of the sample. This inspection affords valuable information on any insoluble and coloured impurities of unknown composition. The following expressions are used in this Pharmacopoeia to denote requirements of this kind.

Clearly soluble means that a certain quantity of a substance is soluble without any residue in a certain quantity of a solvent, as directed in the monograph. A 5 ml portion of the solution and that of the liquid, respectively, must not contain more than 2 to 3 visible floating particles, when poured into a colourless test tube (16 × 160 mm) and viewed in transmittent light. For the testing of solutions for injection particular specifications are given.

Colourless is a liquid and a solution, respectively, if it can not be distinguished from distilled water (or from the pure solvent specified in a monograph) when compared according to *1.1.*

Almost colourless is a liquid and a solution, respectively, if it is not deeper in colour than matching fluid 0, when compared according to *1.1.* The tolerated *tints* of solutions and liquids are checked by comparing those according to *1.1,* with matching fluids specified in the corresponding monograph.

1.1 Limit Test of Colouration

Inspect in a 16 × 160 mm colourless test tube the test liquid or the solution of a prescribed concentration of the test substance, as specified in the monograph, in a column of a height of 10 cm (about 15 ml), simultaneously with the same volume of the matching fluid in another test tube.

The comparison of colours must be performed in diffuse daylight, before a white background or over a white sheet. Use white analytic filter paper for this

purpose. If the prescribed matching fluid is colourless or so faint that its colour is hardly observable (No. 0–1), compare from over the contents of the two test tubes held vertically beside each other over a white sheet (about 10 cm in thickness) or inspect the meniscuses of the liquids over a white sheet and in front of a white background.

If the colour of the prescribed matching fluid is well observable (Nos 2–7), compare the contents of the two test tubes held vertically beside each other before a white background in front view (about 15 mm thickness).

The matching fluids are dilute stable solutions of coloured inorganic compounds prepared with 1 per cent sulphuric acid. The tints of matching fluids are designated by the initials of the tint (reddish = *R*; brownish = *B*; yellowish = *Y*; greenish = *G*), and the intensity of the colour by numbers, from 0 to 7. The tint is not indicated in the monographs, only the number referring to the permitted highest colour intensity is given. In such cases, the intensity of the colour of the test solution must not exceed the colour intensity of the matching fluid of the prescribed number with similar or closely similar tint.

No. 7 of every matching fluid series is at the same time the stock solution of the corresponding colour standard series. Solutions of lower intensity are prepared by proportional (twofold) dilution of the stock solution and of the members, respectively, designed by a number higher by one unit, with 1 per cent sulphuric acid.

The preparation of matching fluids is described under *IV(B)* of the chapter on *Reagents* (Vol. I, p. 327).

2 Tests for Acidity and Alkalinity

The *acidity* or *alkalinity* of aqueous solutions are checked by the addition of specified amounts of indicators, and indicator and volumetric standard solutions, respectively. In some cases the acidity and the alkalinity of the solution is specified as a given pH value.

Exclude the disturbing effect of the carbon dioxide of the air — if necessary — by pouring pentane onto the test solution, to form a 2 to 3 mm thick layer.

3 Tests for Contaminant Matter by Chemical Reactions

Tests for contaminants are performed in some cases by simple qualitative chemical reactions using an amount of the sample specified individually. Apply the reagents for the tests always in the prescribed quantity and order. Unless otherwise directed, evaluate the reactions after the elapse of 15 to 20 seconds.

"The mixture must not change" is prescribed in a monograph when the amount of the contaminant must not exceed the detection limit.

When the upper limit of a contaminant is given numerically, perform a comparative test according to *3.1.*

3.1 Limit Tests

The limit is referred to 1 g of the sample, and the amount of the contaminant is given in micrograms (μg), or in milligrams (mg).

For the comparative tests, 1.00 ml of limit test solution is always prescribed in order to obtain in the reference solution always the same grade of change. For this reason the quantity of the sample in each monograph is specified to limit the tolerated quantity of a certain contaminant.

When the amount of the contaminant must not exceed the detection limit ("the mixture must not change") the 1 ml limit test solution is replaced by an equal volume of water, or solvent; in tests *3.15*, *3.17* and *3.18*, however, the prescribed drops of limit solution should be added to the reagent.

Prepare the samples for the purity tests as specified in the individual monographs. Weigh the samples to 0.01 g accuracy, measure solvents and solutions with volumetric cylinders and with measuring pipets. (The necessary accuracy of the measurements is 0.1 ml up to 10 ml and 0.5 ml for larger volumes.)

When the sample is prepared for the test by digestion with concentrated sulphuric acid (according to *3*, Vol. I, p. 92), reduce the volume of the solution by fuming, to 0.5 ml.

The solution prepared for the test must be clear; thus filter, if necessary, through a moistened, analytical filter paper of 6 cm diameter. In doubtful cases digest the filter paper (Vol. I, p. 92) and test the residue separately.

Perform the tests in colourless test tubes (16×160 mm; about 25 ml) using the prescribed reagents in the given order.

Compare any changes in the test and in the reference solution with naked eyes over white filter paper or over opal glass plate, and over black paper or glass plate, respectively.

The limit test solutions and their preparation are described under IV/A of the chapter on *Reagents* (Vol. I, p. 325).

3.11 Test for Lead

Transfer the solution of the sample, and 1.00 ml of the lead limit solution (= 10 μg Pb) into test tubes. Add to each of both liquids one drop of R-hydrochloric acid, and 1.0 ml of a freshly prepared 1 per cent ascorbic acid solution, and heat them to boiling. Dilute the cooled solutions to 12 ml and add 1.0 ml of a 5 per cent potassium cyanide solution and 2.0 ml of R-sodium hydroxide solution. Filter the liquids, after shaking, through a filter paper of 6 cm diameter. Then add 5 drops of R-sodium sulphide solution to both solutions, and shake the test tubes again.

Perform the tests simultaneously, and compare the solutions after 5 minutes.

Any change in the test solution must not exceed the change in the lead limit solution.

3.12 Test for Heavy Metals (Pb, Fe, etc.)

Prepare the solution of the sample, as specified in the monograph, and transfer into a test tube. Transfer in another test tube 1.00 ml of lead limit solution (= 10 μg of Pb), and in a third test tube 1.00 ml of iron limit solution. Dilute the volumes of the liquids in each of the test tubes to 15 ml, add 0.5 g of ammonium chloride, 2 drops of R-ammonia solution and 5 drops of R-sodium sulphide solution to each test tube, and shake the mixtures. Perform the tests simultaneously, and compare the solutions after 5 minutes. Any change in the test solution must not exceed the brown colour developed in the lead limit solution, and the greyish green colour developed in the iron limit solution, respectively.

If — according to a monograph — the sample is to be digested before the test or the sample is coloured already prior to the addition of sodium sulphide solution to such an extent that the detection is impossible, digest the sample, according to *3*, Vol. I, p. 92, and reduce the volume of the digested solution to 0.5 ml. Dilute the cooled liquid with 2 ml of water, neutralize with a 20 per cent sodium hydroxide solution, transfer into a test tube, and complete the volume to 15 ml. Use this solution for the testing of heavy metals.

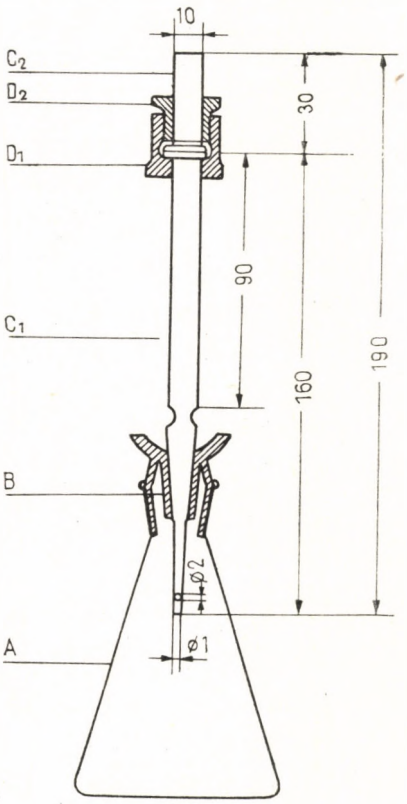

FIG. 8

3.13 Test for Arsenic

The apparatus for the limit test for arsenic (Fig. 8) is a 200 ml ground joint conical flask (*A*) fitted with a reagent tube (C_1) by the aid of a single hole ground stopper (*B*) with a wide collar-like rim. The orifice of the test tube is 1 mm and the side-opening is 2 mm in diameter. The upper end of the tube is a ground rim to which by the aid of a pair of plastic fixing rings (D_1, D_2), also a ground, rimmed annexing tube (C_2) can be fitted. Pack for the test the reagent tube (C_1) through the upper part of the tube with a 15 mm high cotton layer, covered loosely with another cotton plug saturated with lead acetate, and again with a 15 mm high cotton layer. Place then a mercury(II) bromide test paper on the ground upper rim of the reagent tube and attach the annexing tube to it with the aid of fixing rings.

Unless otherwise directed, transfer the sample into the conical flask of the apparatus, dissolve in 30 ml of water or dilute to 30 ml. To this solution add 5.0 ml of a freshly prepared 20 per cent potassium iodide solution, 30 ml of hydrochloric acid containing tin(II) chloride and 5.0 g of granulated zinc. Moisten the ground joints, with water and immediately attach the reagent tube to the flask. Keep the flask immersed in a water bath at 20°. After the reaction has been proceeded for 1 hour, remove the mercury(II) bromide test paper and preserve it in a glass-stoppered vessel until comparison. Compare the mercury(II) bromide test paper with the test papers used in the reference test and in the blank test, respectively.

Any change on the mercury(II) bromide test paper must not exceed that caused by a mixture of 1.00 ml arsenic limit solution (= 0.5 μg) and 29 ml of water in a test performed as directed above. If arsenic contamination over the detectable limit is excluded, perform the reference with 30 ml of water, without added arsenic limit solution.

3.14 Test for Iron

Transfer a solution of the sample, prepared as specified in the monograph, into a test tube, and 1.00 ml of iron limit solution (= 10 μg of Fe) into another one. Dilute both solutions to 13 ml and to each tube, add 1.0 of R-hydrochloric acid, and bromine water until a permanent yellow colour is obtained, and after 1 to 2 minutes 1.0 ml of R-potassium thiocyanate solution, and shake both test tubes.

Perform the tests simultaneously and compare the contents of the test tubes after 5 minutes.

Any change observed in the test solution must not exceed the change in the iron limit solution.

3.15 Test for Calcium

Transfer 4 drops of calcium limit solution into each one of two test tubes, and to each add 1.0 ml of R-ammonium oxalate solution, under shaking. Dilute a solution of the sample, prepared as directed in the monograph, to 14 ml and add 2 drops of R-acetic acid, and pour this solution into one of the test tubes containing the prepared reagent.

Transfer a mixture of 1.00 ml of calcium limit solution (= 50 μg of Ca), 13 ml of water and 2 drops of R-acetic acid into the other test tube. Shake both test tubes and immerse into a hot water bath for 5 minutes.

Perform the tests simultaneously and compare the contents of the test tubes after 4 minutes heating.

Any change observed in the test solution must not exceed the change in the calcium limit solution.

3.16 Test for Ammonia

3.161 Unless otherwise prescribed, dissolve a given quantity of the sample as specified in the monograph, in 15 ml of water in a test tube. In another test tube, mix 1.00 ml of ammonia limit solution (= 10 μg of NH_3) with 14 ml of water. To both solutions add 5 drops of R-Nessler-Winkler solution, and shake the liquids.

Perform the tests simultaneously and compare the contents of the test tubes after 5 minutes.

Any change observed in the test solution must not exceed the change in the ammonia limit solution.

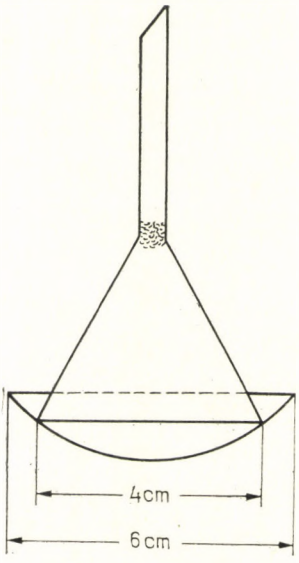

FIG. 9

3.162 To the quantity of the sample, specified by the monograph (0.2 g, if no quantity is specified) on a watch glass of 6 cm diameter, add 2 ml of R-sodium hydroxide solution. Place a small, dry loose cotton plug into a glass funnel of 14 cm diameter, and cover the watch glass (Fig. 9). Close the opening of the funnel leg with a piece of litmus paper (about 5 × 5 mm). Heat the watch glass on a water-bath for 10 minutes; the colour of the litmus paper must not change to blue.

Since test *3.162* is suitable only to detect larger quantities of ammonium salts (about 1 mg), no reference test is necessary.

3.17 Test for Potassium

Transfer 2 drops of potassium limit solution into each one of two test tubes, and add 2.0 ml of R-sodium tetraphenylborate solution and 1.0 ml of 0.1 N acetic acid to each tube. Shake the liquids for 30 seconds. Dilute a solution of the sample, prepared as specified in the monograph, to 12 ml and transfer this solution into one of the test tubes containing the reagent.

Transfer a mixture of 1.00 ml of potassium limit solution (= 100 μg of K) and 11 ml of water into the other test tube. Shake both test tubes.

Perform the tests simultaneously and compare the content of the test tubes after 10 minutes.

Any change in the test solution must not exceed the change in the potassium limit solution.

3.18 Test for Sulphate

Transfer 2 drops of sulphate limit solution into each one of two test tubes, and add 1.0 ml of R-barium chloride solution and 1.0 ml of R-hydrochloric acid. Shake the mixtures for 30 seconds. Dilute a solution of the sample, prepared as specified in the monograph, to 13 ml, and add this solution into one of the test tubes containing the prepared reagent.

Transfer a mixture of 1.00 ml sulphate limit solution (= 50 μg of SO_4) and 12 ml of water into the other test tube. Shake both test tubes.

Perform the tests simultaneously and compare the contents of the test tubes after 10 minutes.

Any change observed in the test solution must not exceed the change in the potassium limit solution.

Note. When silver salts are tested, R-hydrochloric acid and R-barium chloride solution must be replaced by R-nitric acid and R-barium nitrate solutions.

3.19 Test for Chloride

Transfer a solution of the sample, as specified in the monograph, into one test tube, and 1.00 ml of chloride limit solution (= 25 μg of Cl) into another one. Dilute both solutions to 13 ml, add 1.0 ml of R-nitric acid and 1.0 ml of R-silver nitrate solution to each tube, and shake them.

Perform the tests simultaneously, and compare the contents of the test tubes after 5 minutes.

Any change observed in the test solution must not exceed the change in the chloride limit solution.

3.20 Test for Nitrate

Unless otherwise directed in the monograph, dissolve a given quantity of the sample, in 1.0 ml of a 50 per cent sulphuric acid in a test tube. Transfer 1.00 ml of nitrate limit solution (= 100 μg of NO_3) into another test tube. To both solutions add 6 ml of concentrated R-sulphuric acid each, and to each of the cooled mixtures add 2 drops of R-iron(II) sulphate solution.

Perform the tests simultaneously and compare the contents of the test tubes after 1 minute.

Any change observed in the test solution must not exceed the change in the nitrate limit solution.

3.21 Test for Nitrate and Ammonia

For this test, use the distillation apparatus described in the section on *Assay of Organic Nitrogen* (Vol. I, p. 112) with the 50 ml flask with ground joint, lubricated with liquid paraffin.

Dissolve in the distillation flask a given quantity of the sample, as specified in the monograph in 10 ml of R-sodium hydroxide, and add 0.5 g of powdered Dewarda alloy. (Prior to use, boil the alloy with R-sodium hydroxide and rinse with water.) Connect the flask immediately to the condenser. Use a test tube, containing 5 ml of water, as a receiver; let pass the evolving gas through the water for 10 minutes. By gentle warming distil 2 to 3 ml into the test tube, complete the volume of the liquid to 15 ml. Transfer 1.00 ml of ammonia limit solution (= 10 μg of NH_3, or = 36.4 μg of NO_3), and 14 ml of water into another test tube. Add 5 drops each of R-Nessler-Winkler solution to both liquids and shake. Compare the test tubes after 5 minutes.

Any change observed in the test solution must not exceed the change in the ammonia limit solution.

4 Test for Heavy Metals Using Carboxy Cellulose as a Collector

In special cases (if e.g. no heavy metal contamination is permitted, in case if a separate detection of the metals is reguired in case of semi-quantitative test; of water tests and of tests of solvents of high purity) the heavy metal contaminants of the test solution are adsorbed on carboxy cellulose prior to the qualitative test.

The described procedure is not suitable for use directly to test concentrated solutions of inorganic salts, of strong acids and bases, and for compounds forming stable complexes with heavy metals.

4.1 Qualitative Test

Prior to these tests even the traces of heavy metals should be removed from the solvent or from the water.

Solids. Dissolve not more than 1.0 g sample possibly in 30 ml, but in not more than 100 ml of solvent. Use water as the solvent in most cases. For the dissolution of water-insoluble substances use alcohol-water or acetone-water mixtures, dimethylformamide, etc. (Prior to use, purify both water and other solvents from heavy metal ions. For this purpose, shake distilled water or solvents of analytical grade with carboxy cellulose, 0.5 g per litre of solvent, allow to stand for 12 hours and filter.)

Solutions and solvents. Not more than 500 ml may be used in one test. To the test solution, add 5 mg of carboxy cellulose, shake for 5 minutes or allow to stand for a few hours, and collect the dispersed carboxy cellulose fibres on a sintered-glass microrod filter G3 (possibly on a 1 cm diameter filter paper placed on Witt plate) by gentle suction. Rinse the collected carboxy cellulose (usually disc-shaped) with 5 ml of solvent, and remove this disc from the filter with the aid of a tipped glass rod. The heavy metal contaminants of the test solution are adsorbed by this carboxy cellulose disc. Then place the disc on a small watch glass or on a porcelain or glass test plate, and identify the heavy metal contamination by a suitable spot test. If different metal contaminations must be detected in the pres-

ence of each other, the disc is divided into smaller pieces, and the metal ions can be identified separately with specific reactions. A single disc can be used for the detection of three ions.

The intensity of the colour on the carboxy cellulose disc can be used in some cases for the semi-quantitative evaluation of the contaminants.

4.2 Quantitative Test

Collect the metal contaminants on carboxy cellulose in the same manner as directed above. To the filtrate, however, add two successive 5 mg portions of carboxy cellulose to collect the last traces of metal ions.

Transfer the carboxy cellulose disc into a 25 ml volumetric flask with the aid of 10 to 15 ml of water. Add the suitable specific reagents to the suspension (e.g. a carbon tetrachloride dithizone solution, when colourimetric titration with dithizone is applied). Perform a colourimetric titration using a metal ion solution of known concentration.

An alternative procedure is to transfer the carboxy cellulose disc into a separator funnel with the aid of 10 to 15 ml of water, to extract the metal ion with a suitable complexing agent (e.g. dithizone) and to measure its amount by photometry.

5 Test for Readily Carbonizable Substances

Perform the test in a test tube rinsed with 95 per cent sulphuric acid. Unless otherwise directed, add a given quantity of powdered and dried (crystal-water containing or hygroscopic) sample to 5 ml of 95 per cent sulphuric acid and dissolve under shaking. Compare the colour of the sulphuric acid solution with the prescribed matching fluid according to *1.1.*

The colour intensity of the sulphuric acid solution must not exceed that of the matching fluid of equal volume and layer thickness. The number of matching fluid is specified in each single monograph.

(C) QUANTITATIVE TESTS AND ASSAYS

The aim of quantitative tests and assays is on the one hand to check the amount of limit-bound contaminants, using general and quantitative methods, where qualitative tests do not give adequate information, and, on the other hand to establish the contents of limit-bound active substances with specific methods.

The limits given in the monographs were specified on considering the error of the prescribed analytic procedure. Unless otherwise directed in the monographs, the specified and established contents of active substances refer to previously non-dried sample.

The determination of active substances is performed considering the directions of the chapter on *Sampling* (**1** Vol. I, p. 89) generally with air-dry substance, prepared according to the chapter on *Preparation of Material for Measurement* (**2**, Vol. I, p. 91).

In this Pharmacopoeia, the equivalent weights are given as rounded to four decimals in the description of the assay. However, the rounded equivalent weights are followed by the unrounded five-digit logarithms of the equivalent weights.

When the determination of an active substance is not specified and its percentage is not indicated at all or only an approximate value is given, the deviation in the content of active substance determined by an adequate chemical method may not exceed ± 5 per cent from that of the specified approximate value.

(a) GENERAL TESTS

1 Determination of Loss on Drying

Unless otherwise specified in the monograph, use about 0.5 g, powdered sample for the test. Weigh the sample directly in a glass-stoppered, tared, weighing vessel; spread the sample evenly to form a thin layer. Place the unstoppered vessel in the drying oven for 1 hour at 40° to 50°, then elevate the temperature of the oven generally to 100° to 110°, and dry the sample at that temperature to constant weight. Close the vessel before removing it from the drying oven, place the hot vessel into a desiccator over calcium oxide and reweigh it stoppered.

Drying to constant weight means that during the drying the difference between weights determined at 1 hour intervals must not exceed 1 mg per gram referred to the dried sample. When the residue is less than 0.20 g, the difference between the weights must not exceed 0.2 mg, measured at 1 hour intervals.

When the drying is to be performed at room temperature, in desiccator or in vacuo, the sample should be dried at least for 24 hours, unless otherwise specified. The quantity of sample used for this test should be sufficient to determine, after the determination of the loss on drying (water content, moisture), also the residue on ignition or the content of active substance, when required.

The loss on drying of vegetable drugs must be determined according to *3.1*, Vol. I, p. 209.

2 Determination of Residue on Drying

2.1 Determination of Non-volatile Residue

Unless otherwise specified, transfer about 50 g of liquid sample, weighed to mg accuracy, into a 100-ml flask and reduce its volume to about 1 ml by distillation. Rinse the residue, using small portions of the distillate, into a tared glass-stoppered weighing vessel, and evaporate to dryness on a water-bath. Dry the residue at 105° to constant weight.

2.2 Determination of Total Solids

2.21 Unless otherwise specified, transfer into a tared glass-stoppered weighing vessel (50×25 mm) such an amount of the substance (if necessary, in several portions) to obtain a total solid content of about 0.2 g and evaporate the sample to dryness on a water-bath. Unless otherwise specified in the monograph, dry the residue at 100° to 110° to constant weight according to **1**.

Place the bottle into a desiccator over calcium oxide and reweigh it stoppered.

2.22 When the sample is viscous or it contains sugar, dilute the weighed sample with a small portion of an adequate solvent to obtain a continuous liquid layer at the

bottom of the weighing vessel. An alternative procedure is to place about 10 g of sea sand, washed with hydrochloric acid and water, and ignited, into an empty weighing-vessel together with a small glass rod, and to weight it accurately. Pour the viscous liquid sample onto the sea sand, add some water, mix the sand with the liquid by the aid of the glass rod. Evaporate the mixture to dryness on the water-bath by constant stirring, and dry at 100° to 110° in a drying oven to constant weight. Place the bottle into a desiccator over calcium oxide and reweigh it stoppered.

The total solid content of viscous or sugar-containing samples can be determined in a heatable vacuum desiccator, without mixing them with sea sand.

3 Ignition Tests

3.1 Determination of Residue on Ignition

Perform the test, depending on the nature of the sample, in a platinum, porcelain or silica crucible, 25 mm in diameter.* Ignite a crucible, cool in a desiccator, and weigh accurately. Transfer the amount of sample specified in the monograph into the crucible, and weigh accurately. Place the crucible in a triangle, first heat it gently, and then ignite the residue. For the testing of inorganic substances, ignite the sample until the weight of residue does not change any more, when organic substances are tested ignite until all carbon is completely consumed. (Moisten the residue with a few drops of concentrated nitric acid if necessary, and ignite repeatedly.) Allow the crucible to cool in a desiccator and weight accurately. When hygroscopic or carbon dioxide-absorbing substances are tested or when the residue on ignition is hygroscopic, place the crucible into a glass-stoppered weighing vessel before weighing.

For the testing of solutions evaporate the sample to dryness in a prepared crucible, if necessary, in several portions. Proceed further as directed above. The ash content of drugs should be determined according to *4.1*, Vol. I, p. 208.

Note. To test for loss on ignition, proceed as described above.

3.2 Determination of Sulphated Ash

Perform the test according to *3.1*; the organic sample, however, ignite only gently until carbonization, and then cool. Moisten the residue with 1 ml of concentrated R-sulphuric acid, and ignite gently until white fumes cease to evolve, then ignite strongly until the carbon is completely consumed.

4 Determination of Insoluble Residue

Unless otherwise directed, add to an accurately weighed amount of the sample, as specified in the monograph, in a 200-ml conical flask, 100 ml of hot water and heat the flask on the water-bath for 1 hour, inserting a small funnel into the neck of the flask.

* Easily reducible metals and nonmetals (silver, lead, bismuth, antimony, etc.), and alkali hydroxides, etc. corrode platinum crucibles. Fused alkali hydroxides and carbonates attack silica and porcelain crucibles; silver and nickel crucibles are, however, suitable for fusions with alkali hydroxides.

Place a strip of filter paper washed with a suitable solvent, dried and folded, into a weighing vessel, of about 50 mm diameter and 25 mm height. Dry the filter paper at 105° to constant weight. Then allow the vessel to cool over calcium oxide in a desiccator, and weigh it stoppered. Filter the solution containing the insoluble matter through this filter paper and rinse the residue with hot water. Dry the filter paper together with the funnel, then place the filter paper into the weighing vessel and dry it at 105° to constant weight. Determine again the weigh of the vessel and paper filter as described above.

Instead of paper filter a glass filter is also suitable for use.

5 Determination of Water

5.1 Determination of Water by Titration (Karl Fischer Method)

Reagents

dehydrated methanol (it must not contain more than 0.3 mg of water per ml)
dehydrated pyridine (it must not contain more than 1 mg of water per ml)
iodine (Vol. II, monograph No. 191)
sulphur dioxide, gas (it must contain not more than 0.01 per cent of water)
sodium tartrate ($C_4H_4O_6Na_2 \cdot 2H_2O$) (determine its water content by drying at 150° to constant weight)
aquametric iodine standard solution.

Preparation of aquametric iodine standard solution. Mix 760 ml of dehydrated methanol with 270 ml of dehydrated pyridine in a well-stoppered flask. Introduce sulphur dioxide gas into the mixture until the weight increase attains 64 g. In the cooled liquid, dissolve 120 g of iodine under shaking.

The solution is suitable for use after 24 hours. Each ml of this solution is equivalent to about 5 mg of water. Solutions equivalent to 3 mg water or less per millilitre are not suitable for use. The water equivalent of the solution must be checked daily and prior to use, respectively.

Preserve the solution protected from humidity and light.

Determination of the water equivalent of the standard solution. Titrate 10 ml of dehydrated methanol with iodine standard solution to the end point. To this liquid, add quickly about 0.3 g of sodium tartrate, accurately weighed, and titrate the solution with aquametric iodine standard solution. Calculate the water equivalent of the solution by the formula:

$$E = \frac{10 \times b \times g}{a}$$

where b = water content of sodium tartrate in per cent
g = the weight of sodium tartrate in grams
a = millilitres of iodine standard solution consumed for the titration of the water content of sodium tartrate
E = mg of water equivalent to 1 ml of aquametric iodine standard solution.

An alternative procedure is to titrate about 110 ml of dehydrated methanol with aquametric iodine standard solution to the end point. Use this solution to

dilute about 1.00 g of water in a volumetric flask to 100 ml and titrate a 10.00 ml portion of the stock solution with iodine standard solution. Calculate the water equivalent of the solution by the formula

$$E = \frac{100 \times g}{a}$$

where g = grams of water dissolved in 100 ml of methanol
a = millilitres of aquametric iodine standard solution consumed for the titration of the water
E = mg of water equivalent to 1 ml of aquametric iodine standard solution.

Determination of water. Titrate a certain amount of the solvent as directed in the monograph, with aquametric iodine standard solution. Then immediately dissolve or suspend in the solution an accurately weighed sample containing about 50 mg of water. (The amount of the weighed sample must be varied according to the water equivalent of the standard solution.) Titrate the mixture with iodine standard solution. Use a closed titration apparatus or perform the titration quickly to eliminate the effect of air-humidity. Stir the liquid continuously either by passing nitrogen gas bubbles or by using a magnetic stirrer. Apply for the end point detection an electric (dead-stop) method.

Calculate the water content of the sample by the formula:

$$x = \frac{a \times E}{10 \times g}$$

where a = millilitres of aquametric iodine standard solution consumed for the titration of the water content of the sample
E = mg of water equivalent to 1 ml of aquametric iodine standard solution
g = the weight of the sample in grams
x = the water content of the sample in per cent.

Note. The end point can be detected also visually, in this case, however, the weight of the sample must be the double of the amount specified in the monographs. At the end point the orange yellow colour of the solution changes to brownish.

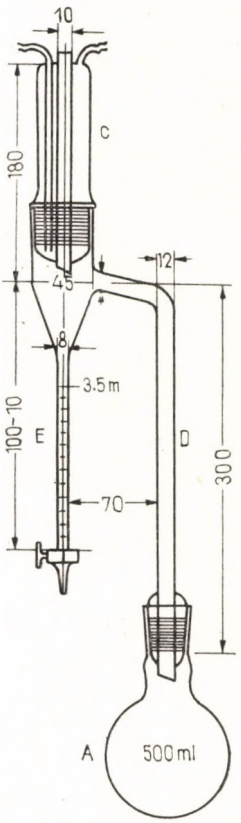

FIG. 10

5.2 Determination of Water by Distillation

Use for determining the apparatus in Fig. 10, consisting of a flask, an adapter and a condenser. Mount a 500 ml standard ground-glass joint flask (*A*), with a conical adapter by the aid of a bent glass tube. A ground joint condenser (*C*) is adaptable to the wider part of the adapter. The lower part of the adapter, serving as the re-

ceiver, is a volumetric tube 3.5 ml in volume, graduated and calibrated to 0.05 ml accuracy, provided with a glass stopcock.

Prior to use, degrease the apparatus (e.g. using peroxysulphuric acid or chromic-sulphuric acid), then rinse with water and dry. Lubricate the glass stopcock of the volumetric tube with silicone fat; wet the other glass ground-joints with xylene.

Agitate xylene to be used in the experiments with about 5 per cent of water, then discard the aqueous layer and distil the xylene.

Transfer into a dry round-bottomed flask an accurately weighed sample containing 2 to 3 ml of water. Add 100 ml of xylene and about 1 g of pumice. Fill the measuring tube and the wider part of the adapter with xylene and add a few mg of sodium lauryl sulphate. Assemble the apparatus, allow the cooling water in the condensor to run, and heat the flask cautiously (*xylene is highly inflammable!*) so that the liquid should start boiling about after 15 minutes. Increase the heating at first slowly, then more rapidly, and keep the liquid boiling for about 40 minutes. After cooling, rinse the condenser tube with a small portion of xylene. Transfer any water droplets adhering to the bottom of the condenser with xylene into the measuring tube.

Read the volume of water in the measuring tube, and calculate the water content of the sample (in per cent) regarding the density of water equal to 1.

6 Determination of Alcohol

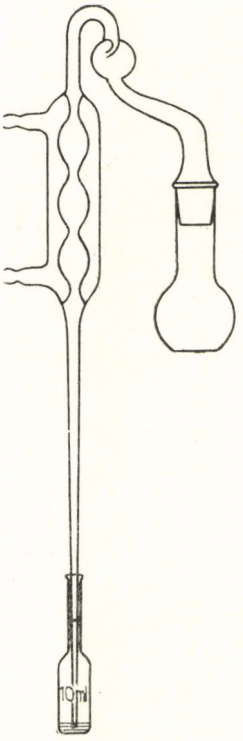

FIG. 11

Determine the alcohol content of medicinal liquids by distillation. Use for this purpose a small distillation apparatus (Fig. 11), consisting of a 50 or a 100 ml distillation flask, a condenser sealed to an adapter fitted by a glass ground joint. The length of the narrow part of the 45 cm long condenser tube, is 15 cm. The length of the cooling mantle is 12 cm. Foam and liquid droplets formed during boiling are retained by a bulb of 2.5 cm diameter. Use as a receiver a 10 ml cylindric glass-stoppered volumetric flask, with a total length of 10 cm with a neck 4 mm in inner diameter.

Perform the determination of alcohol connected with the measurement of density of the liquid. Transfer the liquid sample at 20° into a 10 ml dry receiver, accurately weighed, not filling the flask quite up to the mark. Then insert the receiver into a water-bath at 20°. After 10 minutes, complete the liquid to the mark, using a capillary pipette. Dry the neck of the flask with a strip of filter paper. Remove the flask from the water-bath, clean it scrupulously, place it into the box of the balance or near to it for 15 minutes, and reweigh-accurately. The difference in the two weighings gives the weight of the liquid in the flask. Then transfer the liquid into the distillation flask as specified in *6.1*, *6.2* and *6.3*, respectively, rinse the flask, and perform the above procedure with water of 20° temperature, and boiled the previous day. Calculate the density of the liquid sample according to *6.2*, Vol. I, p. 74.

Two alternative methods are suitable for the determination of alcohol in liquid samples, depending on the probable alcohol content of the liquid.

6.1 Determination of Alcohol below 20 v/v per cent

In the case of liquid samples containing less than 20 v/v per cent of alcohol, rinse the content of the 10 ml receiver into the 50 ml distillation flask of the apparatus with three subsequent 2 ml portions of concentrated R-calcium chloride. Add some pumice, and a few mg of tannic acid (in the test of *Tinctura saponariae* use 0.2 g), to prevent foaming, and assemble the apparatus. Lubricate the ground glass joint of the flask with concentrated R-calcium chloride solution. Rinse the receiver with water and pour 1 ml of water into it. Insert the tip of the condenser tube in the receiver so as to reach under the water level. Distil about 6 ml of the liquid, then lower the receiver so that the tip of the condenser tube should not reach the liquid surface any more. Rinse the tip of the condenser tube and continue distillation until the level of the liquid nearly reaches the circular mark. Then shake the receiver and immerse into a water-bath of 20° temperature. After 10 minutes, complete the liquid with water to the mark. Dry the neck of the receiver with the aid of a filter paper strip; clean the receiver scrupulously and weigh it accurately. Calculate the density of the distillate as directed in Vol. I, p. 74.

Use Table IV in Vol. IV to calculate the alcohol content of the distillate, expressed as v/v per cent.

To express the alcohol content as v/v per cent, divide the alcohol content of the distillate, calculated with the aid of the v/v per cent column of Table IV in Vol. IV, by the density of the liquid sample.

6.2 Determination of Alcohol above 20 v/v per cent

If the liquid sample contains more than 20 v/v per cent of alcohol, rinse the content of the 10 ml receiver, after measuring the density, into a 50 ml volumetric flask, and complete the volume to the circular mark at 20°. Transfer a 10.00 ml portion of the liquid sample thus fivefold diluted, into the distillation flask, add 6 ml of concentrated R-calcium chloride solution, and further continue the test as directed under *6.1.* To obtain the alcohol content of the sample, as v/v per cent, multiply the alcohol content calculated from the density of the distillate with the aid of Table IV, Vol. IV by five.

To express the alcohol content as w/w per cent, divide the v/v per cent data by the density of the liquid sample.

6.3 Determination of Alcohol in Precipitous Liquids

When samples forming congealing precipitate on dilution with water are tested, transfer the content of the 10 ml receiver directly into the 100 ml distillation flask with 25 ml of water and 6 ml of concentrated R-calcium chloride solution and perform the test as specified under *6.1;* use, however, a 50 ml volumetric flask as receiver and distil 30 ml of the liquid. Determine the density of the distillate in a 50 ml flask according to *6.1*, Vol. I, p. 74. Calculate the alcohol content as described in *6.2.*

6.4 Determination of Alcohol in Other Liquids

Unless otherwise directed, determine the alcohol content of liquids containing volatile substances (volatile oils, menthol, camphene, etc.) as follows: to the solu-

tion diluted to 50 ml according to *6.2*, add 2 ml of petroleum ether, shake the mixture, and after separation of the two phases remove the petroleum ether layer by means of a pipet. Repeat this procedure with two 2 ml portions of petroleum ether. Dilute the aqueous solution with water to the mark if necessary, and perform the determination of alcohol as specified under *6.2* and *6.3*.

6.41 Alternative procedure can be performed as follows: after measuring the density of the sample, rinse the content of the 10 ml receiver with three 2 ml portions of concentrated R-calcium chloride solution and 15 ml of water into a separator funnel, and extract the liquid by shaking with three 10 ml portions of petroleum ether (of a boiling point not higher than 50°). Combine the petroleum ether extracts in another separator funnel, and reextract by shaking with two 2 ml-portions of concentrated R-calcium chloride solution. Transfer the calcium chloride solutions and the extracted sample into a 50 ml volumetric flask, complete the volume with water to 50 ml and perform the determination of alcohol according *6.2*. (The test can be performed also according to *6.3*. In this case the sample should be transferred in the course of the extraction with petroleum ether directly into the distillation flask.)

Also the volatile matter can be determined in the combined petroleum ether solutions, after drying with anhydrous sodium sulphate.

Note. Alcohol content expressed in w/w per cent, measured according to *6.1* and *6.2*, can be calculated by measuring the refractive index of the liquids with the aid of a Zeiss immersion refractometer and using Table V in Vol. IV. Both the v/v per cent and the w/v per cent alcohol contents of the distillate can be calculated by using Table IV in Vol. IV.

Example: the density of the liquid sample at 20° ... 0.932
the density of the distillate at 20° ... 0.898.

According to Table IV in Vol. IV, a density value of 0.898 corresponds to 64.80 per cent by volume of alcohol. The alcohol content of the liquid sample expressed in volume ratio equals to 64.80 per cent. This value corresponds to 51.19 w/v per cent. The alcohol content of the liquid sample expressed in weight ratio equals to

$$\frac{51.19}{0.932} = 64.8 \text{ per cent.}$$

(b) ASSAYS

1 Assay of Arsenic

Reagents

concentrated R-sulphuric acid
concentrated hydrogen peroxide solution
hydrazine sulphate
potassium bromide
potassium bromate solutions, 0.1 **N** and 0.01 **N**
I-p-ethoxychrysoidine solution.

Procedure

Weigh a sample accurately, according to *3*, Vol. I, p. 92, as directed in the monograph, in a paper capsule.

Transfer the paper capsule and 1 to 2 glass beads into a 100 ml digesting flask, add 5 ml of concentrated R-sulphuric acid and 3 ml of concentrated hydrogen peroxide solution, and perform the digestion according to *3*, Vol. I, p. 92.

To expel hydrogen peroxide, boil vigorously the concentrated sulphuric acid arsenic solution for 5 minutes after the completion of digestion. Add 200 mg of hydrazine sulphate with the aid of a long necked funnel for powders (Fig. 12), then keep the liquid in vigorous boiling for 20 minutes, and cool. Rinse the neck of the flask, add 30 ml of water and 0.5 g of potassium bromide. Titrate the solution slowly with 0.1 **N** or 0.01 **N** potassium bromate, depending on the estimated amount of arsenic present, using 1 to 2 drops of **I**-p-ethoxychrysoidine solution as indicator. Add the indicator to the liquid near to end-point of the titration. The colour change is from red to faint yellow.

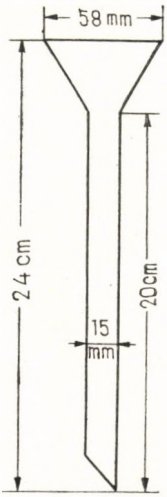

FIG. 12

Each ml of 0.1 **N** potassium bromate solution is equivalent to 3.746 mg (1g .57351) of As; each ml of 0.01 **N** potassium bromate is equivalent to 0.3746 mg (1g. 57351) of As.

Digest arsenic compounds not easily digestible (arzines, cacodyl compounds, arzenobenzene derivatives, etc.) as follows: the digestion must be continued for 20 minutes after permanent decolouration of the sulphuric acid solution by adding to the liquid in every 5 minutes 4 to 5 drops of a concentrated hydrogen peroxide solution.

Note. Take special care with the digestion of samples containing halogen, adding each time a few drops of hydrogen peroxide immediately to decolourize the liquid as soon as its colour changes to brown.

2 Assay of Mercury Following Digestion

Reagents

concentrated R-sulphuric acid
concentrated hydrogen peroxide solution
I-iron(III) nitrate solution
potassium permanganate solution, 0.1 **N**
potassium thiocyanate solution, 0.1 **N** or 0.01 **N**

Procedure

Weigh accurately a sample, as directed in the monograph, if necessary, in a paper capsule, into a 100 ml digesting flask. Add 5 ml of concentrated R-sulphuric acid and 3 ml of a concentrated hydrogen peroxide solution, and perform the digestion according to **3**, Vol. I, p. 92. Proceed with digestion until the turbid liquid becomes clear. Cool, and dilute the liquid with 30 to 40 ml of water, add 1 ml of **I**-iron(III) nitrate solution. Add dropwise 0.1 **N** potassium permanganate solution, until a permanent pink colour is produced. Destroy this colour with 1

drop of hydrogen peroxide solution. Cool the liquid below 15° and titrate it with 0.1 **N** (or 0.01 **N**) potassium thiocyanate solution until a permanent pink colour appears. Each ml of 0.1 **N** potassium thiocyanate solution is equivalent to 10.03 mg (lg .00132) of Hg, each ml of 0.01 **N** potassium thiocyanate solution is equivalent to 1.003 mg (lg .00132) of Hg.

Note. Samples containing mercury should be digested as quickly as possible. A loss in mercury content may occur on prolonged heating with concentrated sulphuric acid. Bismuth, zinc and a small amount of lead do not interfere. If halogens are present, precipitate mercury as mercury sulphide, collect the precipitate on a filter paper, wash until free of halogen and digest with 10 ml of concentrated R-sulphuric acid, adding in small portions a sufficient amount of concentrated hydrogen peroxide.

3 Assay of Iron-Content of Iron(II) and Iron(III) Compounds

Reagents

phosphoric acid, 20 per cent, ironfree
concentrated hydrochloric acid, 37 per cent
dipyridyl solution
alcohol, 96 per cent
ammonium acetate solution, 20 per cent
ascorbic acid.

3.1 Assay of Iron(II) in Preparations Containing Iron(II) Compounds

Weigh accurately a powdered sample corresponding to 0.5 to 10 mg of iron(II), into a 50 ml glass-stoppered flask containing 1 to 2 ml of a 20 per cent phosphoric acid solution. The sample should be dissolved without heating, if necessary, under the addition of 2 ml of 37 per cent hydrochloric acid. Transfer quantitatively the content of the flask into a 100 ml volumetric flask [if the sample contains 0.5 to 1 mg of iron(II)] or into a 1000 ml volumetric flask [if the sample contains 1 to 10 mg of iron(II)]. If the solution is turbid (this is used to occur on testing pills or tablets) it should be filtered through a filter paper.

Into one 100 ml volumetric flask, add 10.00 ml of the solution, 0.04 ml of dipyridyl solution, then 10 ml of a 20 per cent ammonium acetate solution, and dilute with water to 100 ml; into another 100 ml volumetric flask add the same solutions, instead of dipyridil, however, apply 0.40 ml of 96 per cent alcohol. Allow the flask to stand on a dark place for half an hour, and determine the optical density of the solutions in 2 cm cells by a Pulfrich photometer using colour filter No. S 50. (The spectrum has maximum absorption at 520 nm, and for this reason, measurements with a spectrophotometer should be performed at this wavelength.) The solution without dipyridil serves a reference liquid.

Calculate the iron content of the sample, expressed as iron (Fe), from the optical density, and from the calibration curve.

Plotting the calibration curve of iron(II) ammonium sulphate. Transfer 0.702 g of iron(II) ammonium sulphate, accurately weighed, into 100 ml volumetric flask, dissolve it in 50 ml of R-sulphuric acid, and complete the volume to 100 ml with water. (The solution contains 1 mg of Fe per millilitre.)

Dilute this stock solution with water so as to obtain solutions which contain, after the addition of the reagents and 20 mg of ascorbic acid, 2 to 4 μg of iron (Fe) per millilitre. These diluted solutions are used for plotting the calibration curve.

3.2 Assay of the Total Iron Content of Preparations Containing Iron(II) and Iron(III) Compounds

Weigh accurately a powdered sample, corresponding to 0.5 to 10 mg of total iron and perform the dissolution, dilution and filtration according to *3.1.* The sample may be warmed during dissolution.

Transfer 10.00 ml portions of the prepared solution into two 100 ml volumetric flasks; to each flask add 20 mg of ascorbic acid, and allow the liquids to stand in diffuse light. Continue the test further as described in *3.1.* It is necessary to keep the solutions in a dark place.

4 Assay of Small Quantities of Potassium

Reagents

1 **N** acetic acid
sodium tetraphenyl borate solution (approx. 0.1 **M**)
washing liquid.

Procedure

Dissolve 100 mg of potassium chloride in 100 ml of water. Transfer a 5.0 ml portion of this solution into a conical flask and dilute it with 45 ml of water. To this solution, add dropwise and under shaking 5 ml of sodium tetraphenyl borate solution stir the mixture a few times, and allow it to stand for 10 minutes. Collect the bulk of the precipitate on a sintered glass filter No. G4, and wash it with five 5 ml portions of water. Transfer the precipitate into a flask, add 250 ml of water, shake for 30 minutes, then add about 0.5 g of alumina (analytical grade) or aluminium hydroxide, and shake for 5 minutes. Cool the precipitous liquid to 10 to 15°, filter through a dense filter paper, and to the clear filtrate add 10 ml of 1 **N** acetic acid.

Assay

Transfer a sample, as directed in the monograph, into a 200 ml beaker, and dilute it to 100 ml if necessary. Add 1 ml of 1 **N** acetic acid and dropwise 10 ml of sodium tetraphenyl borate solution. Stir the precipitous liquid and allow it to stand for 10 minutes. Collect the precipitate in a sintered glass filter, No. G4, prepared and accurately weighed; wash it with three successive 5 ml portions of the washing liquid, and once with 5 ml of cold water. Dry at 105° for 1 hour, cool and weigh.

Each mg of the precipitate is equivalent to 0.1091 mg (lg .03788) of K, or 0.2081 mg (lg .31819) of KCl.

The apparatus used for the assay of nitrogen is shown in Fig. 13. The distillation flask of the apparatus is a ground-joint glass digestion flask (of 50, 100 or 250 ml volume) which may be attached to a distillation head provided by a ball condenser, and a trap bulb. The condenser tube is 42 cm long, and its tip is smooth-ground. The jacket of the condenser is 16 cm long. A small funnel is attached to the distillation head, which is provided by a three-bore stopcock. To the tip of the three-bore stopcock a rubber hose clogged by a small glass rod is attached. Since boiling with acid is required in some cases (acid amides, urethane, etc.) to split ammonia, a digestion flask can be attached to a ground glass joint reflux ball condenser. Unless otherwise directed in the monograph, weigh accurately a dried and prepared sample corresponding to about 1.5 mg and, respectively, to about 8 mg of nitrogen, when, performing the titration with 0.02 **N** and 0.1 **N** sulphuric acid, respectively (*5.1* and *5.2*). The sample must be weighed in a small nitrogen-free paper capsule (Vol. I, p. 92) or in a small bottle made from a thin-walled glass tube of 5 mm diameter.

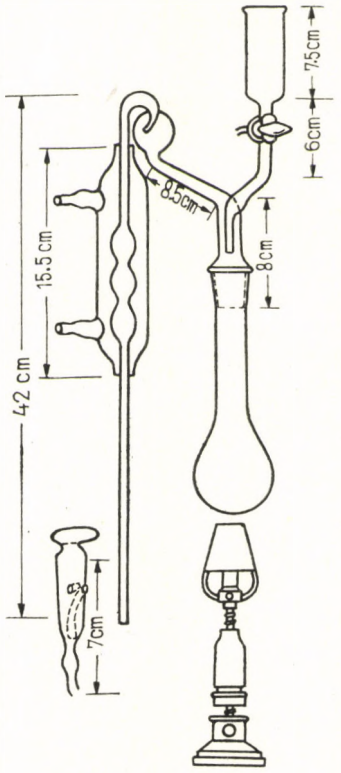

FIG. 13

It is advantageous to prepare a stock solution which contains the quantity to be assayed in 1 millilitre. Use water, dilute alkalies and concentrated sulphuric acid. In the latter case, 50 per cent sulphuric acid is used as diluting agent.

It is necessary to run a blank test when a paper capsule is used. Blank runs should be performed also to check the nitrogen content of the reagents.

Reagents

concentrated R-sulphuric acid
selenium
I-methyl red solution
sodium hydroxide solution, 20 per cent
sulphuric acid, 0.02 **N** or 0.1 **N**
sodium hydroxide solution, 0.02 **N** or 0.1 **N**
sodium thiosulphate solution, 20 per cent
R-sulphuric acid
sodium dithionite.

5.1 Assay on 0.02 N Scale

Transfer the sample into a digestion flask, add 2 ml of concentrated R-sulphuric acid, 20 to 30 mg of selenium, and one glass bead. Heat the flask over a small flame until the solution becomes clear. Continue the digestion for 2 hours

or in special cases for 3 to 4 hours. Barbituric acid derivatives should be digested by attaching a reflux condenser to the digestion flask. (The ground glass joint should be lubricated with concentrated R-sulphuric acid.) After cooling the flask, rinse the ground glass joint of the flask, dilute the liquid with 40 ml of water, add 2 to 3 drops of **I**-methyl red solution and, in order to prevent retarded boiling, some pumice. Lubricate the dry ground joint with liquid paraffin and assemble the apparatus. Fill the leg of the small funnel with water and the funnel itself with a 20 per cent sodium hydroxide solution. Use a 100 ml conical flask as receiver. Unless otherwise directed, pipet 10.00 ml of 0.02 **N** sulphuric acid into the receiver. Press gently the bottom of the receiver to the condenser tube, and begin the distillation. Heat gently the flask over a free flame, then boil to expel air from the apparatus. When the bubbling ceases and the liquid is sucked from the receiver into the condenser tube, turn the stopcock of the small funnel and allow sodium hydroxide to drop into the flask until the red colour of the liquid changes to yellow. Add a few drops in excess. Distil until the distillate attains a volume of 40 ml. Remove the small rubber hose from the tip of the three-bore stopcock, turn the stopcock and allow the sodium hydroxide solution to flow into a small beaker, then through the three-bore stopcock allow air to pass in the apparatus, and finish the distillation. Rinse the condenser tube in the receiver, add some pumice, and heat the liquid to boiling. Then cool, and titrate with 0.02 **N** sodium hydroxide solution using 1 to 2 drops of **I**-methyl red solution as indicator.

Each ml of 0.02 **N** sulphuric acid is equivalent to 0.2802 mg (lg .44741) of N.

5.2 Assay on 0.1 N Scale

Use for digestion, 5 to 10 ml of concentrated R-sulphuric acid and 0.04–0.06 g of selenium. Reduce after digestion the volume of the liquid to 1 to 2 ml. For that purpose, blow an air-stream over the sulphuric acid solution, with the aid of a glass-pipe. (Place a cotton plug soaked in R-sulphuric acid and pressed out by fingers into the wider portion of the pipe.) The tip of the pipe must not reach the liquid. Then cool the solution and continue as directed in *5.1.* Unless otherwise directed, pipet 10.00 ml of 0.1 **N** sulphuric acid into the receiver. Titrate the excess of the acid with 0.1 **N** sodium hydroxide solution using 1 to 2 drops of **I**-methyl red solution.

Each ml of 0.1 **N** sulphuric acid is equivalent to 1.401 mg (lg .14638) of N.

Note. When nitrogen compounds containing mercury are tested, add 1 ml of a 20 per cent solution of sodium thiosulphate to the liquid after making the digested sample alkaline.

Dissolve nitro-, nitroso-, and azo-compounds, prior to digestion, in 1 to 2 ml of R-sulphuric acid in a digestion flask, and reduce by adding 0.10 to 0.20 g of sodium dithionite; then add concentrated R-sulphuric acid and selenium, and digest as described above.

6 Assay of Halogens in Organic Bond

6.1 Assay with Preceding Saponification

Reagents

R-potassium hydroxide, halogen-free
R-propanol
R-nitric acid
silver nitrate solution, 0.1 **N** or 0.01 **N**
I-iron(III) nitrate solution
potassium thiocyanate solution, 0.1 **N** or 0.01 **N**
R-potassium nitrate, halogen-free.

Place 1 g of halogen-free potassium hydroxide granules in a 25 ml test tube. Heat the upper part of the test tube in a flame, draw it apart to provide a neck of 3 mm diameter. Cut the test tube with a 2.5 to 3 cm long capillary. Seal the capillary on the upper part of the original test tube, and use it as cover for the weighing tube. Pour 5 to 8 ml of propanol onto the solid potassium hydroxide in the test tube, and weigh it accurately.

Draw in a flame the tip of a pipet graduated to 0.01 ml to a capillary so as to shift it in easily into the capillary ending of the test tube. Suck into the pipet a quantity of the preparation as directed in the monograph and hold the pipet in horizontal position without closing its upper ending with a finger. Then shift the capillary ending into the test tube, close the ending of the test tube with the finger, and force in this way the liquid, by its own vapour, into the test tube.

When solid samples are tested, prepare a solution of known concentration, possibly with R-propanol, and pipette a portion (1.00 to 5.00 ml) of the solution into a prepared test tube containing potassium hydroxide.

Seal the capillary ending of the test tube as quickly as possible, and weigh it accurately. The weight of the sample is the difference between the two weighings. Shake the sealed test tube until potassium hydroxide dissolves, then immerse it in a boiling water-bath for a period depending on the nature of the sample.

Cool, and open the test tube with the aid of a rasper and transfer the liquid quantitatively into a 200 ml conical flask with about 50 ml of water. Add about 10 ml of R-nitric acid; 20.00 ml of 0.1 **N** silver nitrate solution and 2 g of potassium nitrate, then boil the mixture for 3 minutes. (Performing the assay on micro scale, 20 ml of water, 10 ml of R-nitric acid, 10.00 ml of 0.01 **N** silver nitrate solution and 1 g of potassium nitrate should be used.) Cool the precipitous liquid below 15°, and titrate with 0.1 **N** (or 0.01 **N**) potassium thiocyanate solution, using 0.5 to 1 ml of **I**-iron(III) nitrate solution as indicator.

Each ml 0.1 **N** silver nitrate solution is equivalent to 3.546 mg (lg .54970) of Cl; 7.992 mg (lg .90263) of Br and 12.69 mg (lg .10353) of I, respectively.

Each ml of 0.01 **N** silver nitrate solution is equivalent to 0.3546 mg (lg .54970) of Cl, 0.7992 mg (lg .90263) of Br, and 1.269 mg (lg .10353) of I, respectively.

6.2 Assay of Organic Iodine with Preceding Alkali-fusion

Reagents

R-potassium hydroxide, halogen-free
R-ethanol
sodium hydroxide solution, 20 per cent

I-methyl red solution
sulphuric acid, 50 per cent
hydrochloric acid, 1 **N**
R-chlorine water
pumice
R-phosphoric acid
potassium iodide
sodium thiosulphate solution, 0.005 **N**
I-starch solution
R-sodium hydroxide solution
potassium cyanide, 5 per cent
I-methyl orange solution.

Weigh accurately a sample as directed in the monograph, in an about 50 ml nickel crucible (the quantity of the organic matter must not be more than 0.1 g). Cover the sample with 2 g of potassium hydroxide granules, and add 2 to 3 ml of R-ethanol. Evaporate the mixture to dryness over a small flame, and fuse the residue. For that purpose, held the crucible 5 cm above the flame, expell the moisture, and then place the crucible slowly in the flame. Fuse the content of the crucible by constant swinging; stop this operation when the evolution of bubbles ceases, and the melt becomes colourless. Cool the crucible, dissolve the melt in water, and filter the solution into a 200 ml flask through a cotton plug, treated previously with 10 to 20 per cent sodium hydroxide. (Place the cotton plug into a funnel of 5 cm diameter, wash it with a 10 to 20 per cent solution of sodium hydroxide and finally with water.)

Neutralize the clear and colourless solution with 50 per cent sulphuric acid in the presence of **I**-methyl red solution. Boil the solution to expel carbon dioxide, neutralize again with 1 **N** hydrochloric acid, and add another 1 ml of 1 **N** hydrochloric acid. To this solution add freshly prepared chlorine water (1–10 ml) until the liquid which eventually became yellow, due to liberation of iodine, turns colourless, and an odour of chlorine is strongly perceptible. Then add some pumice and boil vigorously to expel excess chlorine. Cool the liquid, add 5 ml of R-phosphoric acid and 0.2 g of potassium iodide. Titrate the liberated iodine with 0.005 **N** sodium thiosulphate solution using 1 ml of **I**-starch solution as indicator.

Each ml of 0.005 **N** sodium thiosulphate solution is equivalent to 0.1058 mg (lg .02435) of I.

Alternative procedure: after adding 1 ml of 1 **N** hydrochloric acid and chlorine water to the liquid in a 200 ml conical flask, allow it to stand for 10 minutes. Then add 1 ml of R-sodium hydroxide solution and a volume of freshly prepared 5 per cent potassium cyanide solution, half as much in millilitres as that of chlorine water added. Shake the flask a few times, loosen the glass stopper of the flask to allow the liquid to renew on the stopper. After 5 minutes, add 0.2 g of potassium iodide and 10 ml of R-phosphoric acid to the mixture. Titrate the liberated iodine with 0.005 **N** sodium thiosulphate solution using 1 ml of **I**-starch solution as indicator. In the case of samples (or reagents) containing also bromide, add 5 ml of R-sodium hydroxide to the solution, after adding the potassium cyanide solution. After 10 minutes add 20 ml of R-phosphoric acid and continue the test as described above.

Note. Test the liquid for chlorine after boiling, by adding one drop of a twenty-fold diluted **I**-methyl orange solution. The liquid must show a faint red colour. If the red colour disappears, dilute the liquid to 100 ml and boil again.

6.3 Assay of Organic Iodine Following Combustion in Oxygen Atmosphere

Use for this test a 500 ml glass-stoppered, thick-walled conical flask of a glass resistant to heat and chemicals, the neck of which is widened to a "collar" above the ground joint (so-called Schöniger flask). The top of the stopper is flat, and a platinum wire of about 10 cm length and of 1 mm diameter is sealed to its bottom, the lower end of the wire is provided with an U-shaped platinum net of 1.5 cm width and 2 cm length.

Cut out a piece of about 4×4 cm from a sheet of ash-free and halogenfree filter paper in a way that one corner should end in a strip of about 1.5×1.5 cm. Weigh a sample to 0.01 mg accuracy as directed in the monograph, on this filter paper, and wrap the sample into it. For this purpose, fold the upper third of the filter paper onto the next third and coat this by the lower third. Close the formed "capsule" at both ends, and place it into the previously ignited and cooled platinum net. The strip should point upwards, obliquely.

Transfer 10 ml of water and 2 ml of 1 **N** sodium hydroxide solution into the flask, and moisten its ground joint with water. With the aid of a glass tube placed into the flask, introduce oxigen gas for 2 to 3 minutes from an oxigen flask through a pressure regulator. Then, holding the neck of the flask in the left hand and the glass stopper in the right hand, ignite the strip, and seal the flask immediately with the stopper. Press the stopper with the left thumb until the ignition ceases. Then allow the flask to stand for 10 minutes, and shake it vigorously. Pour some water into the "collar" of the flask and pull out the stopper cautiously. Rinse the stopper, the platinum net and the walls of the flask with water. To the liquid, add 10 ml of concentrated acetic acid containing 1 g of powdered sodium acetate, and 3 drops of bromine. After three minutes add 3 to 4 drops of concentrated formic acid to reduce excess bromine, and after 15 minutes expel oxygen from the inside of the flask by allowing carbon dioxide to pass through. Add 0.2 g of potassium iodide and 10 ml of 25 per cent sulphuric acid to the liquid, and after 10 minutes titrate the liberated iodine with sodium thiosulphate, using **I**-starch solution as indicator.

7 Assay of Sulphate

Reagents

I-methyl red solution
R-ammonia solution
R-hydrochloric acid
Ammonium chloride
hydrochloric acid, 1 **N**
barium chloride solution, 5 per cent
ethyl alcohol, 96 per cent.

Weigh accurately a quantity of the preparation, as directed in the monograph, corresponding to 0.10 to 0.40 g of barium sulphate. Dissolve the sample in about 50 ml of water in a 200 ml beaker, and neutralize the solution against **I**-methyl red solution with R-hydrochloric acid. Dilute the solution to 100 ml, add 1 g of ammonium chloride and 1 ml of 1 **N** hydrochloric acid, and place a small nickel plate (about 5×5 mm) into it. Boil the liquid and add dropwise (1 to 2 drops/sec) 10 ml of a 5 per cent barium chloride solution from a precipitating burette (see Fig. 14).

Allow the precipitous liquid to stand for 12 hours and then collect the precipitate on a sintered glass filter crucible No. G4 (see Fig. 15) or on a micro filter crucible. Wash, dry and weigh the precipitate in the way described below for filter crucibles.

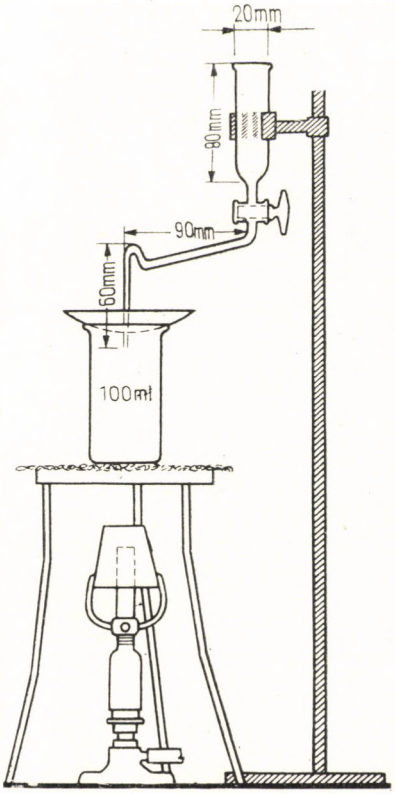

Fig. 14

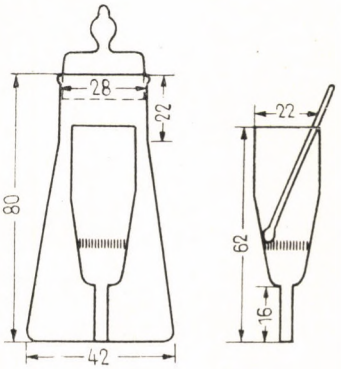

Fig. 15

Attach the filter crucible, by means of a small rubber hose, to the glass tube of a bell-shaped suction equipment. Place a beaker in the suction bell placed on a ground glass plate. The glass tube should touch the side of the beaker. Connect the suction bell to a water jet pump. Pour off first most of the supernatant liquid, then stir the precipitous liquid, and with the aid of a "policeman" (a white feather cut to adequate shape or a glass rod with a rubber hose on its tip) collect the precipitate on the glass filter crucible. Rinse the beaker a few times with small portions of the filtrate. Wash the precipitate with four successive portions of 5 ml hot water and then with four portions of 5 ml cold water. Prior to each washing, interrupt the suction, pour the washing liquid on the precipitate and stir vigorously with a glass rod (see Fig. 15). Wash the walls of the filter crucible with the aid of the glass rod. Finally, wash the precipitate with three successive portions of 4 ml of 96 per cent alcohol. Rinse both the walls of the filter and the glass rod with alcohol. Then cover the filter crucible with a glass cylinder provided with a side tubing. Place a small cotton plug into the latter, take out the beaker, and, for 20-25 minutes, allow a strong air stream to pass through the

precipitate. Clean the filter crucible, place it into a weighing bottle and, after 10 minutes, weigh it accurately.

The precipitate can also be dried at 120 to 130° for 2 hours.

If the barium sulphate precipitate was dried in an air stream or in a drying over at 130°, multiply its weight by 1.006 to obtain the accurate value.

Each mg of barium sulphate ($BaSO_4$) is equivalent to 0.1374 mg (lg .13789) of S.

Note. Since of the ions occurring frequently, nitrate and iron(III) ions interfere with the determination, remove nitrate by evaporating the solution thrice with hydrochloric acid, and reduce iron(III) to not-interfering iron(II) ions by adding 0.5 g of hydroxylammonium chloride to the solution, prior to precipitation.

8 Assay of Alkaloids

Reagents

R-hydrochloric acid
R-sulphuric acid
sulphuric acid, about 0.1 **N**
tragacanth
R-chloroform
R-ammonia solution
ammonia solution, 10 per cent
sodium sulphate, andhydrous
sulphuric acid, 0.02 **N**
sodium hydroxide solution, 0.02 **N**
I-methyl red solution
I-methyl red—methylene blue solution
R-iodine solution
R-Mayer's solution
R-ether
benzene
ammonia solution, concentrated
sulphuric acid, 2 per cent
R-sodium hydroxide solution
acetic acid, dehydrated
I-gentian violet solution
perchloric acid solution, 0.02 **N**

8.1 Assay of Alkaloid Content of Alkaloid Salts

8.11 Simplified Procedure for Alkaloid Extraction

Weigh accurately a quantity of the alkaloid salt, as directed in the monograph, in a 100 ml conical flask, and dissolve it in 2 ml of water, if necessary, by the addition of 1 to 2 drops of R-sulphuric acid. Place a small strip of litmus paper into the solution, and add about 70 ml of chloroform. Close the flask with a cork stopper, and shake it vigorously. Unless otherwise directed, add ammonia in marked excess. Shake again and add 5 g of finely powdered anhydrous sodium sulphate, stopper and shake again vigorously. If a turbid chloroform phase is obtained, add 2 to 3 g of anhydrous sodium sulphate and shake. Filter the liquid

into a dry conical flask through a defatted cotton plug (of about 0.1 g) moistened with chloroform and placed into a small filter funnel. Rinse the first flask and filter with 3 successive portions of 10 ml of chloroform.

8.12 Complete Procedure for the Extraction of Alkaloids

Weigh accurately a quantity of alkaloid salt, as directed in the monograph, in 5 ml of water, if necessary, add a few drops of R-hydrochloric acid. Transfer the solution into a separator funnel (see Fig. 16), and moisten the stopcock with water. Place a small strip of litmus paper into the solution, and, unless otherwise directed, add R-ammonia in excess. Extract the aqueous chloroform. Collect the chloroform portions in a 200 ml conical flask by filtering them through a layer of about 2 g of powdered anhydrous sodium sulphate spread over a defatted cotton plug (about 0.05 g), placed in a filter funnel.

Filter 2 to 3 ml of the last chloroform portion into a glass cup of 3 cm diameter. Add 2 to 3 ml of water acidified with 1 drop of R-hydrochloric acid and evaporate the mixture until the odour of chloroform is not perceptible. Transfer the aqueous residue into a small test tube, and unless otherwise directed, perform an alkaloid test by adding 2 to 3 drops of R-iodine solution or 2 to 3 drops of R-Mayer's solution to the liquid. If a positive reaction is obtained, repeat the extraction with further 10 ml portions of chloroform, until complete extraction is achieved.

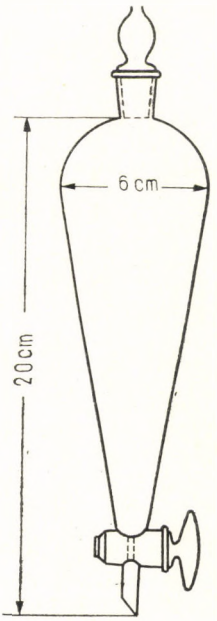

FIG. 16

8.13 Volumetric Assay of Alkaloids

To the combined chloroform portions (*8.11* and *8.12*, respectively) in a 200 ml conical flask add some (about 100 mg) pumice. Remove the bulk of chloroform by distillation. To the residue (5 to 10 ml chloroform solution) pipet 10.00 ml of 0.02 **N** sulphuric acid and expel chloroform completely by heating the liquid on a water-bath. Cool and titrate the excess acid with 0.02 **N** sodium hydroxide solution, using 1 to 2 drops of **I**-methyl red solution or **I**-methyl red—methylene blue solution as indicator.

8.14 Gravimetric Assay of Alkaloids

Weigh accurately the conical flask serving to collect the chloroform portions prior to extraction of the alkaloid together with some pumice. Remove the bulk of chloroform by distillation, and expel chloroform completely by heating the liquid on water-bath. Unless otherwise directed in the monograph, dry the residue at 110° for 2 hours, allow it to cool in a desiccator over calcium oxide, and weigh it accurately.

8.2 Assay of Alkaloid Content of Extracts and Tinctures

8.21 Preparation of Extracts for Test

Weigh accurately the quantity of extract directed in the monograph, in a 100 ml conical flask, add 2 ml of about 0.1 **N** sulphuric acid and shake, or heat on the water-bath, until the complete dissolution or complete dispersion of the sample. Lumps should not remain in the solution.

8.22 Preparation of Tinctures for Test

Weigh accurately the quantity of tincture directed in the monograph, in a 100 ml conical flask, add 1 to 2 drops of about 0.1 **N** sulphuric acid and 2 ml of water, and evaporate the liquid on the water-bath to 2 to 3 ml.

8.23 Assay of Alkaloid Content after the Simplified Procedure for Alkaloid Extraction

To the cooled liquid prepared according to *8.21* or *8.22*, add 70 ml of R-chloroform and 1 ml of a 10 per cent ammonia solution. Close the flask with a cork stopper and agitate it mechanically for 30 minutes. To the liquid add 1 g of powdered tragacanth and agitate vigorously for 1 minute. Then filter the solution into a 200 ml conical flask through 4 to 5 g of anhydrous sodium sulphate spread ower a defatted cotton plug (of about 0.1 g). Wash the flask, tragacanth and filter with three successive portions of 10 ml of R-chloroform and combine them with the filtered chloroform solution. Test the combined chloroform filtrates according to *8.13*.

8.24 Assay of Alkaloid Content in Preparations Containing Volatile Bases

To the cooled liquid, prepared according to *8.21* or *8.22*, add 50 ml of R-ether and 1 ml of a 10 per cent ammonia solution. Close the flask with a cork stopper and shake it mechanically for 15 minutes. To the liquid add 1 g of powdered tragacanth and shake vigorously for 1 minute. Then filter the solution into a 200 ml conical flask through a layer of 4 to 5 g of anhydrous sodium sulphate spread over a defatted cotton plug (of about 0.1 g). Wash the flask, tragacanth and filter with three successive 10 ml portions of R-ether, and combine the ether portions with the filtered chloroform solution. Reduce the volume of filtrate to 1 to 2 ml by distillation, and blow off the last portion of ether by an air stream. Dissolve the residue in 10 ml of R-chloroform, expel the bulk of chloroform on the water-bath and blow off the last portion by an air stream. Repeat this procedure with two 10 ml portions of chloroform.

Dissolve the residue by adding 10.00 ml of 0.02 **N** sulphuric acid and 2 ml of chloroform, and expel chloroform on the water-bath. Titrate the solution according to *8.13*.

In order to check that the titrated solution is free of volatile bases, add 1 drop of R-sulphuric acid, and evaporate to 2 to 3 ml on the water-bath. To the cooled solution add 50 ml of chloroform and 0.4 g of ammonia-free, finely powdered crystalline sodium carbonate, and shake. (The yellow colour of methyl red indicator or the green colour of the mixed indicator indicates that the solution is alkaline.) Then close the flask with a cork stopper and agitate mechanically for 15 minutes. To the liquid add 1 g of powdered tragacanth, and agitate vigorously for 1 minute. Then filter the liquid into a 200 ml conical flask through 4 to 5 g spread over a defatted cotton plug (of about 0.1 g). Wash the flask, tragacanth and filter with three successive portions of 10 ml of R-chloroform, and combine the chloroform portions with the filtered chloroform solution. Test the combined chloroform filtrates according to *8.13*.

If the deviation between the two titrations is not more than ± 2 per cent, use the result of the first titration to calculate the alkaloid content. If the deviation exceeds this limit, repeat the assay.

Preparation of the drug for the test. Powder the air-dry drug of known moisture content, and sift the total quantity through sieve No. IV. Powder any remaining coarse particles again and sift. Repeat this procedure until the total quantity is passed through the sieve. Scrupulously homogenize the sifted drug powder.

Assembling the apparatus. Pack the extraction socket (*1*) of the apparatus with cut-up glass wool (see Fig. 17). Lubricate stopcock (*2*) and the ground joint of circulation tube (*3*) with solvent-resisting grease, preferably with glycerol ointment and stopcock (*6*) with white soft paraffin.

Extraction of alkaloids. In a mortar triturate a certain quantity of drug, as directed in the monograph, with 0.5–1 ml of concentrated ammonia solution, and transfer the drug quantitatively on the glass wool in the extractor socket (*1*). Open the cock (*2*) connecting the solid-liquid (*A*), and liquid-liquid extractors (*B*), and pour into the liquid-liquid extractor (*B*) 10 ml of 2 per cent sulphuric acid through charging tube (*7*). Into the solid-liquid extractor (*A*) pour a sufficient amount of benzene so as the benzene level in (*B*) should reach the circulation tube (*3*), and after closing cock (*2*), a 3 to 4 cm benzene layer should cover the drug. Then start the circulation of benzene either by a continuous gentle air current through pressure pipe end (*4*) or by suction through air-discharge cock (*8*). To produce an overpressure for the circulation of the solvent a laboratory air pump or a water jet pump can be used. Buffer flasks should be connected to the apparatus when a pump is used. When the apparatus is operated by overpressure through pipe end (*4*), the air-discharge cock (*8*) should be opened. It is advisable to insert a gas trap to saturate air with solvent vapours.

Benzene in (*B*) is sprayed onto the drug through the circulation tube, penetrates through the drug, attains the sulphuric acid phase and accumulates on the surface of the latter. Then the whole process is repeated. This keeps the circulation of the benzene phase in motion. The alkaloid content of drugs is extracted by benzene, and on the contact of benzene with the sulphuric acid solution, alkaloids are resolved in the latter.

Repeat the extraction procedure described above once for 2 hours, and thrice for 1 hour each. After the end of each period stop the circulation by ceasing both the pressure and suction by closing stopcock (*2*). Collect the acid layer in a 50 ml volumetric flask. Then pour 10 ml of a 2 per cent sulphuric acid into the apparatus through charging tube (*7*). On completing the extraction procedure, the volume of the solution in the volumetric flask will be about 40 ml, and this should be completed to 50.00 ml with 2 per cent sulphuric acid.

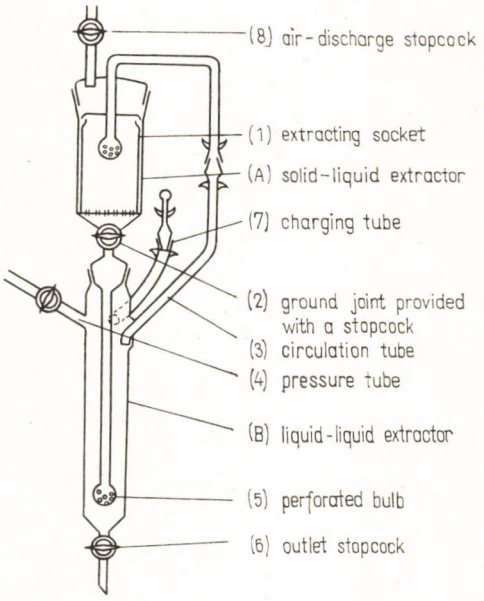

FIG. 17

Assay of alkaloid content. Transfer a 20.00 ml portion of the aqueous acidic solution into a separatory funnel, adjust the pH value 8 to 9, and extract alkaloids by shaking with two successive portions of 20 ml R-chloroform and then with two successive portions of 10 ml R-chloroform. Filter the chloroform extracts into a conical flask through anhydrous sodium sulphate. Distil the chloroform. Dissolve the cooled residue in 10 ml of R-chloroform, add 5 ml of dehydrated acetic acid, and titrate the liquid with 0.02 **N** perchloric acid, using 1 to 2 drops of **I***-gentian violet solution as indicator.

Multiply the result of the titration by 2.5 to obtain the alkaloid content of the drug, which should be expressed in per cent.

9 Assay of Morphine in Opium and Opium Preparations

9.1 Opium and Opium Preparations (Opium nativum, Pulvis opii, Extractum opii, Tinctura opii)

Reagents

R-Sodium hydroxide solution
R-Chloroform
Sodium sulphate, anhydrous
Litmus paper, blue and red
R-Hydrochloric acid
Isopropanol
Sodium hydrogen carbonate solution, 4 per cent
Hydrochloric acid, 0.1 **N**, or Sulphuric acid, 0.02 **N**
Sodium hydroxide solution, 0.1 **N** or 0.02 **N**
I-Methyl red solution
I-Methyl red—methylene blue solution
Sodium plumbite solution
(dissolve 1.64 g of lead acetate in 100 ml of R-sodium hydroxide solution)
Sulphuric acid, 50 per cent
R-Hydrochloric acid
Hydrochloric acid, 1 **N**
R-Iodine solution.

The following quantities should be measured from the preparations:

9.11 Opium Nativum, Pulvis Opii

Weigh accurately about 0.5 g from the finely powdered preparation into a glass vessel of 4 cm diameter.

9.12 Extractum Opii

Weigh accurately about 0.2 g of the finely powdered preparation into a glass vessel of 4 cm diameter.

9.13 Tinctura Opii

Weigh, with mg accuracy, about 5.00 g in a small glass vessel covered with a watch glass. Evaporate the opium tincture to the consistency of honey and allow it to cool.

Triturate the weighed and prepared sample with 2.0 ml of R-sodium hydroxide solution to a homogeneous pulp. (Opium powder should be triturated until it does not crackle when rubbed.) Transfer the pulp into a 50 ml volumetric flask with five successive portions of 1 ml of sodium plumbite solution and then with water and make up the volume with water. Shake the liquid and immediately filter it through a dry, folded filter paper. Pipet 40.00 ml of the filtrate into another 50 ml volumetric flask, add 1.2 ml of 50 per cent sulphuric acid, and complete the volume with water to 50 ml. Filter the precipitous liquid through a dry, folded filter paper of 6 cm diameter to obtain a clear solution. Pipet 40.00 ml of the filtrate into a 50 ml beaker, and neutralize the solution with R-sodium hydroxide solution in the presence of a small strip of litmus paper, then add 1 drop of R-hydrochloric acid and evaporate the liquid to about 5 ml. If a precipitate separates during the procedure, filter the liquid into a 100 ml separatory funnel through a cotton plug, rinse the beaker and the filter with three successive portions of 1 ml water containing 1 drop of R-hydrochloric acid. To this solution add 3 ml of R-sodium hydroxide solution and perform extraction with three successive portions of 20 ml chloroform. Collect the chloroform portions in another separatory funnel, and shake the liquid with 5 ml of dilute sodium hydroxide solution (3 drops of R-sodium hydroxide solution in 5 ml of water). Neutralize the combined aqueous phases with 50 per cent sulphuric acid, and add a few drops of R-sodium hydroxide solution. Accurately neutralize the slightly alkaline solution with 1 drop of **N** hydrochloric acid in the presence of a small strip of litmus paper. The volume of the liquid in the separatory funnel should not exceed 20 to 22 ml. (In case of a larger volume, reduce it by evaporation.) To the liquid in the separatory funnel add 25 ml of a mixture of R-chloroform and isopropanol (3 vol. chloroform + 1 vol. izopropanol) — then 5 ml of a 4 per cent solution of sodium hydrogen carbonate, and shake the liquid immediately: filter the chloroform layer into a 200 ml conical flask through anhydrous sodium sulphate spread over a small cotton plug. Repeat the extraction with three successive 25 ml portions of the mixture of R-chloroform and isopropanol and combine the extracts in the conical flask. (At the last extraction evaporate 3 ml of the chloroform phase in a small glass cup, dissolve the residue in 2 ml of water and 1 drop of R-hydrochloric acid, and add 0.5 ml of R-iodine solution; the liquid must not change.) Place some pumice into the flask and reduce the volume of the liquid to about 10 ml by distillation. Evaporate the residue to dryness on a waterbath. To the obtained morphine base add 10.00 ml of 0.02 **N** sulphuric acid, if the morphine content of the sample is less than 15 per cent, or 20.00 ml of 0.02 **N** sulphuric acid at higher morphine contents. Heat the liquid to avoid dissolution or add 1 to 2 ml of chloroform, and expel it by heating. Cool the liquid, and titrate excess sulphuric acid with 0.02 **N** sodium hydroxide solution, using 1 to 2 drops of **I**-methyl red or 1 to 2 drops of **I**-methyl red—methylene blue solution as indicator. (Using the latter one, the transition colour is dirty green.)

Each ml of 0.02 **N** sulphuric acid is equivalent to 5.707 mg (lg .75638) of anhydrous morphine base ($C_{17}H_{19}O_3N$).

Note. The consumed millilitres of 0.02 **N** sulphuric acid are equivalent to 64 per cent of the total morphine content of the sample. According to this, use for calculation the following formula:

$$\text{anhydrous morphine, per cent} = \frac{a \times 5.707 \times 0.1}{b \times 0.64}$$

where a = consumed millilitres of 0.02 **N** sulphuric acid
b = the weight of sample in grams.

At this procedure of calculation of morphine content, the volume of the alkali-insoluble portion of the sample and the volume of the precipitated lead sulphate is neglected intentionally.

9.2 Pulvis Opii et Ipecacuanhae (Dover's Powder)

Reagents

Propanol, in addition to the reagents listed in *9.1.*

Procedure

Weigh accurately about 1 g into a small glass cup, triturate it with a mixture of 5 ml of water and 5 drops of R-hydrochloric acid, and evaporate to dryness on the water-bath. To the sample prepared in this way add 2.0 ml of R-sodium hydroxide, triturate, and proceed further according to *9.1.*

An alternative method is as follows: weigh accurately about 1 g, and perform extraction by triturating it with four successive portions of 5 ml of hot propanol containing 1 per cent of hydrochloric acid. Filter the extracts through a small filter paper, combine the extracts, and evaporate the liquid to dryness. Add 2.0 ml of R-sodium hydroxide solution, triturate and continue the assay according to *9.1.*

10 Non-Aqueous Perchloric Acid Titrations

Reagents

Dehydrated acetic acid
(it must not contain more than 0.5 per cent of water).

Determine the water content of concentrated acetic acid according to *5.1*, Vol. I, p. 104. If the water content exceeds 0.5 per cent, add calculated amount of acetic anhydride to the concentrated acetic acid, and heat the mixture in a drying oven at about 60° for 6 hours. An alternative method is to mix concentrated acetic acid with acetic anhydride and allow the mixture to stand at room temperature (about for 1 week) until the water content decreases to 0.5 per cent.

The quantity of acetic anhydride needed to dehydrate the concentrated acetic acid can be calculated by the formula:

$$x = a \times 5.67$$

where a = the water content of concentrated acetic acid in per cent
x = grams of acetic anhydride needed to dehydrate 100 g of concentrated acetic acid.

Prior to use, titrate dehydrated acetic acid with 0.1 **N** or 0.02 **N** perchloric acid to transition colour in the presence of the applied indicator.

Test

Basic impurities, water. To 10 ml of dehydrated acetic acid add 2 drops of **I***-gentian violet solution and 0.05 ml of 0.1 **N** perchloric acid. The liquid must acquire a green or bluish green colour.

Acetic anhydride. (This test should be performed when dehydrated acetic acid is used for the determination of amines readily acetylable on the nitrogen atom.) To 5 ml dehydrated acetic acid add 2 ml of a 2 per cent solution of benzinide in acetic acid. No crystalline precipitate must separate from the solution within 2 hours. (Sulphate and perchlorate ions interfere.)

R-Acetic Anhydride

Test

Basic impurities. To 20 ml of acetic anhydride add 3 drops of **I***-gentian violet solution and 0.02 ml of 0.1 **N** perchloric acid; the liquid must acquire a yellow or green colour.

Perchlorid acid, 0.1 **N**

Each ml of the solution contains 10.0470 mg of $HClO_4$.

To about 1 g of perchloric acid solution, accurately weighed, add 50 ml of freshly boiled and cooled water. Titrate the liquid with 0.1 **N** sodium hydroxide solution, using a few drops of **I**-phenolphthalein solution.

Each ml of 0.1 **N** sodium hydroxide solution is equivalent to 10.047 mg (lg .00204) of perchloric acid ($HClO_4$).

In a 1000 ml volumetric flask mix a certain volume of perchloric acid solution, corresponding to 10.05 g of $HClO_4$, with about 600 ml of dehydrated acetic acid.

To this solution add a quantity of acetic anhydride calculated by the formula:

$$x = 5.67(a - 10.05)$$

where a = grams of perchloric acid solution corresponding to 10.05 g of $HClO_4$
x = grams of acetic anhydride needed for the preparation of 1000 ml of standard solution.

Make up the volume of the cooled solution to 1000 ml with dehydrated acetic acid.

Store the standard solution in a buret with container bulb protected from air humidity (traps packed with silica gel should be attached to the buret).

Standardization of 0.1 **N** *perchloric acid*

Weigh accurately about 0.2 g of potassium hydrogen phthalate, dried at 120° for 2 hours, and dissolve in 15 ml of dehydrated acetic acid by gentle warming. Cool the liquid and titrate it with 0.1 **N** perchloric acid, using 2 to 3 drops of **I***-gentian violet solution as indicator, to a bluish green colour.

The titre of the standard solution can be calculated by the formula:

$$T_b = \frac{g \times 1000}{b \times 20.423}$$

where g = weight of potassium hydrogen phthalate in grams
b = millilitres of 0.1 **N** perchloric acid consumed
(log 20.423 = 1.31012).

Perchlorid acid, 0.02 **N**.

Each ml of the solution must contain 2.0094 mg of $HClO_4$.

Dilute 200 ml of 0.1 **N** perchloric acid with dehydrated acetic acid to 1000 ml

Standardization of 0.02 **N** *perchloric acid*

Weigh accurately about 0.4 g of potassium hydrogen phthalate, dried at 120° for 2 hours, in a 100 ml volumetric flask and make up the volume with dehydrated acetic acid. Titrate a 10.00 ml portion of the solution with 0.02 **N** perchloric acid, using 2 to 3 drops of **I***-gentian violet solution as indicator, to a greenish blue colour.

The titre of the standard solution can be calculated by the formula:

$$T_b = \frac{g \times 100}{b \times 4.0846},$$

where g = grams of potassium hydrogen phthalate dissolved in 100 ml of dehydrated acetic acid
b = millilitres of 0.02 **N** perchloric acid consumed
(lg 4.0846 = .61115).

Dehydrated acetic acid solution of mercury(II) acetate

Dissolve 3 g of mercury(II) acetate in dehydrated acetic acid to 100 ml, under frequent shaking. Prior to use, titrate the solution with 0.1 **N** (or with 0.02 **N**) perchloric acid in the presence of the applied indicator to bluish green or blue colour.

Assay

The assay of preparations is specified in the respective monographs. If the temperature at the titration deviates by more than $\pm 1°$ from that of the standardization, the titer of standard solution should be multiplied by T_1:

$$T_1 = 1 + 0.0011(t_0 - t_1)$$

where t_0 = temperature at standardization,
t_1 = temperature at titration.

11 Bromatometric Determinations by Measuring the Excess of Bromine by Iodometry

Perform the assay in a bromination flask. This is a 500 ml (or 250 ml) conical flask (see Fig. 18), with a widened neck above the ground joint. A 30 ml cylindrical funnel provided by a glass stopper, and a ground stopcock can be joined to the flask.

Weigh accurately a quantity of preparation as specified in the monograph, and dissolve it in water or in another solvent. To the neutral or slightly alkaline solution add the prescribed quantity of 0.1 **N** potassium bromate solution, and 0.5 to 1.0 g of powdered potassium bromide. Close the bromination flask with the cylindrical funnel. (Moisten the ground joint with water.) Open the cock of the funnel and reduce the pressure in the flask by suction using a water jet pump. Pour 10 ml of R-hydrochloric acid into the funnel and suck up the liquid into the flask. (The pressure in the flask should remain lower than the atmospheric pressure.) Shake the flask, and allow it to stand in a dark place. If two-phase bromination is performed, shake the mixture frequently. After the

bromination period, pour 10 ml of a freshly prepared 10 per cent potassium iodide solution into the funnel, and suck up the liquid into the flask. Shake and dilute with water to 100 ml, and after 5 minutes, titrate the liberated iodine with 0.1 **N** sodium thiosulphate solution, using **I**-starch solution as indicator.

If the pressure has been eventually compensated in the flask, the potassium iodide solution can be introduced into the flask by filling it into the cup-like collar of the flask andloosening the stopper (with tunnel) of the flask.

The assay can be performed also in a glass-stoppered conical flask. In this case the mixture should be acidified rapidly and the flask closed immediately with the stopper.

Add solid potassium iodide also with a rapid movement, to avoid loss in bromine.

The factors of sodium thiosulphate solutions should be checked by blank tests when organic solvents are applied.

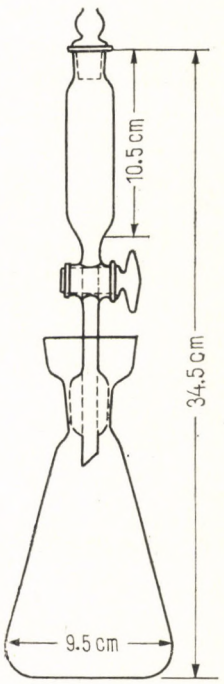

FIG. 18

(c) CHEMICAL INDEXES

1 Determination of Acid Value

Acid value (A) is the quantity of base expressed in mg of KOH needed for the neutralization of free acids in 1 g sample

Unless otherwise directed, weigh about 3.0 g of the sample with mg accuracy into a 100 ml glass-stoppered conical flask. Filter the preparation if necessary. To the sample, add 20 ml of alcohol, 96 per cent, neutralized against phenolphthalein, and 10 to 20 mg of phenolphthalein. Add dropwise 0.1 **N** sodium hydroxide to the liquid until a permanent (for 30 seconds) pink colour is obtained.

If solid samples (lard, wool fat, wax, etc.) are tested, melt the sample by warming it on the water-bath and titrate the still warm liquid.

Each ml of 0.1 **N** sodium hydroxide solution is equivalent to 5.610 mg (lg .74899) of KOH;

Calculate the acid value (A) by the formula:

$$A = \frac{v \times T \times 5.61}{m}$$

where v = millilitres of 0.1 **N** sodium hydroxide solution consumed
T = titre of 0.1 **N** sodium hydroxide solution
m = weight of sample in grams
A = acid value.

2 Determination of Saponification Value

Saponification value (S) is the quantity of base expressed in mg of KOH needed for the complete saponification of 1 g of sample

Unless otherwise directed, weigh about 1.5 g of the sample with mg accuracy into a 100 ml flask. Filter the preparation, if necessary. To the sample add 10 ml of propanol and 20.00 ml of 0.5 **N** propanolic potassium hydroxide solution. Cover the flask with a small beaker. Heat the flask on the water-bath for 1 hour. Titrate the hot liquid with 0.5 **N** hydrochloric acid, using 20 to 30 mg of phenolphthalein as indicator. If the liquid becomes jelly-like during titration, melt the reaction mixture by gentle warming.

If the colour change in the end point is not sharp enough, add 30 ml of hot, concentrated sodium chloride solution to the dark coloured reaction mixture, and continue titration until the pink colour of the separated sodium chloride phase perfectly disappears.

In order to eliminate the necessity of the standardization of the 0.5 **N** propanolic potassium hydroxide solution, perform a blank test. The difference in millilitres of 0.5 **N** hydrochloric acid consumed in the two titrations is just the quantity of 0.5 **N** propanolic potassium hydroxide solution, of the same titre as that of 0.5 **N** hydrochloric acid, needed for the saponification of the sample.

Each ml 0.5 **N** propanolic potassium hydroxide solution is equivalent to 28.05 mg (lg .44796) of KOH.

Calculate the saponification value by the formula:

$$S = \frac{(a—b) \times T \times 28.05}{m}$$

where a = millilitres of 0.5 **N** hydrochloric acid consumed in the blank test
b = millilitres of 0.5 **N** hydrochloric acid consumed in the assay
T = titre of 0.5 **N** hydrochloric acid
S = saponification value
m = weight of sample in grams.

3 Determination of Ester Value

Ester value (E) is the quantity of base expressed in mg of KOH needed for the saponification of the substance in ester bonds in 1 g of sample

Ester value is the difference between the saponification value (S) and the acid value (A). If the sample is neutralized previously, the assay according to 2 yields directly the ester value (E).

4 Determination of Hydroxyl Value

Hydroxyl value (H) is the quantity of base expressed in mg of KOH, equivalent to the amount of acetic acid consumed for the acylation of 1 g sample

Both the quantity of the sample to be weighed and the acylation mixture (Vol. I, p. 266) depend on the hydroxyl value expected (see Table 14).

Transfer the preparation weighed to mg accuracy into a 100 ml acylation flask (Fig. 24; Vol. I, p. 191), add the prescribed quantity of acylation mixture, attach a reflux condenser to the flask and heat on the water-bath for 60 minutes.

TABLE 14

Hydroxyl value (H)	Weight of sample, g	Acylation mixture, ml
10–100	2.100–1.900	5.00
101–150	1.600–1.400	5.00
151–200	1.050–0.950	5.00
201–250	0.790–0.720	5.00
251–300	0.630–0.570	5.00
	or	or
	1.260–1.140	10.00
301–350	1.050–0.950	10.00
351–700	0.790–0.720	15.00
701–950	0.530–0.480	15.00
951–1500	0.320–0.290	15.00
1501–2000	0.210–0.190	15.00

The acylation flask should be immersed into the water-bath in a way that the reaction mixture should be 2 cm below the level of the water-bath. Cool the mixture to 20°, pour 1.00 ml of water into the condenser and rinse it with 5.0 ml of R-pyridine into the acylation flask. Stir the mixture vigorously and heat it for 10 minutes on the water-bath, then cool to 20°. Rinse the liquid between the ground joint and the condenser tube into the acylation flask with 5.0 ml of alcohol 96 per cent, previously neutralized and mixed with 1.0 ml of **I**-phenolphthalein solution. Titrate the liquid with 0.5 **N** propanolic potassium hydroxide solution to a pink colour. For this titration use a 50 ml burette. Perform a blank test under identical conditions and determine the acid value of the preparation according to **1**, Vol. I, p. 127. Calculate the hydroxyl value by the formula:

$$H = \frac{28.05\ (b—a)\ T}{m} + A,$$

where a = millilitres of 0.5 **N** propanolic potassium hydroxide solution consumed in the assay
b = millilitres of 0.5 **N** propanolic potassium hydroxide solution consumed in the blank test
T = the titre of 0.5 **N** propanolic potassium hydroxide solution
m = weight of the sample in grams
A = acid value
H = hydroxyl value

5 Determination of Iodine Value

The iodine value (X) represents the number of grams of halogen expressed in iodine consumed under prescribed conditions by 100 g of the tested substance (fat, wax, oil, etc.).

Reagents

Methanolic bromine solution (about 0.2 **N**). Shake about 150 g of powdered sodium bromide, dried previously at 140°, with 1 liter of dehydrated methanol to saturation. Decant (filter) the solution, and add 5.2 ml of bromine per litre. The solution is suitable for use for a few months it is preserved in a glass-stoppered brown bottle.

Sodium thiosulphate solution, 0.1 N

chloroform

potassium iodide solution, 10 per cent (freshly prepared)

I-Starch solution.

Assay

Transfer the quantity of sample prescribed in Table 15, accurately weighed, into a 250 ml bromination flask (Fig. 18, Vol. I, p. 127) or into a glass-stoppered conical flask. It is advisable to weigh the sample into a small glass thimble, and then throw it into the flask. An alternative method is to weigh the substance in a small glass vessel (10 ml in volume) together with a small glass rod, to transfer the sample into the flask by means of the glass rod and to reweigh the bottle.

Pour 10 ml of chloroform into the bromination flask or into the glass-stoppered conical flask and after the complete dissolution of the sample (if necessary, under gentle heating) add 25.00 ml of methanolic bromine solution. (A part of sodium bromide precipitates as white crystals.) Shake the flask and place it immediately in a dark place.

TABLE 15

Sample		Weight of sample g
Name	Iodine value	
Acidum oleinicum	80–90	0.18–0.20
Adeps lanae	18–36	0.40–0.50
Adeps solidus	0–7	1.0
Alcohol cetylstearylicus	0–4	2.0
Butyrum cacao	3–38	0.40–0.50
Cetaceum	0–4	2.0
Glycerinum monostearinicum	0–3	2.0
Lanalcolum	43–45	0.60
Oleum helianthi	122–135	0.10–0.12
Oleum jecoris aselli	140–170	0.10–0.11
Oleum lini	160–190	0.09–0.10
Oleum ricini	82–86	0.18–0.20
Oleylum oleinicum	75–90	0.18–0.20
Stearinum	0–5	2.0

Agitate repeatedly for a few times in the first five minutes. (Care must be taken to avoid daylight during bromination.) After 2 hours add 20 ml of a freshly prepared 10 per cent potassium iodide solution and 100 ml of water to the flask through the cock of the funnel of the bromination flask and shake. Rinse the stopper of the funnel and titrate the liberated iodine with 0.1 **N** sodium thiosulphate solution. Near the end point add 5 ml of **I**-starch solution to the liquid.

If no bromination flask is used, after the addition of methanolic bromine solution, close the flask immediately with a wetted glass stopper. After the elapse of the bromination period add 1 g of solid potassium iodide to the liquid by a rapid movement, close the flask, shake and dilute the liquid with 100 ml of water. Then continue the test as directed above. Standardize the methanolic bromine solution against 0.1 **N** sodium thiosulphate solution.

Each ml of 0.1 **N** sodium thiosulphate solution is equivalent to 12.69 mg (lg .10353) of iodine (*I*). To perform calculations, substract the volume of 0.1 **N** sodium thiosulphate solution consumed from the volume of 0.1 **N** sodium thiosulphate solution equivalent to 25.00 ml of methanolic bromine solution, and multiply the difference by the titre of 0.1 **N** sodium thiosulphate. Each ml of the difference is equivalent to 12.69 mg (lg .10503) of iodine (*I*). Calculate the iodine value (*X*) by the formula:

$$X = \frac{(a-b) \times T \times 12.69 \times 0.1}{m}$$

where a = millilitres of 0.1 **N** sodium thiosulphate solution equivalent to 25.00 ml of methanolic bromine solution
b = millilitres of 0.1 **N** sodium thiosulphate solution consumed
T = titre of 0.1 **N** sodium thiosulphate solution
m = weight of the sample in grams
X = iodine value.

6 Determination of Unsaponifiable Matter

The unsaponifiable matter (CN) is the portion of the sample which is unsaponifiable with alkali and extractable by ether determined according to the following procedure, and expressed as per cent.

Transfer 5 to 10 g of the substance, weighed to 10 mg accuracy, into a 200 ml ground joint flask, and add 50 ml of R-alcohol 96 per cent, and 4 to 5 g of potassium hydroxide. Attach a reflux condenser to the flask and boil the liquid for 1 hour. Pour the warm liquid into a separatory funnel and rinse the flask with water so as to obtain about 100 ml liquid. Shake the mixture cooled to room temperature, with 57 ml of ether for 1 minute. Transfer the heavier layer (the soap solution) into another separatory funnel and shake it again with 50 ml of ether. Repeat the procedure. Combine the ethereal extracts in the first separatory funnel. Rinse the walls of the separatory funnel without shaking the liquid. Repeat the procedure until the wash water remains colourless on the addition of phenolphthalein solution. Then shake the ether extracts vigorously with 10 ml of water, to remove the last traces of soap. Filter the ethereal solution through anhydrous sodium sulphate spread on a cotton plug into a tared flask. Expel ether by distillation and dry the residue at 105° to constant weight.

Express the amount of unsaponifiable matter (N) in percentage of the sample:

$$N = \frac{a \times 100}{m}$$

where a = weight of the residue in grams
m = weight of the sample in grams
N = unsaponifiable matter.

(D) INFORMATIVE TEST

If the complete pharmacopoeial test of the drug has been previously performed by a central control laboratory, it is sufficient to check the identity and quality of the preparations in the pharmacies by means of the official informative tests. All those drugs, however, which are used for the preparation of injections or which might contain some contaminants hazardous to the health, should be subjected to a complete pharmacopoeial test. Drugs should be tested first through inspection (colour, odour, consistency and other properties), and the informative tests should be performed thereafter.

Informative tests are prescribed by the Pharmacopoeia only for drugs which are used in the compounding of medicines in the pharmacies.

If it becomes necessary in a pharmacy to perform informative tests for a drug, the monograph of which gives no specifications for informative tests, the identification and qualitative tests should be applied respectively.

If any uncertainty arises concerning the identity or quality of the drug after performing the identification tests, the preparation should be subjected to a complete pharmacopoeial test.

IV BIOLOGICAL AND MICROBIOLOGICAL TESTS AND ASSAYS

(A) GENERAL PRINCIPLES

In this Pharmacopoeia biological tests and assays are specified to determine the measure of the activity and the active ingredient content of drugs, respectively, when chemical or physico-chemical control methods are not available or do not afford due security with respect to the specific biological effect of the drug. Biological tests and assays are performed on intact animals, when possible on isolated animal organs, or by microbiological methods.

All biological assay methods are based on the dependency of a biological response on the magnitude of the dose. However, the magnitude of the response is dependent not only on the dose, but also on the sensitivity of the biological substrate (animal, animal organ, microorganism). Hence all biological assay methods involve comparison of the responses of the substrate on the effects of the sample and those of the standard preparation.

Some fixed amount of the standard preparation is accepted as a measure of the effect and is termed as the "Unit".

The principle of the comparative biological assay methods is as follows: That amount of the sample which causes similar responses of the substrate as a determined amount of the standard preparation is established in simultaneously performed parallel runs. These doses are regarded as biologically equivalent and the activity of the sample is expressed as a percentage of the standard preparation; i.e. the relative activity value of the sample with respect to the standard preparation is established.

If the assay is performed in this manner, then the response of the substrate will depend both in the case of the sample and of the standard preparation equally on the sensitivity of the biological substrate, hence the result of the comparative assay will be real. However, only identical responses attained with both the standard and with the sample may be accepted as indication of biologically equivalent quantities of the standard and of the sample. The errors involved in biological assay methods are substantially higher than those involved in chemical methods, and the biological methods are subjects to a wider range of variation. Therefore in most cases the statistical evaluation of the results are prescribed by the Pharmacopoeia. Tolerance limits are set by which the responses produced by the sample may deviate from those produced by the standard

TABLE 16

International Standards and International Units

Standard	Valid since	One I.U. per mg
Antibiotics		
Neomycin (Neomycin sulphate)	1958	0.00147
Nystatin	1963	0.000333
Oxytetracycline (Oxytetracycline dihydrate)	1955	0.00111
Penicillin (Benzylpenicillin sodium)	1952	0.0005988
Streptomycin (Streptomycin sulphate)	1958	0.001282
Tetracycline (Tetracycline hydrochloride)	1957	0.00101
Hormones, Vitamins, Enzymes		
Vitamin-D_3 (an oily solution of cholecalciferol)	1949	0.000025
Heparin (Heparin sodium)	1958	0.0077
Insulin	1958	0.04167
Chorionic gonadotrophin	1939	0.1
Corticotrophin (formerly Adreno-Corticotrop Hormone, ACTH)	1962	1.0
Oxitocic, Vasopressant and Antidiuretic Substances (formerly Posterior Lobe of Hypophysis) Aceton dehydrated and powdered cattle hypophysis	1957	0.5
Other Substances		
Digitalis (powdered Digitalis purpurea leaves)	1949	76.0

preparation; also the required accuracy of the measurements is indicated by limiting the fiducial limits of the assay (cf. Vol. I, p. 135). The standard preparations for biological tests and assays are specified mostly by international agreements.

Individually established quantities of International Standards are termed as International Units (I.U.).

National Standards are established by the National Institute of Public Health. Manufacturers' control laboratories are compelled to prepare working standards ("home standards") which are checked periodically by comparing to the National Standard Preparation.

In addition to quantitative tests and assays, qualitative tests are also specified by the Pharmacopoeia, mainly for parenteral preparations which must be sterile and free of pyrogens. Similarly inocuity tests are specified for the determination of the maximum dose of an antibiotic which is not yet lethal to mice. Neither must contain the parenteral preparations blood pressure lowering contaminants causing higher decrease of the blood tension than is caused by 0.1 μg of histamine administered intravenously to cats.

Animals used in biological tests and assays may show rather variable responses on drug doses. This variance should be maintained as low as possible. Homogenous strains of animals raised under uniform circumstances and held in individual cages, especially animals of the same litter, respond usually rather more evenly on the same dose of a drug, than do animals of unhomogenous origin, which may come from several sources, or those kept under improper conditions — as e.g. on not identical diet, at changing temperatures, in noisy surroundings, etc.

(B) STATISTICAL EVALUATION OF BIOLOGICAL TESTS AND ASSAYS*

(a) GENERAL

Compared to the errors of chemical tests, the errors of biological tests and assays on the one hand highly exceed the former. On the other hand, they are subject to great variation, even for the identical kind of assay thought to be executed under similar conditions. Consequently, it does not suffice to know "in general" the error committed: the error has to be computed for each assay performed. Neglecting this precaution, the risks of faulty decisions concerning the acceptance of the preparation tested would be highly increased.

For this end, the methods of estimations are discussed, with special respect to the conditions rendering these — in the bulk simplified — methods reliable and thus, applicable. For defining these conditions the pharmacological claims and possibilities are to be met. Declining their application the pharmacological consequencies were to be taken into consideration. If the circumstances do not allow the application of these simplified methods, the exact procedures are to be followed. (More exact methods of statistical analyses are presented in statistical, pharmacological and laboratory manuals and text books.)

* In this chapter, the original Hungarian text has been slightly altered by changes in some signs, symbols and forms of ratios, etc. as those used in Hungarian are not always quite identical to those in English, for the convenience of the reader being accustomed to the latter ones, they have been applied. In fact, it resulted in just transplanting, besides the words and sentences — the signs, etc. too.

No infallible verdict concerning acceptance or rejection could be even thus achieved. The decisions on acceptance or rejection must be based on probability. For example, in an assay 9 I.U. potency is estimated for a preparation for which 10 I.U. are required by the Pharmacopoeia with a tolerated lower limit of 8 I.U. A statement is aimed at on the probability of preparation having potency less than 8 I.U. exhibiting (owing to experimental errors) 9 units or more. If this probability is low, the preparation should be accepted.

Fiducial limits. The prescriptions of the Pharmacopoeia for the biological assays are mainly twofold. Viz. 1) they prescribe the tolerated deviations of the "estimated" potency from the "standard" one; 2) the precision of the test itself is prescribed, too. The estimated relative potency is prescribed as percentage of the Standard preparation (e.g. for Digitalis 90–111 per cent) and the precision, by fixing the fiducial limits. The Pharmacopoeias prescribe the 0.95 fiducial limits. Fiducial limits are estimated to state those "true" values to which the estimated value is not contradictory. That highest "true" value is computed which — owing to experimental errors — could generate such a low value as the "estimated" one (or even a lower one); this one serves for the upper limit (F_U). The lower limit (F_L) is found in a similar way. According to the accepted symbolics in statistics — the "0.95" means: the highest "true" value which — owing to experimental errors — may yield with 0.025 probability such low (or even lower) values as the "estimated" one. Similar for the lower limit. $1.00 - 2(0.025) = 0.95$. As most Pharmacopoeias do so, this Pharmacopoeia prescribes the fiducial limits as percentages of the "estimated" potency, too. For example, for Digitalis 80 to 125 per cent.

The estimated potency is dependent on the average value of the replicates, the fiducial limits on their dispersion and of their number. For measurement of the average, the arithmetic mean is used,* and of the dispersion, the standard deviation.

The arithmetic mean is estimated by summing all the individual entries dividing this sum by the number of all the entries:

$$\bar{x} = \frac{\Sigma' x}{n} \tag{1}$$

$\bar{x}$ = arithmetic mean
x = values of the individual entries
n = number of entries
Σ = "summation",** i.e. all the x-values added are up. (Cf. Examples 1 to 5.)

* In the Pharmacopoeias practically always the log doses are prescribed. Their arithmetic mean corresponds to the geometric mean of the doses.

** For the exact symbolics at summation, indices are requested: the limits of summation have to be denoted. E.g. $\sum_{i=1}^{n}{}' x_i$. However, for the statistical analyses in this Pharmacopoeia, it is evident that all entries are to be summed. In accordance with the statistical conventions, to avoid sophisticated looking formulae, the limits and the indeces are omitted. Exceptions are Formulae (3) and (4) where the limits had to be stated. There the summation is to be executed first within groups then to sum up all these sums for all the groups.

Standard deviation is estimated as follows. The difference of each individual value is computed from their mean. These differences are squared and the squares summed. The sum is divided by one less than the number of entries and square-root of this quotient is taken.

$$s = \sqrt{\frac{\Sigma'(x-\bar{x})^2}{n-1}} \tag{2}$$

s = standard deviation.

$(x-\bar{x})^2$ is called *squared deviation* and $\Sigma(x-\bar{x})^2$ *sum of squares*. The expression under the square-root is the *variance*.

Generally the mean has more digits than the entries do, rendering the computation of the squared deviations tedious. For this reason it is more feasible to compute the sum of squares as follows: sum up the squares $-\Sigma x^2$ — subtract from it the quotient of the square of the sum of the entries — $(\Sigma x)^2$ — and the number of entries.

$$s = \sqrt{\frac{\Sigma' x^2 - \frac{(\Sigma' x)^2}{n}}{n-1}} \tag{2/a}$$

Note. Σx^2 stands for the sum of the squares of the individual entries and $(\Sigma x)^2$ for the square of the sum of the individual entries.

If there is no access to computer apt for squaring, tables of squares may be consulted. The use of a slide-rule should be avoided and for formulae like *2a* it is strictly forbidden.

Provided the variance being the same for all the test preparations and for the standard and also for all their doses applied in the assay, the "pooled" standard deviation is estimated. Pooling all the square deviations (each computed from their own group-means) their sum is divided by the overall number of entries minus the number of groups (e.g. in case of 2×2 doses "$N-4$").

$$s = \sqrt{\frac{\sum_1^l{}' \sum_1^n{}' (x-\bar{x})^2}{N-l}} \tag{3}$$

where N = number of entries of the whole assay

l = number of groups

$\sum_1^l{}'$ = summation is for all the (l) groups

$\sum_1^n{}'$ = summation is for all the (n) entries of each group.

In case of having the same number of entries for all the groups, it is more convenient to apply

$$s = \sqrt{\frac{\sum_1^l{}' \sum_1^n{}' x^2 - \frac{\sum_1^l{}' \left(\sum_1^n{}' x\right)^2}{n}}{n-1}} \tag{4}$$

Having the standard deviation, the standard error of the mean is estimated by

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} \tag{5}$$

where $s_{\bar{x}}$ = standard error for the mean.

It is often more convenient and by lowering the rounding-off-errors may yield more accurate result to divide s^2 by n and then to take the square-root.

Having the mean and the standard deviation in hand, the fiducial limits are estimated by applying Student's "t"-table. The "t" values with appropriate degree of freedom, corresponding to the 0.05 probability give the multiplyer. The standard error of the mean multiplied by it yields the quantity to be added to the mean to have the upper limit and to be subtracted from it to get the lower one (Table 17).

TABLE 17

Values of t corresponding to P = 0.05

d.f.	*t*	*d.f.*	*t*
1	12.71	11	2.20
2	4.30	12	2.18
3	3.18	14	2.14
4	2.78	16	2.12
5	2.57	20	2.09
6	2.45	30	2.04
7	2.36	40	2.02
8	2.31	60	2.00
9	2.26	120	1.98
10	2.23	∞	1.96

d.f. = degrees of freedom

$$F = \bar{x} \pm t\, s_{\bar{x}} \tag{6}$$

where F = fiducial limits (F_U is used to denote the upper limit and F_L the lower one)
$\bar{x}$ = mean
$s_{\bar{x}}$ = standard error of the mean
t = Student's t-value (Table 17).

The degree of freedom (d.f.) in the following example will be the number of entries minus one ($n - 1$). In some cases it is even less. For each type of assay of the Pharmacopoeia, the degree of freedom is indicated.

For example, having in an experiment 12 parallels yielding 110 I.U. for the mean and 6 I.U. for its standard error. According to Table 17, in the 11 d.f. row for 0.95 (i.e. 1.00—0.005) we have t = 2.20. The fiducial limits will be $6 \times 2.20 =$ $= 13.20$ I.U. from the mean. 13.20 is 12 per cent of 110. This renders the fiducial limits 88 to 112 per cent.

Note. Most of the prescriptions in all the Pharmacopoeias are not for doses but for log-doses. Consequently, the lower limit will be nearer to the estimated potency than the upper one. Often will be the estimated potency (compared to itself 100 per cent) the geometric mean of the two limits: the product of their percentage values rendering 10 000 (i.e. 100^2). E.g. 80 to 125 per cent, or 67 to 150 per cent, i.e. 4/5 and 5/4 or 2/3 and 3/2.

However, our aim concerning the errors committed does not estimate that for the means of the preparations. Our interest lies in the quantity of the active substance in the test preparation, compared to that of the standard. As the computations are executed on log-doses, their difference stands for this proportion. For methods applied in the Pharmacopoeia the *standard error of the difference*

$$s_{\text{diff}} = \sqrt{s_S^2 + s_T^2} \tag{7}$$

where s_{diff} = standard error of the difference of the means,
s_S = same for the standard preparation,
s_T = same for the test preparation.

For the methods of the Pharmacopoeia the preparations to be matched have to be administered in doses containing the active principle in closely similar quantity. Consequently, the variances could be supposed to be the same. In such cases it is better and more feasible to apply the formula:

$$s_{\text{diff}} = \sqrt{\frac{\Sigma'(x_S - \bar{x}_S)^2 + \Sigma'(x_T - \bar{x}_T)^2}{n_S + n_T - 2} \times \frac{n_S + n_T}{n_S\, n_T}} \tag{8}$$

where s_{diff} = the standard error of the difference of the means
$\Sigma(x_S - \bar{x}_S)^2$ = sum of squares for the standard
$\Sigma(x_T - x_T)^2$ = sum of squares for the test
n_S = entries for the standard
n_T = entries for the test.

In most cases the number of entries (n) are the same for all the preparations so

$$s_{\text{diff}} = \sqrt{\frac{\Sigma'(x_S - \bar{x}_S)^2 + \Sigma'(x_T - \bar{x}_T)^2}{n(n-1)}} \tag{9}$$

or

$$s_{\text{diff}} = \sqrt{\frac{\Sigma' x_s^2 + \Sigma' x_T^2 - \frac{(\Sigma' x_S)^2 + (\Sigma' x_T)^2}{n}}{n(n-1)}} \tag{9a}$$

(cf. Example 1, Vol. I, p. 140).

As mentioned above, fiducial limits may be attached also to the estimated relative potency. These limits are estimated on principles alike though not in such simple as given by Equation 6. The scheme of estimation depends on the type of the assays. The computational scheme for each type will be indicated there (Table 18).

The computational schemes mentioned already and those to follow are relatively simple as the responses were supposed obeying the *normal (Gaussian)* law. For the biological assays this presumption is warranted in the majority of cases if log-doses are applied; the responses do so at least with fairly good

approximation. However, if any doubt arises, the type of distributions is to be checked.

Prior to the assay itself the *validity* is to be checked to ensure that all preparations to be tested are chemically identical. Provided validity, any difference in the mean responses could be ascribed exclusively to quantitative (dilutional) differences.

In direct assays the grade of response to be achieved is prescribed by Pharmacopoeiase. The required doses to achieve it are sought by the assay for each preparation. The difference of the log-doses serve to estimate the *relative potency*. Here the potency is inversely proportional to the required dose. For example, 4/5 of the dose required for the standard preparation proved sufficient for that of the test: the estimated relative potency is 125 per cent. In the indirect assay prefixed doses of the standard and test preparations are administered. The responses are read and the dose of the standard to induce the same response as the test doses is estimated. Here the proportionality is direct: the increase in the response is proportional to the increase of the log-dose,* i.e. the regression of the response is rectilinear on the log-dose. It is presumed that the rate of increase is the same for all the preparations tested in the assay. I.e. the regression lines run parallel (Fig. 19).

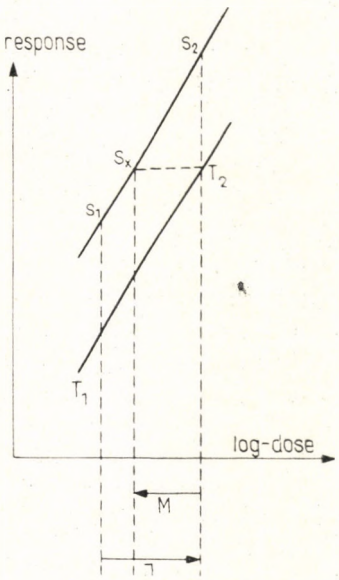

FIG. 19

The aim of the analysis is to estimate the shift along the log-dose (x)-axis of the $T_1 - T_2$ regression line, compared to the $S_1 - S_2$ line. (In Fig. 19 the potency of T_2 corresponds to that of S_x.) The log-difference yields the logarithm of the quotient, so its antilog gives how many times more (or less) potent the test is than the standard: i.e., the *relative potency*. As mentioned above, the validity being presumed (and if necessary, checked, too) i.e. the slope of the lines running parallel all the data are combined to estimate the regression coefficient. On the other hand, the position of the standard line is estimated using all the data concerning the standard and alike for the test.

The assumption of *rectilinear* regression of the response on the log-dose is generally righteous for the assays prescribed in the Pharmacopoeia. Nevertheless, for too high or too low doses this presumption does not hold. Consequently, caution is to be exercised applying high doses multiple of the lower one. Having variances highly differing for the different doses, the reliability of the estimate may be highly vitiated.

Due randomization is also one of the indispensable prerequisites of the reliable assay.

* In case of the measured effect being less for the greater dose (e.g. blood sugar level and insulin), the proportionality is inverse.

(b) PROCEDURES OF CALCULATION

The biological assay methods official in the Pharmacopoeia are divided, according to the route of computation, into the following groups:

1 Direct Estimation

The doses of the standard (S) and of the test (T) are determined which are necessary to cause a certain response; the potency of the test is expressed as related to that of the standard preparation by the ratio of the doses. In similar cases the quotients of the doses required — the difference of the logarithms — are to be computed (cf. Example 1) and the fiducial limits are to be determined by this method. Procedures of this type are the assay methods of digitalis potency, of adrenaline, of vasopressin and the appraisal of the histamine-like activities.

Note. Since the potency of that preparation is higher of which a lower dose is enough to exert an identical response, i.e. an *inverse proportionality* exists between relative potency and log-dosis, with computation of the relative potency *the doses of the test is substracted from that of the standard* and not the standard from the preparation to be tested.

Direct Assay

EXAMPLE 1

Digitalis Assay pigeon method (ml/100 g)

Standard Preparation			Test Preparation		
x	x (log 10 x)	x^2	x	(log 10 x)	x^2
1	2	3	4	5	6
0.854	0.931	0.866761	1.021	1.009	1.018081
0.972	0.988	0.976144	1.034	1.015	1.030225
0.919	0.963	0.927369	1.000	1.000	1.000000
0.950	0.978	0.956484	0.990	0.996	0.992016
1.073	1.031	1.062961	0.933	0.970	0.940900
1.116	1.048	1.098304	1.000	1.000	1.000000
Σ	5.939	5.888023	Σ	5.990	5.981222

$$\bar{x}_S = \frac{5.939}{6} = 0.990 \qquad (\Sigma' x_S)^2 = 35.271721 \qquad 5.981222 = \Sigma x_T^2$$

$$5.888023 = \Sigma x_S^2$$

$$\bar{x}_T = \frac{5.990}{6} = 0.998 \qquad (\Sigma' x_T)^2 = \frac{35.880100}{71.151821} \qquad 11.869245 = \Sigma' x_T^2 + \Sigma' x_S^2$$

$$-11.858637 = \frac{(\Sigma' x_S)^2 + (\Sigma' x_T)^2}{6}$$

$$M = -0.008$$

$$\frac{(\Sigma' x_S)^2 + (\Sigma' x_T)^2}{6} = \frac{71.151821}{6} = 11.858637 \qquad 0.010608 = \sum_1^1{}' \sum_1^n{}' (x + \bar{x})^2$$

Relative potency (R) = antilog (2—0.008) = **98.2 per cent of the Standard Preparation**

$$s_{\text{diff}} = \sqrt{\frac{0.010608}{6\ (6-1)}} = \sqrt{0.0003536} = 0.0188$$

d.f. $= n_T + n_T - 2 = 10$

According to Table 17. $t_{[10]}$ (0.05) = 2.228

Log fiducial limits = $2 \pm (0.0188)\ (2.228) = (2 \pm 0.0419) = 1.9581$ to 2.0419

Fiducial limits (F_L, F_U) = **90.8 to 110.1 per cent of the estimated potency**

In columns 1 and 4 the doses required to induce lethal effect, in columns 2 and 5 the logarithms of their tenfolds are indicated. Tenfolds are taken to facilitate computational work: the characteristics will be zero for the majority of the entries and in no case will they be negative. Consequently, there are in most of the entries only 3 digits, instead of 4. For such assays it generally suffices to have only 3-digit mantissae. (Where only 4-digit-tables are available, it is better to use 4 digits. The disadvantages of using 4 digits — more laborious computational work, higher risk of pen-slips, etc. — are less than those due to rounding-off labour and mistakes.) The end-result is not at all influenced by this multiplication by 10. It is equivalent of taking the values for 1000 g instead of the usual 100. The squares in columns 3 and 6 are given to 6 mantissa-digits. Though the reliability of the end-results would disallow such sham-precision, we do it to lower the errors due to rounding-off. Nevertheless, more digits imply higher risk of pen-slips and faults alike.

The means were computed according to Equation 1. However, as the number of entries is the same, it would have sufficed to compute direct their differences

$$M = \frac{\Sigma x_S - \Sigma x_T}{n} = \frac{5.939 - 5.990}{9} = \frac{-0.051}{6} = -0.008$$

This difference equals the "log relative potency" (M). Its antilog yields the potency ratio (R) of the test to the standard.

The standard error of the difference is computed according to Equation 9*a*.

According to the computational results, for estimated potency (R) we have 98 per cent as compared to the potency of the standard preparation, with fiducial limits 91 to 110 per cent of the estimated potency.

Note. Though, for the computations more digits were written (working digits), the results should be presented only to the whole numbers (significant digits).

2 Indirect Estimation

Prefixed doses of both the standard and the test are administered and the responses are determined. From these data the dose of the standard yielding response equivalent to the given dose of the test is computed. From this relative potency is estimated.

2.1 "Four-point" Method (Simple Design)

This is the most often employed design of the Pharmacopoeia (cf. Example 2). For each preparation, 2 doses are used. The doses of the standard are taken to have the higher dose 1.5 to 3 times higher than the lower one. The responses

due to the test doses should be about the same as those of the standard doses. (In most cases the same by weight as the standard.) From the responses the combined slope of the regression lines is computed (the regression of the responses on the log-dose), further the horizontal shift of the test-regression-line is calculated from that of the standard, and with these results the relative potency is estimated. (For computational scheme see Table 18.)

EXAMPLE 2

Simple Four-point Method

Chorionic Gonadotrophin (Diczfalussy's method) Assay (mg prostate + vesiculae seminales/100 g body weight)

	Standard Preparation		Test Preparation	
	2 I.U.	4 I.U.	2 I.U.	4 I.U.
$n = 11$	217.1	254.9	186.5	222.2
	190.5	236.2	219.0	259.6
	198.4	264.6	182.7	228.2
	197.8	220.2	207.1	255.5
	197.9	252.2	249.0	312.3
	200.0	283.1	220.5	241.7
	225.6	247.9	199.5	276.8
	175.0	263.2	204.5	301.0
	193.2	211.3	178.0	184.9
	176.1	270.2	237.2	258.6
	201.5	297.5	190.0	223.5
Mean	$S_1 = 197.55$	$S_2 = 254.66$	$T_1 = 206.73$	$T_2 = 251.30$

$$E = \frac{(T_2 - T_1) + (S_2 - S_1)}{2} = \frac{44.57 + 57.11}{2} = 50.84 \qquad b = \frac{E}{I} = \frac{50.84}{0.301} = 168.90$$

$$F = \frac{(T_2 - S_2) + (T_1 - S_1)}{2} = \frac{-3.36 + 9.18}{2} = 2.91 \qquad M = \frac{F}{b} = \frac{2.91}{168.90} = 0.0172$$

$$h = \frac{(T_2 - S_2) - (T_1 - S_1)}{2} = \frac{-3.36 - 9.18}{2} = -6.27 \qquad I = \log 2 = 0.3010$$

Relative potency (**R**) = antilog 2.0172 = **104.0 per cent of the Standard Preparation**

$s^2 = 689.66$ $\qquad A = H = V = 62.6964$

$$V = \frac{s^2}{n} = \frac{689.66}{11} = 62.6964 \qquad g = \left(\frac{t}{b}\right)^2 \times B\, t_{[40]}\,(0.05) = 2.021$$

$$B = \frac{V}{I^2} = \frac{62.6964}{(\log 2)^2} = \frac{62.6964}{0.0906} = 692.0132 \qquad t_h = \frac{(h)}{\sqrt{H}} = \frac{6.27}{\sqrt{62.6964}} = 0.79$$

$$g = \frac{(2.021)^2}{(168.90)^2} \times 692.0132 = \frac{4.084}{28\,527} \times 692.0132 = \frac{2826.18}{28\,527} < 0.1$$

As $g < 0.1$, it can be neglected and the simplified equation can be applied to estimate fiducial limits.

$$\text{Log fiducial limits} = 2 \pm \frac{t}{b}\sqrt{A + B \times M^2} = 2 \pm \frac{2.021}{168.90}\sqrt{62.6964 + 692\ (0.0172)^2} =$$

$$= 2 \pm 0.012\ \sqrt{62.6964 + (692) \times (0.0003)} = 2 \pm 0.012 \times \sqrt{62.9040} = 2 \pm (0.012) \times$$

$$\times (7.93) = 2 \pm 0.0952 = 1.9048 \text{ to } 2.0952$$

Fiducial limits (F_L, F_U) = **80.3 to 124.5 per cent of the estimated potency**

The s^2 is computed according to Equation 4. The degree of freedom equals the denominator (here 44 — 4).

The estimated potency (R) is good, the fiducial limits are acceptable. The value of g is low, the parallelity (validity) is good. In case of no disturbing factor or condition, the preparation is to be accepted. However, if in other cases doubt has arisen concerning accuracy (e.g. lack of due homogenity of the animal stock), all the conditions are to be checked.

In general, the prescriptions of the Pharmacopoeia warrant due safe-guard for the difference of the standard and the test to be taken as only quantitative, i.e. dilutional. Nevertheless, it may occur the sample having suffered chemical changes resulting in not at all unessential changes in biological efficacy. (This can occur in spite of the change being indetectable by the prescribed chemical tests.) In most of such cases the regression lines cease to run parallel, consequently, the estimated values may be highly biassed. Accordingly, if suspicion has arisen concerning validity, the parallelism is to be checked; i.e. it should be ascertained whether the regression coefficients differ significantly.

The "t"-test for parallelism:

$$t_h = \frac{h}{\sqrt{H}}; \qquad (10)$$

for h and H see Table 18.

Having the t_h value exceeding one given in Table 17, the difference in the regression coefficients is significant: the lines do not run parallel. In such cases the above computational scheme is prohibited. In this example we have 0.79 for t_h, this is much less than the tabular value (for 40 d.f. it is 2.021), i.e. having the **P**-value highly over the critical 0.05: the two slopes are not differing significantly.

2.2 Twin Cross-over Method

The sensitivity of the 4-point assay can be increased by applying the Twin Cross-over Method. Both the standard and the test are administered to the same animals. This design is feasible and advantageous for tests having on the one hand no long after-effect and on the other the variation between test-animals being high compared to within animal variations in time. The computational scheme is rather similar to that of the simple design (cf. Table 18). However, the computations for the standard and the test are omitted, only the intra-animal S—T differences (y) are used. (The sign of the values is inverse here as the greater insulin doses induce lower blood-sugar levels.)

For the allocation of the animals according to doses and preparations the following scheme is used:

TABLE 18

Computational schemes for indirect assays

S = standard preparation, T = test preparation, y = groups treated alike, Q = probit, S_1, T_2, y_3, etc. denote group means. Lower index 1 stands for the lowest dose and 3 for the highest.

	4-point design			6-point design
	Simple	Twin cross-over	Probit	
E = Dose difference	$\frac{(T_2 - T_1) + (S_2 - S_1)}{2}$	$\frac{y_1 - y_2 - y_3 + y_4}{4}$	$\frac{(Q_{T_2} - Q_{T_1}) + (Q_{S_2} - Q_{S_1})}{2}$	$\frac{(T_3 - T_1) + S_3 - S_1)}{4}$
F = Preparation difference	$\frac{(T_2 - S_2) + (T_1 - S_1)}{2}$	$\frac{y_1 + y_2 + y_3 + y_4}{4}$	$\frac{(Q_{T_2} - Q_{S_2}) + (Q_{T_1} - Q_{S_1})}{2}$	$\frac{(T_3 - S_3) + T_2 - S_2) + (T_1 - S_1)}{3}$
h = Slope difference	$\frac{(T_2 - S_2) - (T_1 - S_1)}{2}$	—	$\frac{(Q_{T_2} - Q_{S_2}) - (Q_{T_1} - Q_{S_1})}{2}$	$\frac{(T_3 - S_3) - (T_1 - S_1)}{2}$
C_1 = Curvature (1)	—	—	—	$(T_1 + T_3 - 2T_2) + (S_1 + S_3 - 2S_2)$
C_2 = Difference in curvatures (1)	—	—	—	$(T_1 + T_3 - 2T_2) - (S_1 + S_3 - 2S_2)$
I = Log ratio of doses (2) difference of log-doses	I	I	I	I
b = Slope	$\frac{E}{I}$	$\frac{E}{I}$	$\frac{E}{I}$	$\frac{E}{I}$
M = Log potency ratio	$\frac{F}{b}$	$\frac{F}{b}$	$\frac{F}{b}$	$\frac{F}{b}$
R = Relative potency (T per cent of the S)	antilog $(2 + M)$	antilog $(2 + M)$	antilog $(2 + M)$	antilog $(2 + M)$

V = Variance of mean [3]	$\frac{s^2}{n}$	$\frac{s^2}{2\,n}$	$\frac{4}{\Sigma\,(wn)}$	$\frac{s^2}{n}$
B = Variance of b	$\frac{V}{I^2}$	$\frac{V}{2\,I^2}$	$\frac{V}{I^2}$	$\frac{V}{4\,I^2}$
B = Variance of F	V	$\frac{V}{2}$	V	$\frac{2V}{3}$
H = Variance of h	V	—	V	V
C = Variance of c [1]	—	—	—	$12\,V$
t_h = Test of significance of h	$\frac{h}{\sqrt{H}}$	—	$\frac{h}{\sqrt{H}}$	$\frac{h}{\sqrt{H}}$
t_{C_1} = Test of significance of C_1 [1]	—	—	—	$\frac{C_1}{\sqrt{C}}$
t_{C_2} = Test of significance of C_2 [1]	—	—	—	$\frac{C_2}{\sqrt{C}}$
g = Index of significance of b	$\frac{t^2}{b} \times B$	$\frac{t^2}{b} \times B$	$\frac{t^2}{b} \times B$	$\frac{t^2}{b} \times B$
d.f = degrees of freedom [4]	$N - 4$	$N - 4$	∞	$N - 6$

$$g \geq 0.1 \quad \text{antilog} \left[2 + \frac{Mg}{1-g} \pm \frac{t}{b(1-g)} \times \sqrt{A \times (1-g) + B \times M^2} \right]$$

$$g \quad 0.1 \quad \text{antilog} \; (2 \pm \frac{t}{b} \times \sqrt{A + B \times M^2})$$

[1] As used only for t_{C_1} and t_{C_2}, the cancelling divisors omitted.
[3] For s^2 see Equation 4, n = number of entries for each group, for w see Table 20.
[3] For the 6-point assay for the neighbouring doses.
[4] N = number of entries for all the groups ($N = \Sigma\, n$).

Group	1st day	2nd day
I	S_1	T_2
II	S_2	T_1
III	T_1	S_2
IV	T_2	S_1

where S_1 stands for the lower dose of the standard (1 I.U.), S_2 for the higher one (2 I.U.), T_1 and T_2 having the same meaning for the test.

EXAMPLE 3

Twin Cross-over Method

Insulin Assay (1 1/2 hour blood sugar values, mg/100 ml)

Group I			Group II			Group III			Group IV		
1st day	2nd day	Difference	1st day	2nd day	Difference	1st day	2nd day	Difference	1st day	2nd day	Difference
S_1	T_2		S_2	T_1		T_1	S_2		T_1	S_2	
88	69	19	76	68	8	87	76	—11	82	82	0
108	86	22	93	85	8	107	86	—21	94	89	—5
90	86	4	85	90	—5	96	72	—24	76	66	—10
112	94	18	68	77	—9	86	78	— 8	66	61	— 5
90	87	3	70	63	7	101	79	—22	96	94	— 2
88	74	14	—	—	—	103	92	—11	69	74	5
106	88	18	87	82	5	76	60	—16	76	78	2
105	85	20	85	92	—7	83	64	—19	87	84	— 3
92	72	20	66	73	—7	84	66	—18	63	64	1
$S_1 - T_2 = y_1$			$S_2 - T_1 = y_2$			$S_2 - T_1 = y_3$			$S_1 - T_2 = y_4$		
15.33			0			—16.67			—1.89		

$$s^2 = 39.1290$$

$$n = \frac{35}{4} = 8.75$$

$$E = \frac{y_1 - y_2 - y_3 + y_4}{4} = \frac{30.11}{4} = 7.5275$$

$$F = \frac{y_1 + y_2 + y_3 + y_4}{4} = \frac{-3.23}{4} = -0.8075$$

$$b = \frac{E}{\log^2} = \frac{7.5275}{0.301} = 25.0083$$

$$M = \frac{F}{b} = \frac{-0.8075}{25.0083} = -0.0323$$

Relative potency (R) = antilog $(2 + M)$ = antilog 1.9677 = **92.8 per cent of the Standard Preparation**

$$V = \frac{s^2}{2\,n} = \frac{39.129}{17.50} = 2.2359$$

$$A = \frac{V}{2} = 1.1180$$

$$B = \frac{V}{2\,I^2} = \frac{2.2359}{2.\ (\log)^2} = \frac{2.2359}{2.\ (0.0906)} = \frac{2.2359}{0.1812} = 12.3394$$

$t_{[31]}\ (0.05) = 2.040$

$$g = \left(\frac{t}{b}\right)^2 \times B = \left(\frac{2.040}{25.0083}\right)^2 \times 12.3394 = (0.0816)^2 \times 12.3394 = (0.0067) \times (12.3394) = 0.0827$$

As $g < 0.1$, it can be neglected and the simplified equation can be applied to estimate fiducial limits.

$$\text{Log fiducial limits} = 2 \pm \frac{t}{b}\sqrt{A + B \times M^2} = 2 \pm 0.0816\sqrt{1.1180 + \sqrt{+\,12.3394\ (-0.0323)^2}} = 2 \pm 0.0816\sqrt{1.1180 + 0.0129} = 2 \pm 0.0816\sqrt{1.1309}$$

$$= 2 \pm (0.0816) \times (1.0635) = 2 \pm 0.0868 = 1.9132 \text{ to } 2.0868$$

Fiducial limits (F_L, F_U) = **81.9 to 122.1 per cent of the estimated potency**

The s^2 is generally computed according Equation 4. However, as in this case the number of entries differ, Equation 3 is to be applied. The number of entries was not the same for all the groups. In group 2 one of the rabbits convulsed, consequently, both its responses were discarded. As only few data are missing, the average number of entries can be used: here $n = 8.75$ (p. 152). The degree of freedom is also reduced by the number of missing animals. Though according to the prescription of the Pharmacopoeia 24 rabbits would be sufficient, here 36 were used. From the previous assays it could be seen that the stock was rather heterogeneous. With only 24 rabbits it would have been but little hope to have tolerable fiducial limits. Even now, with 35 of them, the limits are just acceptable (82 to 122 per cent). Had the operator attempted with the prescribed 24 animals, neither the assay itself nor test preparation could have been accepted. Consequently, repetition with 24 others would have to follow, to pool the results of the two assays (cf. p. 153). In this case the operator saved undue labour: 36 rabbits in one assay, instead of 48 in two. As the relative potency is also tolerable (93 per cent) the sample is to be accepted.

However, in such cases it is highly advisable to ameliorate the homogeneity of the stock. Taking this 36 as best scheme, for the present, 50 per cent of undue labour is inevitable. Scrutinizing the data, the 1st day readings show decreased sensitivity (a not at all rarely met phenomenon). Though the same animals, the same preparations, the same doses were applied, instead of practically the same responses we had 87 mg per ml on the first day and 78 for the second as the daily means. This is one of the main advantages of the twin cross-over design. The inter-day variations in sensitivity do alter but little the results of the assay. The design is "balanced", i.e. such changes and others due to chance exert practically the same influence for all the preparations and all the doses.

2.3 *Probit Analysis*

There exist responses, like "decease" where graduation is impossible, only "presence or absence" of a response can be registered. Such *quantal responses* are involved, e.g. in toxicity tests (cf. Example 4). In such cases the same doses of the standard and of the test are administered and the relative frequencies (per cent) of positive responses serve the basis of estimation. Applying probit analysis, the relative potency or toxicity can be estimated, provided *the number of test-objects* being *sufficient* for estimation (i.e. at least the number prescribed in the Pharmacopoeia or quoted in the literature). The exact probit analysis is highly time consuming, hence a simplified method may be used, similar to the simple "four-point method", provided the underlying conditions are fulfilled. The operator should have good practice of the specified assay he executes; there should be no essential change in the circumstances compared to the previous ones; the potency ratio may deviate but little from 1.0. These prerequisites existing, the computational scheme (Table 18) is similar to that of the simple design. Instead of the group means the corresponding probits are taken from Table 19. The variance of the mean is computed using the number of entries and the "weights" (Table 20). For example, having for dose S_1 the objects numbering n_1, this n_1

TABLE 19

Probits corresponding to Percentages

%	0	1	2	3	4	5	6	7	8	9
0	—	2.67	2.95	3.12	3.25	3.36	3.45	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33

TABLE 20

Weights corresponding to Probits (w)

Probit	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
2.0	0.015	0.019	0.025	0.031	0.040	0.050	0.062	0.076	0.092	0.110
3.0	0.131	0.154	0.180	0.208	0.238	0.269	0.302	0.336	0.370	0.405
-4.0	0.439	0.471	0.503	0.532	0.558	0.581	0.601	0.616	0.627	0.634
5.0	0.637	0.634	0.627	0.616	0.601	0.581	0.558	0.532	0.503	0.471
6.0	0.439	0.405	0.370	0.336	0.302	0.269	0.238	0.208	0.180	0.154
7.0	0.131	0.110	0.092	0.076	0.062	0.050	0.040	0.031	0.025	0.019

is multiplied by the weight (w_1) of the probit values found. These $w.n$ products are computed for all the four groups, summed and divided by four. The reciprocal of this average serves the estimate of the variance of the mean. (For probit analysis the degree of freedom is infinite.)

EXAMPLE 4

"Four-Point" (Probit) Design

Toxicity Test (4 × 32 animals)

Dose I.U.	Deceased	per cent	Probit	Weight
S_1 1.8	14	43.750	4.84	0.630
S_2 2.4	17	53.125	5.08	0.635
T_1 1.8	10	31.250	4.51	0.583
T_2 2.4	21	65.625	5.40	0.601

$$E = \frac{(5.40 - 4.51) + (5.08 - 4.84)}{2} = \frac{0.89 + 0.24}{2} = \frac{1.13}{2} = 0.565$$

$$F = \frac{(5.40 - 5.08) + (4.51 - 4.84)}{2} = \frac{0.32 - 0.33}{2} = -0.005$$

$$h = \frac{(5.40 - 5.08) - (4.51 - 4.84)}{2} = \frac{0.32 + 0.33}{2} = 0.325$$

$I = \log 2.4 - \log 1.8 = 0.3802 - 0.2553 = 0.1249$

$I^2 = 0.01560$

$$b = \frac{0.565}{0.1249} = 4.524$$

$$M = \frac{-0.005}{4.524} = -0.001105 \quad M^2 = \emptyset$$

R = antilog (2—0.0011) = antilog 1.9989 = **99.7 per cent of the Standard preparation**

$$V = A = H = \frac{4}{\Sigma\ (w \times n)} = \frac{4}{32\ (0.630 + 0.635 + 0.583 + 0.601)} = \frac{1}{8 \times (2.449)} =$$

$$= \frac{1}{19.59} = 0.05105$$

$$\sqrt{H} = 0.2259$$

$$B = \frac{0.05105}{0.0156} = 3.272$$

$t_{[\infty]}\ (0.05) = 1.960$

$$g = \left(\frac{1.96}{4.524}\right)^2 \times (3.272) = (0.4332)^2 \times (3.272) = (0.1877) \times (3.272) = 0.6142$$

$1 - g = 0.3858$

$$\text{Log fiducial limits} = \left[2 + \frac{(-0.001105)\ 0.6142}{0.3858} \pm \frac{0.4332}{0.3858} \times \right.$$

$$\left. \times \sqrt{(0.05105) \cdot (0.3858) + (3.272) \cdot (\emptyset)}\right] = \left[2 - 0.0018 \pm 1.123 \times \sqrt{0.01970}\right] =$$

$$= 1.9982 \pm (1.123) \times (0.1404) = 1.9982 \pm 0.1577 = 1.8405 \text{ to } 2.1559$$

Fiducal limits (F_L, F_U) = **69.3 to 143.2 per cent of the estimated potency**

$$t_h = \frac{0.325}{0.2259} = 1.439$$

$p_h > 10$ per cent

The estimated relative toxicity (R) is 100 per cent but the fiducial limits are too loose: 69 to 143 per cent, in spite of having had 4×32 animals for the test. As the test concerns toxicity and the risk of the toxicity being one-and-a-half times higher compared to that of the standard, the assay is to be repeated and the results duly combined according to the method described on p. 153.

The test of parallelism yielded here $t_h = 1.4$ (Equation 10). This is not near the critical 1.96 value (Table 17). If in the similar assays the parallelism proved acceptable, further more, no circumstance exists which could vitiate parallelism then this somewhat higher t-value bears no importance. On the other hand, having other signs for the suspicion of non-parallelism then this t-value should be seriously taken. The parallelism is disturbed mostly by qualitative difference in the preparations (validity). Consequently, this 1.4 t-value should be gravely pondered in case of chemical analysis (or some other facts) already indicating suspicion.

It has to be repeatedly stressed, this simplified computational scheme is feasible only for operators having good routine in techniques, if no essential changes occur compared to the previous tests, no essential differences in the potency of the preparations for the doses applied.

2.4 *"Six-point" Method*

For some assays the Six-point Method is prescribed in the Pharmacopoeia instead of the Four-point one. For such cases there exist simplified schedules, too, provided identical proportionality of the doses. Viz. the log-differences being equal both for the standard and the test: $\log S_3 - \log S_2 = \log S_2 - \log S_1$, the same for the test. The Six-point design is the method of choice where either high precision is required or the linearity is often vitiated, so it is to be checked on each occasion.

Such method is prescribed for the ACTH-assay.

EXAMPLE 5

"Six-point" Design

ACTH-Assay (Hamburger's method, mg Ascorbic acid/100 g adrenal gland)

Standard Preparation			Test Preparation		
50m I.U.	150m I.U.	450m I.U.	50m I.U.	150m I.U.	450m I.U.
396	394	356	409	384	360
534	375	358	450	418	366
405	366	331	422	429	375
459	414	391	480	500	326
461	409	330	444	386	291
476	391	340	441	415	285
Means $S_1 = 455.17$	$S_2 = 391.50$	$S_3 = 351.00$	$T_1 = 441.00$	$T_2 = 422.00$	$T_3 = 333.83$

$$E = \frac{(T_3 - T_1) + (S_3 - S_1)}{4} = \frac{(333.83 - 441.00) + (351.00 - 455.17)}{4} =$$

$$= \frac{-107.17 - 104.17}{4} = \frac{-211.34}{4} = -52.835$$

$$F = \frac{(T_3 - S_3) + (T_2 - S_2) + (T_1 - S_1)}{3} = \frac{(333.83 - 351.00) + (422.00 - 391.50) +}{3}$$

$$\frac{+ (441.00 - 455.17)}{3} = \frac{-17.17 + 30.50 - 14.17}{3} = \frac{-0.84}{3} = -0.28$$

$$h = \frac{(T_3 - S_3) - (T_1 - S_1)}{2} = \frac{(333.83 - 351.00) - (441.00 - 455.17)}{2} =$$

$$= \frac{-17.17 - (-14.17)}{2} = \frac{-17.17 + 14.17}{2} = -1.500$$

$C_1 = (T_1 + T_3 - 2\,T_2) + (S_1 + S_3 - 2S_2) = (441.00 + 333.83 - 844.00) + (455.17 +$
$+ 351.00 - 783.00) = -69.17 + 23.17 = -46.00$

$C_2 = (T_1 + T_3 - 2T_2) - (S_1 + S_3 - 2S_2) = 441.00 + 333.83 - 844.00) - (455.17 +$
$+ 351.00 - 783.00) = -69.17 - 23.17 = -92.34$

$I = \log 3$

$$b = \frac{E}{I} = \frac{-52.835}{0.4771} = -110.74$$

$$M = \frac{F}{b} = \frac{-0.28}{-110.74} = 0.0025$$

Relative potency (R) = antilog (2 + M) = antilog 2.0025

$R = 100.6$ per cent of the Standard Preparation

$$V = \frac{s^2}{n} \qquad s^2 = 1224.05 \qquad n = 6 \qquad V = \frac{1224.05}{6} = 204.008$$

$$B = \frac{V}{4\,I^2} = \frac{204.008}{4 \times (\log 3)^2} = \frac{204.008}{4 \times (0.2276)} = \frac{204.008}{0.9104} = 224.086$$

$$A = \frac{2\,V}{3} = 136.005 \qquad H = V = 204.008 \qquad C = 12\,V = 2448.096$$

$$t_h = \frac{|h|}{\sqrt{H}} = \frac{1.500}{\sqrt{201.008}} = \frac{1.50}{14.28} = 0.105 \qquad s_{[30]}\,(0.05) = 2.042$$

$$t_{C1} = \frac{|C_1|}{\sqrt{C}} = \frac{46.00}{2448.096} = \frac{46.00}{49.48} = 0.930 \qquad t_{C2} = \frac{|C_2|}{\sqrt{C}} = \frac{92.34}{49.48} = 1.866$$

$$g = \left(\frac{t}{b}\right)^2 \times B = \left(\frac{2.042}{-110.74}\right)^2 \times 224.086 = (-0.0184)^2 \times 224.086 = (0.00034) \times$$

$\times\,(224.086) = 0.0762$

As $g < 0.1$, it can be neglected and the and the following simplified equation can be applied to estimate fiducial limits.

Log fiducial limits =

$$= 2 \pm \frac{t}{b} \times \sqrt{A + B \times M^2} = 2 \pm (-0.0184) \times \sqrt{136.005 + 224.086 \times 0.0025^2} =$$

$$= 2 \pm (-0.0184 \sqrt{136.005 + 224\,086 \times 0.00000625} = 2 \pm 0.0184 \times \sqrt{136.005 + 0.0014} =$$

$= 2 \pm (0.0184) \times (11.662) = 2 \pm 0.2146 = 1.7854$ to 2.2146

Fiducial limits (F_L, F_U) = **61.0 to 163.9 per cent estimated potency**

Note: s^2 is computed according to Equation 4.

For ACTH the less ascorbic acid is in the adrenals, the greater is the effect. Consequently, the proportionality is inverse just as for the direct assays or for insulin. Nevertheless, the signs are not to be changed at subtraction as the regression coefficient being negative, converts the signs at division.

In the example the R is excellent: the test exhibiting practically the same potency as the standard dose. The fiducial limits and the g-value are acceptable, too.

Scrutinizing the responses themselves, they are found not so unambiguous. Provided linearity, the two lines run parallel: the t-value for h is very far from proving significant. However, for the curvature the data are far not so reassuring. The divergence in curvature is rather high though not yet significant. This relatively high divergence is due to the Test seeming to diverge from linearity while the standard values fit the straight line very well. This divergence is of negative sign: the straight line $T_1 - T_3$ being below the T_2 point. As in this assay the proportonality is inverse, it has to be thought of the decrease between T_1 and T_2 being less than it should be, i.e. T_1 being below the log-linear stretch of the response curve. (In similar cases of direct proportionality, the increase would prove insufficient.) However, for this test T_1-effect being too little may be excluded by two facts: the relative potency was estimated to be 100 per cent and — being the main argument — T_1 yielded higher or at least the same effect as S_1 did. Consequently, it should be accepted that T_2 response proved to be too low merely owing to random fluctuations.

3 Other Determinations

In some monographs (e.g. of serobiological preparations) the Pharmacopoeia defines but the lower limits, i.e. the potency of the test preparation should *surpass a certain prescribed value*. For such preparations there is no need for very accurate assays. Nevertheless, for the due dilution of too concentrated preparations the exact statistical analysis proves a good guide.

The estimation could be made more reliable and the reliability itself can be checked and improved by applying 2 or more doses instead of one. In some cases increasing the number of the replicates would suffice for these aims.

4 Varia

The Pharmacopoeia prescribes the *same number of test objects* for each group within one assay, (i.e. the lower dose of the standard is administered to as many animals as its higher dose is, etc.). However, it occurs not at all rarely that some of the test animals fall out, e.g. succumb (Example 3). For such cases the exact scheme of computation is tedious. However, good approximation can be achieved by taking the average number of animals for groups. For example, assay was started with 4×6 animals, of which 2 fell ill and had to be discarded, leaving 22, yielding 5.5 for average ($\bar{n}$). This simple correction is not permitted for cases having groups of missing entries exceeding the quarter of those originally allocated there. Discarding a greater number, or when more precise estimation is required, then this simple procedure is unsuitable.

In all the examples in this Pharmacopoeia it is supposed that identical doses are applied for both the Standard and the Test, hence equal results are to be expected. For example, both for the Standard and for the Test the lower dose was

1 mg and the higher one 2 mg. If for any reason the application of this rule seems not feasible, a correction factor is to be applied, e.g. on the ground of previous determinations (or of other information), the relative potency highly differing from 100 per cent. For a 4-point design having different doses, of the preparations but the ratio of the larger to the smaller stays constant, (e.g. 30 : 20 mg for the Standard and 24 : 16 for the Test), the correction is relatively simple. The logarithm of S_2 is subtracted from that of T_2 and the difference is subtracted from the log-relative potency (M). Certainly, this correction factor could be computed from the lower doses, too. The same rule holds for the 6-point design. Having the ratios of doses different for the Standard and the Test, the use of this simple correction factor is unsuitable.

Combination of results of repeated assays. Pooling the results is required if the assays taken one-by-one do not yield results supplying reliable informations about the potency. In such cases the weighted ($\overline{M}$) mean of the means in the individual assays (M) is the best estimate for the log-relative potency, weighing is accomplished by estimating the reliability of the individual means to be combined. The relative potency being — in fact — a mean value, the weights are the reciprocals of the variances of the means (called *invariance)*. For the direct aysass it is $\frac{l}{2s^2}$ and for the indirect ones $\frac{b^2}{A + BM^2}$ (Table 18). The combined estimate of relative potency is computed, therefore, by multiplying each mean by its own invariance, summing these products and dividing this sum by the sum of the weights:

$$\overline{M} = \frac{\Sigma' (MW)}{\Sigma' W} \tag{11}$$

where $\overline{M}$ = combined mean
M = means of the individual assays
W = weights.

The standard error of the combined mean is, however, estimated by

$$s_{\overline{M}} = \sqrt{\frac{1}{\Sigma' W}} \tag{12}$$

It has to be borne in mind, that these equations are simplified and yield only approximate estimates. They are valid for most of the prescriptions in the Pharmacopoeia, provided g being less than 0.10. If not, deciding on acceptance great caution should be exercised.

(C) BIOLOGICAL AND MICROBIOLOGICAL TESTS AND ASSAYS

(a) BIOLOGICAL ASSAYS

1 Assay of Chorionic Gonadotrophic Hormone

The potency of a sample of chorionic gonadotrophic hormone is estimated by comparing its effect on the weight increase of the *prostate* and *seminal vesicle* of male rats with that of the standard preparation of chorionic gonadotrophic hormone. The extent of increase is linearly proportional to the logarithm of the hormone doses.

Animals

Use at least 40 immature male rats weighing 25 to 40 g each for a single assay.

Procedure

Divide animals in four equal groups in such a way that not more than 5 animals are placed into one cage. Administer by subcutaneous injection to two groups two dilutions of the standard chorionic gonadotrophic hormone dissolved in isotonic sodium chloride solution and to two other groups two different dilutions of the sample being tested. Prepare the dilutions in such a way that the desired doses could be administered in equal volumens for 3 subsequent days (e.g. 2 resp. 4 intern. units dissolved in 0.3 ml isotonic sodium chloride solution are injected in 0.1 ml doses per day). Narcotize the animals on the fourth day with ether and dissect the *prostate* and the *seminal vesicle* together. Weigh the organs freed from connecting tissue on a torsion-balance with the accuracy of one mg. Calculate the potency relative to the standard preparation according to the chapter: *Statistical evaluation of the results of biological assays* (*2.1* Vol. I, p. 141).

The labelled potency meets the requirement if the estimated potency is not less than 80 per cent and not more then 125 per cent of the potency of the standard preparation. Fiducial limits of the assay are at $P = 0.95$, 75 per cent and 133 per cent of the estimated potency.

2 Assay of Powdered Digitalis Purpurea Leaf

The potency of a Digitalis Purpurea sample is estimated on pigeons by comparing the dose which arrests the heart function with that of the Standard Powdered Digitalis leaf. The International Standard is a mixture of dried and powdered Digitalis leaves. The international unit, (1 I.U.) is 0.076 g of the dried and powdered Standard Digitalis Leaf.

2.1 Preparation of Alcoholic Extract from Standard Powdered Digitalis Leaf

Weigh 2 to 3 grams of Standard Powdered Digitalis Leaf from a freshly opened vial into a glass stoppered 100 ml conical flask with mg accuracy. Pour 10.0 ml of diluted alcohol per gram powder, prepared from 4 volume of alcohol 96 per cent and 1 volume of water. Agitate the mixture in a suitable rocking machine at 20 to 30° for 22 to 26 hours, then centrifuge or filter through a glass filter. Preserve the extract in a well stoppered glass bottle in the refrigerator. The properly preserved alcoholic extract is suitable for use within 30 days.

2.2 Preparation of Alcoholic Extract from the Powdered Digitalis Leaf to Be Tested

Prepare the extract from the sample by the same method used for the preparation of the standard extract.

2.3 Preparation of Diluted Extract

Dilute tenfold with isotonic sodium chloride solution both the standard extract and the sample being tested.

Test animals. Use for the assay healthy pigeons weighing 300 to 500 g each.

2.4 Description of the Assay

Withold food from the pigeons 16 to 17 hours prior to assay. Weigh the pigeons on the day of assay. Use for the narcosis of pigeons 5 per cent solution of phenobarbital sodium. When 0.25 ml/100 g body weight of this solution is injected a superficial narcosis sets in within 20 to 30 minutes; the pupillary and corneal reflexes are retained. Immobilize the pigeons and insert a cannula into one of the alair veins exposed previously. Connect the cannula with rubber tubing to a burette of narrow diameter calibrated to 0.005 ml (Fig. 20). Fill both the burette and the rubber tubing with the diluted extract of the Digitalis leaf; ensure the abscence of air bubbles. Provide the burette with appropriate capillary tubing to ensure a uniform pressure of fluid inflow. With the aid of a screw-clamp mounted on the rubber tubing, infuse the digitalis extract with a uniform rate of about 1.0 ml per 10 minutes into the vein of the pigeon until the heart is arrested. Control the fluid inflow such that the heart arrest is accomplished between 30 and 60 minutes after the start of the infusion. Note the amount of millilitres of the digitalis extract used from the burette and calculate the lethal volume of the extracts per kilogram bodyweight.

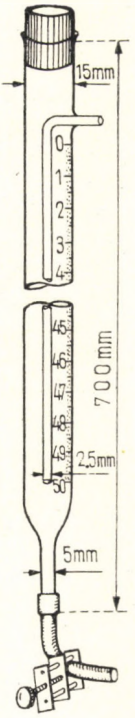

FIG. 20

Establish the lethal amount pro kg bodyweight of both the standard and the sample being tested on at least 6 pigeons each. Calculate the average of the lethal amount per kg bodyweight for both the standard powder and the sample being tested. Calculate the potency relative to the standard preparation according to the chapter: *Statistical evaluation of the results of biological assays* (*1.* Vol. I, p. 140). The labelled potency meets the requirement if the potency relative to the standard preparation is not less than 90 per cent and not more than 110 per cent. Fiducial limits of the assay are at $P = 0.95$, 80 per cent and 125 per cent of the estimated potency.

3 Assay of Vitamin D

The antirachitic potency of vitamin D preparations (cholecalciferol, ergocalciferol) is estimated by comparing it with that of the Standard Vitamin D preparation. The international standard preparation is a sample of crystalline vitamin D_3 0.025 μg of which represents 1 International Unit (I.U.).

3.1 Test Animals

Divide young rats of both sexes ranging in weight from 35 to 55 g into 5 groups by randomisation in such a way that each group should include one or two rats of the same litter. Each group should consist of 15 rats. The fifth group which serves as a control group can be completed with rats of other litters but of comparable weight in case when the number of rats from the same litters is not sufficient. — Keep the rats from the start of the experiment separated in individual cages. Rickets is induced by feeding the animals on the following diet:

ground yellow maize	76 per cent
wheat gluten	20 per cent
calcium carbonate	3 per cent
sodium chloride	1 per cent

3.2 Description of the Assay

After 3 weeks of restricted feeding administer to two groups two doses — of the Standard Vitamin D_3 preparation dissolved in *Arachis-* or *Helianthus oil* — differing by 100 per cent in potency and to two other groups two doses of the sample being tested. The doses of the sample being tested should contain presumably the same amounts of vitamin D as those of the standard and differing also by 100 per cent in potency from each other. (The concentration of the solutions should be chosen in such a way, that the volume of the administered fluid should be 0.1 to 0.2 ml.) Since for the purposes of estimating the healing effect, the administration of vitamin doses, producing only partial healing is more suitable than the administration of vitamin doses producing marked effect or even total healing, 0.5 to 1.0 I.U. should be given as the lower dose and 1.0 to 2.0 I.U. as the higher one. The dose must be administered in a single portion by means of a blunt injection-cannula mounted on a tuberculin-syringe. The cannula should be introduced into the stomach of the rats.

Sacrifice the animals two weeks after the administration of vitamin D and dissect the *radius* and *ulna* bones. Maintain the bones prior to evaluation, in a 4 per cent formaldehyde solution for 24 hours. Cut each pair of bones in two equal parts by the aid of a razor blade and place both parts with the cut surface upward in a 1.5 per cent silver nitrate solution. Expose the bones to bright daylight or to ultraviolet light for a few minutes. The time of exposition must be long enough to make possible the observation of calcification, but not so long as to render the picture too dark and thus impede the clear observation of the finer morphological details under the microscope. Wash the bone sections with distilled water and place them while wet in two transverse grooves of a Bürker's erythrocyte counting chamber. Cover the bones with a 2.5 mm thick coverslip. Use coverslips into the side of which facing the bones two grids are etched to form squares of 0.1 cm^2 area. If no such etched grid is available a so-called net-micrometer eye piece is also suitable for use.

Place the Bürker's chamber on the stage of a microscope and using 50 to 70 fold magnification and illumination from above (stereomicroscopes are very suitable for this purpose), measure the diameter of the freshly calcified cartilage with the aid of the grid or of the grid-micrometer at 7 about equidistant points of the *epiphyseal* cartilage from one end up to the other end. Calculate the averages per bones and per groups. Regard that portions of the calcified cartilage as fresh calcification and being due to the effect of the administered vitamin D, which

have a honey-comb structure or a smoke-like picture of homogeneous appearance. The portions should stain intensely deep-dark or black and must have no larger gaps than 0.05 mm in diameter.

In case of the administered vitamin doses, the group mean diameters of the calcified cartilage are linearly proportional to the logarithm of the doses. Rarely it happens that instead of a minor-degree healing complete healing is occurred. In this case the diameter of the calcified cartilage is no longer linearly proportional to the logarithm of the administered doses. Thus, the actually observed measure of calcification can not be considered as the base of calculation. In this case the calculation should be based on the largest diameter of the calcified cartilage of incompletely healed bones of the group receiving the same dose of vitamine D. As signs for completely healed rickets are regarded the following: the diameter of the epiphyseal cartilage measured in the middle of the *radius* and *ulna* bones are 0.2 to 0.4 mm, and the calcified cartilage facing the *epiphysis* is completely smooth, free from incisions. The average diameter of the calcified cartilage frequently measures 0.1 to 0.2 mm only. In cases of incomplete heeling, diameters of calcificications of 0.3 to 0.4 mm frequently occur.

The control group, not receiving vitamin D should show the typical microscopic picture of rickets. Characteristic for that is the enlarged diameter of the *epiphyseal* cartilage, which when measured medially on the *radius* and *ulna*, is not more than 0.6 to 0.7 mm, and in well developed rickets reaches 1.0 to 1.8 mm. The small areas of calcified cartilage tissue can be stained faintly and there are generally gaps of more than 0.05 mm diameter between the calcified portions of the cartilage.

Calculate the potency of the sample being tested according to the chapter: *Statistical Evaluation of the results of biological assays* point 2.1 ("four-point method"). The result meets the requirements if the potency of the sample is not less than 85 per cent and not more than 120 per cent of that of the standard preparation. Fiducial limits of the assay are at $P = 0.95$, 60 per cent and 170 per cent, respectively, of the estimated potency.

4 Assay of Heparin

The potency of heparin is determined by comparing its effect to prevent blood clotting to that of a standard preparation.

4.1 Reagents

Isotonic, sterile sodium chloride solution

sterile sodium citrate solution, 8 per cent

calcium chloride solution, 3 per cent [containing 3 g of anhydrous calcium chloride ($CaCl_2$)] per 100 ml water.

4.2 Preparation of Blood Plasma

Transfer for each litre of plasma 50 ml of a 8 per cent sodium citrate solution into a vessel sterilized previously at 120° for one hour. Let sheep blood run into the vessel under continuous agitation and keep it in a refrigerator until centrifuging. Centrifuge in sterile tubes at 10° for 30 minutes. Decant the plasma and filter through sterile cotton gauze into sterile bottles of 100 ml, and store at —8°

in a frozen state. Filter again the plasma before use through a sterile gauze after it had melted at room temperature. To each ml of the plasma add 0.8 ml of isotonic sodium chloride solution and 0.1 ml of a 3 per cent calcium chloride solution, and mix carefully. The plasma is suitable for use if its clotting time is not more than 5 minutes.

4.3 Preparation of the Standard Solution

Prepare parallel solutions with isotonic sodium chloride solution from the "Heparin Standard Preparation" containing 50 international I.U. per ml.

From this stock solutions prepare dilutions with isotonic sodium chloride solution containing 1.0, 1.5, 2.0, 2.5, and 3.0 I.U. per ml of the "Heparin Standard".

4.4 Determination of Plasma Activity

Make up 0.50 and 0.72 ml of each diluted standard solution with isotonic sodium chloride solution to 0.8 ml in two test tubes each. Add 1.0 ml of citrated sheep plasma and 0.1 ml of 3 per cent solution of calcium chloride and mix the content of the test tubes carefully. Read the degree of clotting after 1 hour.

Use for the assay those diluted standard solutions in which the test tube containing 0.5 ml shows complete clotting and the test tube containing 0.72 ml fluid shows complete prevention of clotting. (When preserved plasma is used, determine the plasma activity prior to each assay.)

4.5 Description of the Assay

Prepare from the sample a solution containing presumably about as much I.U. of heparin as the chosen dilution of the standard solution.

Use test tubes of 12×140 mm for the assay defatted by rinsing with chromic-sulfuric acid and carefully washed.

Transfer into each of the 12 test tubes of two simultaneous series decreasing volumes of the diluted standard solution (0.80, 0.76, 0.72, 0.68, 0.62, 0.59, 0.56, 0.53, 0.50, 0.47, 0.45 ml). Prepare another two similar simultaneous series and dispense in the test tubes the same amounts of the solution of the preparation being tested. (The difference in the heparin content between any of two adjoining test tubes is thus 5 per cent.) Fill the content of each test tube up to 0.8 ml with isotonic sodium chlorid solution. Add 1.0 ml of plasma and 0.1 ml of a 3 per cent calcium chloride solution, and mix thoroughly the content of the test tubes by inverting three times. After the lapse of one hour note the tube the content of which is clotted and respectively that other one the content of which remained fluid. Thus determine the smallest amount of heparin which prevents clotting of the recalcified plasma. (Note completely clotted plasma with "+", and the unclotted one with "—".)

Express the potency of sample relative to the potency of the standard preparation in International Units (I.U.).

The potency of the preparation meets the labelled one if it is not less than 90 per cent and not more than 110 per cent relative to the standard preparation. Fiducial limits of the assay are at $P = 0.95$, 80 per cent and 125 per cent of the estimated potency.

5 Assay of Insulin Solution

The potency of an insulin solution is estimated by comparing its hypoglycemic effect on rabbits to that caused by a standard preparation. Each mg of crystalline "insulin standard" contains 24 International Units (I.U.).

5.1 Solvent

Dilute 1.0 g of liquefied phenol *(phenolum liquefactum)* and 10 ml of 0.1 **N** hydrochloric acid with water to 1000 ml (Hydrogen exponent of the solvent: pH 2.5 to 3.5).

5.2 Standard Solution

Dissolve a portion of the "insulin standard" weighed with an accuracy of 0.1 mg, and prepare with adequate amount of the solvent a standard solution containing in each ml exactly 20.0 I.U. of insulin. — The standard solution is suitable for use for 6 months if stored at a cold place.

5.3 Preparation of Solution for the Assay

Dilute the standard solution with the solvent in the ratio of 1 + 9 and 1 + 19, respectively. (The diluted standard solutions should contain 2.0 and 1.0 I.U. per ml respectively.)

Prepare similarly two dilutions from the insulin solution being tested with the solvent; one of the solutions should contain presumably 1.0 I.U. and the other 2.0 I.U. per millilitre.

5.4 Test Animals

Use for the assay healthy rabbits weighing 2.0 to 3.0 kg. Keep the animals for at least a week prior to the assay in the animal house of the laboratory under constant environmental conditions, separately, in individual cages. Feed the rabbits daily on a uniform diet consisting of oat, vegetables and water.

5.41 Selection of the Test Animals

In a preliminary experiment, test each rabbit for insulin sensitivity. — Those rabbits whose normal bloodsugar level does not decrease by at least 10 per cent, after the subcutaneous administration of 0.2 to 0.6 I.U. insulin are not suitable for the assay. Animals in which a dose of 0.4 to 1.0 I.U. insulin produces an excessive decrease of the bloodsugar level with consequent convulsions, should not be used either.

5.5 Description of the Assay

Place the selected 24 rabbits 18 hours prior to the assay in the individual cages of the blood-sampling laboratory and withhold food and water until the test is finished. Divide the rabbits at random into four equal groups and determine their weight before the assay.

Select a suitable volume of the diluted solution of the insulin standard and of the sample being tested (generally 0.2 to 0.5 ml per rabbit are adequate), of which previously had been established that it will produce an adequate decrease of the bloodsugar level. Inject this volume subcutaneously into each of the 6 rabbits each of the below enumerated 4 groups:

Group 1. Standard insulin solution containing 1.0 I.U. per ml
Group 2. Standard solution containing 2.0 I.U. per ml
Group 3. Insulin solution being tested, containing an estimated 1.0 I.U. per ml
Group 4. Insulin solution being tested, containing estimated 2.0 I.U. per ml.

One and a half hours after the injection withdraw about 2.0 ml of blood from the marginal ear-vein of the rabbits and determine the blood sugar content expressed as percentages according to point 5.75. After the withdrawal of blood, the rabbits should be allowed to drink water for half an hour and then water is withheld again until the second part of the assay is finished. (The animals must not be given food even now, they should be starved further until the blood sampling on the second day is finished.)

Repeat the assay on the next day according to the "twin cross-over" method. Inject solutions of the same volume which were used on the first day exactly after 24 hours lapse into each of the 6 rabbits of the below enumerated 4 groups:
Group 1. Insulin solution being tested containing estimated 2.0 I.U. per ml
Group 2. Insulin solution being tested, containing an estimated 1.0 I.U. per ml
Group 3. Standard solution, containing 2.0 I.U. per ml
Group 4. Standard solution containing 1.0 I.U. per ml.

The twin cross-over assay can be performed instead of 24 hours after the first part of the assay also after a lapse of 6 days. Proceeding in the latter way, the animals must not be starved in the intermediate time. Only 18 hours prior to the beginning of the second part of the twin cross-over assay water and food must be withheld.

Use the same animals for a further assay only after at least 9 to 14 days of recovery.

5.6 Estimation of the Results

Calculate the potency of the insulin solution being assayed by comparing the blood sugar content, expressed in percentage, of the blood samples according to the chapter: *Statistical evaluation of the results of biological tests and assays* (*2.1* Vol. 1, p. 141).

The potency of the preparation meets the labelled requirement if the potency relative to the "insulin standard" is not less than 90 per cent and not more than 110 per cent. Fiducial limits of the assay are at $P = 0.95$, 80 per cent and 125 per cent of the estimated potency. If the fiducial limits of the assay are worse than that, the assay must be repeated using additional rabbits until the required fiducial limits are met.

5.7 Determination of Blood Sugar (Hagedorn–Jensen Method)

5.71 Reagents

Sodium oxalate.

Sulphuric acid, 0.0803 (1/12) **N** (Dilute 500 ml 0.2 **N** sulphuric acid with 700 ml water).

Sodium tungstate solution 10 per cent (dissolve 10 g sodium tungstate in a volumetric flask to 100 ml with water).

Potassium iron(III) cyanide solution, alkaline, 0.005 **N** (dissolve 1.66 g of potassium iron(III) cyanide and 10.6 g of anhydrous sodium carbonate in water to 1000 ml. (Protect from light).

Sodium chloride and zinc sulphate solution (dissolve 500 g of sodium chloride and 100 g of zinc sulphate in a volumetric flask in water to 2 litre).

Potassium iodide, dissolved in solution of sodium chloride and zinc sulphate (dissolve 2.5 g of potassium iodide in a volumetric flask in the solution of sodium chloride and zinc sulphate to 100 ml. Prepare the solution directly prior to use recently).

Acetic acid solution, 3 per cent (dissolve 3 g of concentrated acetic acid in water in a volumetric flask to 100 ml).

Starch mucillage, 1 per cent (Vol. I, p. 354).

Sodium thiosulphate solution 0.005 (1/200) **N** (dilute a 0.01 **N** solution of sodium thiosulphate with equal volume of water. Check the factor of the solution with due frequency with 0.005 **N** solution of potassium hydrogen iodate).

Potassium hydrogen iodates solution, 0.005 (1/200) **N** (dilute 100.00 ml of a 0.01 **N** of potassium hydrogen iodate solution in a volumetric flask with water to 200 ml).

TABLE 21

Table of blood sugar

Volume in ml of the volumetric solution consumed

Whole ml	Tenths of ml	Hundredths of ml									
		0	1	2	3	4	5	6	7	8	9
0	0		0.002	0.003	0.005	0.007	0.008	0.010	0.012	0.014	0.015
0	1	0.017	0.019	0.020	0.022	0.024	0.025	0.027	0.029	0.031	0.032
0	2	0.034	0.036	0.039	0.041	0.043	0.043	0.045	0.047	0.048	0.050
0	3	0.052	0.054	0.056	0.057	0.059	0.061	0.063	0.065	0.066	0.068
0	4	0.070	0.072	0.074	0.075	0.077	0.079	0.081	0.083	0.084	0.086
0	5	0.088	0.090	0.092	0.093	0.095	0.097	0.099	0.101	0.102	0.104
0	6	0.106	0.108	0.110	0.111	0.113	0.115	0.117	0.119	0.120	0.122
0	7	0.124	0.125	0.127	0.129	0.131	0.132	0.134	0.136	0.138	0.139
0	8	0.141	0.143	0.145	0.146	0.148	0.150	0.152	0.154	0.155	0.157
0	9	0.159	0.161	0.163	0.164	0.166	0.168	0.170	0.172	0.173	0.175
1	0	0.177	0.179	0.181	0.182	0.184	0.186	0.188	0.190	0.191	0.193
1	1	0.195	0.197	0.199	0.200	0.202	0.204	0.206	0.208	0.209	0.211
1	2	0.213	0.215	0.217	0.219	0.221	0.222	0.224	0.226	0.228	0.230
1	3	0.232	0.234	0.236	0.238	0.240	0.241	0.243	0.245	0.247	0.249
1	4	0.251	0.253	0.255	0.257	0.259	0.260	0.262	0.264	0.266	0.268
1	5	0.270	0.272	0.274	0.276	0.278	0.280	0.282	0.284	0.286	0.288
1	6	0.290	0.292	0.294	0.296	0.298	0.300	0.302	0.304	0.306	0.308
1	7	0.310	0.312	0.314	0.316	0.318	0.321	0.323	0.325	0.327	0.329
1	8	0.331	0.333	0.336	0.338	0.341	0.343	0.345	0.348	0.350	0.352
1	9	0.355	0.358	0.361	0.364	0.367	0.370	0.373	0.376	0.379	0.382

Resulting mg glucose in 0.1 ml of blood.

5.72 Procedure

Determine the blood sugar content in several simultaneous experiments.

Collect about 2 ml of blood from the marginal ear vein of the animal in a small vessel containing 6 mg of sodium oxalate. For the determination of the blood sugar transfer 0.60 ml of blood from each animal into two test tubes containing 4.8 ml 1/12 **N** sulphuric acid solution. Rinse the pipette several times by drawing up and letting out the fluid. Dispense into each test tube 0.60 ml of the 10 per cent sodium tungstate solution and shake the content vigorously. Immerse the tubes for 1 minute into a boiling water bath and cool. Filter the cold solution through a small dry paperfilter. Mix a 1.00 ml portion of the clear filtrate in a larger test tube with 10 ml of water and 2.00 ml 0.005 **N** alkaline potassium iron(III) cyanide solution. Keep the test tube in a boiling water bath for 15 minutes and cool rapidly. Add to the cold liquid 3 ml of potassium iodide dissolved in Sodium chloride and zinc sulphate solution, 2 ml 3 per cent acetic acid, 2 to 3 drops of starch mucillage and mix.

Titrate the liberated iodine with 0.005 **N** of sodium thiosulphate solution. Perform simultaneously two blank experiments as described above, but instead of the blood sample use distilled water.

Calculate the amount of blood sugar as follows. Subtract from the mean volume of the 0.005 **N** sodium thiosulphate solution consumed in the blank titration, the mean of the ml's of the 0.005 **N** sodium thiosulphate solution consumed in the two simultaneous titrations of the same blood sample. Correct the difference with the titer of the volumetric solution. Look up the value of the blood sugar in Table 21 corresponding to the value thus obtained. This value represents the sugar contained in 0.1 ml of blood expressed in mg of glucose.

6 Test for Prolongation of the Effect of Protamine Zinc Insulin

6.1 Preparation of the Solution for the Test

Precipitate the active ingredient of the sample being tested according to the direction for use and test the precipitous fluid without dilution.

Dilute the "Standard Solution" with the solvent in such a proportion that the insulin concentration of the diluted standard solution should be equivalent to that of the sample being tested.

6.11 Test Animals

Use for the test healthy rabbits weighing not less than 3 kg each.

6.2 Description of the Test

Distribute the rabbits kept for 24 hours without food and water in two groups of six rabbits each and take about 2 ml of blood from the marginal ear vein. Determine the blood sugar content expressed in percentage according to point *5.72*. Inject subsequently with the aid of a microsyringe subcutaneously the six rabbits of the 1st group with 0.5 I.U. per kg of body weight of the precipitous fluid to be tested; and the six rabbits of the 2nd group with 0.5 I.U. per kg of body weight of the dilution of the standard solution. 1 1/2, 3, 5 and 7 hours after injection withdraw blood and determine the concentration of blood sugar in each withdrawn sample.

6.3 Estimation of the Results

Sum up separately the blood-sugar values of the rabbits of the 1st group prior to injection and the values of the single determinations after injection at the given intervals. Summarize similarly the blood-sugar values of the 2nd group. Express the extent of the lowering of the blood-sugar content as percentage of the blood-sugar value established prior to the injection. The retardation effect of the preparation is satisfactory if at the time, when the blood-sugar value of the rabbits injected with the standard solution already reached the initial value found prior to injection (i.e. 100 per cent), the blood-sugar value of the rabbits injected with the sample preparation of prolongated effect does not exceed 80 per cent of the initial value found prior to injection. If the blood-sugar value of the rabbits injected with the standard solution does not return to 100 per cent even at the 7th hour, but attains not less than 90 per cent of the initial value, the blood-sugar value of the preparation of prolongated effect ought to be at least 20 per cent less than the value of the standard preparation — determined at the 7th hour — expressed in percentage of the initial value found prior to the injection.

If the blood-sugar value of the rabbits injected with the standard does not attain 90 per cent of the initial blood-sugar value, the test must be repeated with a lower injected dose.

If in the above described test the blood-sugar value of the preparation of prolongated effect decreases only a few per cent instead of the expected decrease of 20 per cent with respect to the initial blood-sugar value, the test can be repeated after a week with the same rabbits and with the same doses used in the first test, by a crossing over of the animals. In this second test those rabbits receive the preparation of prolongated effect which have received the standard preparation in the first test, however the standard preparation will be injected into those animals which were injected with the preparation of prolongated effect in the first test. Calculate the result of the test from the pooled values of both tests.

7 Assay of Corticotrophin

The corticotrophic potency is estimated on rats after administration of prednisolon by comparing the effect of the sample on the decrease of the adrenal ascorbic acid content with that of a "corticotrophic standard" hormone preparation. The decrease of the ascorbic acid content is linearly proportional to the logarithm of the corticotrophin doses.

7.1 Test Animals

Use for one assay 60 rats of the same sex weighing 90 to 110 g each.

7.2 Reagents

Trichloroacetic acid solution, 4 per cent

Activated charcoal (Boil 1000 g of activated charcoal *(carbo activatus)* in a wide mouthed flask with 1000 ml of 10 per cent hydrochloric acid and filter. Suspend the charcoal collected on the filter in 1000 ml water and refilter. Repeat this procedure until 10 ml of the filtrate mixed with 1.0 ml of R-hydrochloric acid, a few drops of bromine water and 1.0 ml of R-potassium-rhodanide solution develops a red colour).

Dinitrophenylhydrazine, solution of (dissolve 2 g of 2,4-dinitrophenylhydrazine in 100 ml 35 per cent sulphuric acid, and filter the solution).

Sulphuric acid, 85 per cent

Thiourea solution, 10 per cent (dissolve 10 g of thiourea in 50 per cent alcohol to 100 ml).

7.3 Description of the Assay

Perform the assay on animals kept separately at least for a week prior to the beginning of the assay in a place of constant room temperature, exposed neither to strong light nor to noise. On the day of assay inject subcutaneously each rat with 3.5 mg of prednisolon. Divide the animals at same time at random into six groups; each group consisting of 10 animals, but not more than 5 animals should be placed in one cage. Administer injections at 2 minute intervals to the test animals.

After exactly two and a half hours, inject subcutaneously 3 groups with three different doses of the "corticotrophin standard" preparation and the three other groups with the same dilutions of the sample being tested. The dilutions should form a geometric series (e.g. 0.05, 0.15, 0.45 I.U.) and keep 2 minutes intervals between two subsequent injections.

After the lapse of another two and a half hours narcotize the animals with ether and remove both adrenals. Clean the adrenals from the connective tissue and weigh them on a torsion balance to the nearest mg. Homogenize with 4 ml of a 4 per cent trichloroacetic acid solution and after adding a further 4 ml of the 4 per cent trichloro-acetic acid and 0.5 g of activated charcoal, filter the liquid. To 2 ml portions of each filtrate add in a test tube 2 ml of 4 per cent trichloroacetic acid, 1 drop of 10 per cent thiourea solution and 1 ml of dinitrophenylhydrazine solution and mix. Add finally to the content of the test tubes chilled in ice water drop by drop 5 ml of 85 per cent sulphuric acid shaking cautiously. After a lapse of 30 minutes, determine the extinctions at 540 nm, taking care that the determinations should be finished within 60 minutes. Calculate the ascorbic acid content of the adrenals from the ascorbic acid concentration extinction plot expressed in g per 100 mg adrenal.

Calculate the potency relative to the standard preparation from the decrease of ascorbic acid content according to the chapter: *Statistical Evaluation of Biological Tests and Assays* (*2.4*, Vol. 1, p. 150) ("Six-point" method).

The potency of the preparation meets the labelled one's, if it is not less, than 80 per cent and not more than 125 per cent of the standard preparation. Fiducial limits of the assay are at $P = 0.95$, 50 per cent and 200 per cent of the estimated potency.

8 Assay of Oxitocin

The potency of an oxitocin preparation is estimated on fowl by comparing the depression of the blood pressure induced by the preparation with the effect of the standard preparation. The extent of the depression of the blood pressure is linearly proportional to the logarithm of the dose.

8.1 Test Animals

Use healthy Leghorn hens weighing 1.5 to 2 kg for the assay.

8.2 Description of the Assay

Narcotize the animal with an intramuscular injection of 4 ml of a 5 per cent phenobarbital sodium solution. Fix the deeply narcotized hen to the operating board and after removing the plumage over the thigh bone, make a 4 to 5 cm long skin-cut. After cutting through the *musculus gluteus primus*, dissect the *arteria poplitea* and insert a cannula filled with 5 per cent solution of sodium citrate. Record the blood-pressure on a kymograph fitted to a mercury manometer. Insert a venous cannule into the *vena brachialis* and for prevention of blood clotting inject 0.1 ml of heparin solution. Then using the cannule, inject two different dilutions of both the standard preparation and the sample being tested. — The volume of dilution should not exceed 0.4 ml (e.g. 0.04 and 0.05 I.U. in 0.1 ml volume) and the smaller dose should cause a blood pressure depression of 20 to 30 torr (mmHg). The time interval between the injections should be 3 to 10 minutes. Take care that the blood pressure should return to its original level and that the time intervals should be uniform within an experiment. Inject the standard preparation and the sample being investigated alternately, at least four times each. Express the extent of blood pressure depression in torr. Calculate the potency relative to the standard preparation according to the chapter: *Statistical evaluation of Biological Tests and Assays (2.1* Vol. 1, p. 141 "Four-point method").

The resulting potency meets the labelled one, if it is not less than 80 per cent and not more than 125 per cent. Fiducial limits of the assay are at $P = 0.95$, 90 per cent and 111 per cent of the estimated potency.

(b) MICROBIOLOGICAL TESTS AND ASSAYS

1 Assay of Antibiotics

The potency of a sample of an antibiotic is determined by comparing the dose which inhibits the growth of an adequately susceptible microorganism with the dose of the standard of that antibiotic which produces the same degree of inhibition.

It is suitable to use the method of diffusion into agar from the holes bored in the medium in Petri dishes. The main points of the assay are as follows. Molten nutrient agar medium previously inoculated with a susceptible microorganism placed into Petri dishes. In the medium bore cylindric holes with a sterile cork borer and fill these with uniform amounts of antibiotic solutions. The antibiotic — while diffusing into the zone around the holes — inhibits the growth of the microorganism in a ring-like pattern. After the lapse of a suitable incubation time, measure the diameters of the zones of inhibition accurately. The composition of the nutrient agar media to be used, the solvents for the solution of the individual antibiotics, the description of the method of growth of the test organism used for the preparation of the inoculum, the dilutions to be prepared, are described in the respective monographs of antibiotics. Standard preparations of antibiotics are issued in Hungary by the National Institute of Public Health.

Procedure

1.1 One-level, Standard Plot, Agar Diffusion Plate Method

If there is no other instruction in the respective monograph, place in each of 15 planparallel Petri dishes of 90 mm diameter and 15.0 mm depth — on a horizontal table — 21 ml molten nutrient agar medium cooled to 48° to 50°. After solidification, spread over the medium 4 ml of medium holding the inoculum and by continuous tilting the Petri dish back and forth procure an even distribution.

According to the prescription of the monograph *Nystatinum*, (Vol. II, monograph No. 274) however, for the assay of this antibiotic add 1 per cent v/v of the inoculum to the whole volume of the agar medium, cooled to 48 to 50° in advance and dispense the inoculated medium into Petri dishes.

Bore with a sterile cork borer in the solidified layer of agar 6 holes of 6 to 8 mm diameter, spaced at equal distance from each other, and deliver 0.1 ml portions of both the solution of the standard and of the sample being tested in these holes with a sterile pipette.

Prepare a stock solution of appropriate concentration from the standard preparation and on the day of the assay prepare 5 dilutions from this stock solution according to a suitable geometrical series. The quotient 1.25 is often adequate for preparing a suitable geometrical progression. Design these dilutions with the letters *a, b, c, d, e*. Dilution *c* is called the "median dilution" and its concentration for the single antibiotics is given in the monographs.

Similarly to the standard, prepare a stock solution from the sample being tested. On the day of the assay, produce such a dilution of this stock solution that its concentration should be presumably equivalent to the concentration of the "median dilution" of the standard preparation.

Fill 3 of the 15 Petri dishes with the "median dilution" of the standard preparation and with the dilution of the sample being tested, the concentration of which is presumably equal with the "median dilution" of the standard. Dispense correspondingly in each of 3 Petri dishes alternately in 3 holes the dilution of the sample being tested and in the other the "median dilution" of the standard preparation.

Deliver into the 12 remaining Petri dishes the 5 different dilutions of the standard preparation. Dispense correspondingly in 3 holes of each of the 12 dishes the "median dilution" (*c*) of the standard preparation. Allocate the plates at random into 4 groups, consisting each of 3 dishes and deliver into the 9 vacant holes of a group one of the 4 other dilutions (*a*, or *b*, or *d*, or *e*) of the standard preparation. Incubate the Petri dishes at 35° to 37° for 16 to 18 hours and measure the diameters of the zones of inhibition with 0.5 mm accuracy.

Preparation of standard plot and evaluation of measurements. Compute the average of the 36 measured diameters of the "median dilution" in the 12 plates containing the different dilutions of the standard preparation. This average is called "the grand average" and is designated with the letter *c*. Then for each set of 3 plates, determine the correction factor. Subtract correspondingly from the value of the "grand average" *c* the average of the values of 9 zones of inhibition of the "median dilution" pertaining to the 3 Petri dishes of one set. This value may be either positive or negative.

Determine subsequently the average separately for each set of 3 dishes of the zones of inhibition of the other dilution dispensed in the same dish and add to it algebraically the factor of correction of the same groups.

Designate the so corrected averages of the zones of inhibition with the letters: a, b, d, e, while c is the "grand average". Subsequently, plot on a semilogarithmic paper on the logarithmic scale (x-axis) the concentrations of the dilutions of the standard preparation and on the arithmetic scale (y-axis) the averages of the corrected diameters of the zones of inhibition belonging to the individual concentrations. Draw through the 5 points so obtained the line of the best fit.

If the 5 points seem to define a straight line, then the calculated values of the lowest and the highest points of the line of the best fit are

$$y_L = (3\,a + 2\,b + c - e)/5$$

and

$$y_H = (3\,e + 2\,d + c - a)/5\,,$$

where y_L the calculated diameter of the zone of inhibition for the smallest concentration and

y_H the calculated diameter of the zone of inhibition for the highest concentration.

Designate the average value of the recorded zones of inhibition of the standard preparation in the 3 Petri dishes used for the sample being investigated as $\overline{y}_s$ and designate in the same dishes the recorded average value of the zones of inhibition of the sample being tested as $\overline{y}_i$. Subtract $\overline{y}_s$ from the value c, i.e. the value of the "grand average" of the standard curve. Add this difference algebraically to the average value of the sample being investigated, i.e. to $\overline{y}_i$. Using this corrected average value for the sample being tested, read the concentration of the sample on the logarithmic scale.

Calculate the slope of the standard plot (the regression coefficient) by the following equation:

$$b = (Y_H - Y_S)/(X_H - X_L)\,,$$

where X_H is the logarithm of the highest concentration of the standard preparation, and

X_L is the logarithm of the lowest concentration of the standard preparation.

The potency of the preparation of antibiotic being investigated is given by the antilogarithm of the following equation:

$$M = [\,(\overline{y}_i - \overline{y}_s)\,/\,b] + \lg R$$

where R = the concentration of the test preparation.

1.2 Three-level Agar Diffusion Plate Method

Into each of at least 4 Petri dishes bore 6 holes in the manner described in the one-level method, and fill 3 holes in each dish with 3 concentrations — increasing according to a geometrical progression — of the standard antibiotic preparation. Dispense in the other 3 holes of each dish 3 concentrations of the antibiotic preparation being assayed presumably equivalent to the concentrations of the standard preparation. Often the quotient 1.33 is suitable for the preparation of the geometrical progression. Fill the 3 dilutions of both preparations at random into the holes. After incubation of 16 to 18 hours measure the diameters

of the zones of inhibition accurately. From the values of diameters with the aid of mathematical statistical calculations not only the potency and the fiducial limits of the preparation can be computed, but also the validity of the assay can be checked.

(D) SAFETY TESTS

1 Test for Pyrogens

Use rabbits weighing at least 1500 g for the test. Keep the animals a week prior to the performance of the test in individual cages on the same adequate diet in order that they do not lose weight.

For establishing the normal temperature of the rabbits record the rectal temperature 1 to 3 days prior to the test 4 times a day at 1 hour intervals. Use for this purpose accurate thermometers graduated into 0.1 degrees or any other suitable instrument of at least equal precision. Insert the thermometer in the rectum beyond the internal *sphincter* muscle (to a depth of 6 to 8 cm). If the instrument is left in the rectum of the rabbit throughout the test, the rabbits may be fixed only with loosely fitting neckstocks in the cage. Use for the test only those rabbits the temperature of which is between 38.5° and 39.8° in these days and on the day of the test. The temperature of the animals of the same group should agree within 1.0°.

House the animals 48 hours preceding the test protected from noise in a room where the temperature fluctuation does not exceed $\pm 3°$ and where humidity is constant, too.

Twelve hours before the first temperature measurement withhold food and water from the animals until the test is finished.

The result of the first temperature measurement recorded on the day of the test is regarded as the base temperature of the rabbit. Compare the change in temperature observed during the test to this base temperature.

This method is suitable for the testing of preparations tolerated by the rabbits in a dose of 10 ml/kg. If only lower doses than 10.0 ml are tolerated, the volume that can be used or the degree of dilution is to be established in advance separately for each preparation. If the volume to be injected exceeds 10 ml warm the solution to 30° to 37°.

Render syringes and needles used for the test free of pyrogens. The most appropriate method for this purpose is heating at 250° for 30 minutes. Hypotonic solutions (diluent fluids, distilled water, etc.) should be made isotonic before the test with the aid of suitable amount of pyrogen free sodium chloride (*natrium chloratum pro infusione*). Only pyrogen-free water for injection is suitable for this test.

Procedure

Inject the preparation to be tested warmed to 37° into the ear veins of 3 rabbits within 30 minutes after the establishment of the base temperature in a dose prescribed per kg of body weight. Record the temperature 1, 2, and 3 hours after the injection. Calculate the results from the difference between the base temperature and the maximum temperature for each rabbit. Carry out the test first on 3 rabbits and evalutae the result according to Tables 22 and 23, respec-

tively. If the values found are falling between the limit values of the two tables, repeat the test on 3 further rabbits. If the results of tests performed on 6 animals are lying also within the limits, repeat again the test on 3 further rabbits.

Accept the preparation as free of pyrogens if the result of the test complies with Table 22 or reject as pyrogenic, if they comply with Table 23.

If performing the test on 9 rabbits, at least 4 rabbits exhibit a minimum of 0.6° temperature rise, but the summed response of temperature rises does not exceed 4.4°, or inversely the summed response of temperature rises exceeds 4.4° but less than 4 rabbits have a temperature rise of at least 0.6°, repeat the test on 9 further rabbits and reject the preparation if at a repeated test at least 4 rabbits have a temperature rise with a minimum of 0.6° each or if the summed response of temperature rises of the rabbits exceeds 4.4°.

Rabbits once used for a test can be re-used for a new test after a rest of at least 48 hours if no pyrogenic reaction was observed, but in case of pyrogenic reaction only after an interal of two weeks.

TABLE 22

Number of rabbits	Number of rabbits with a temperature rise of 0.6° or more (not exceeding)		Summed temperature rise of the rabbits (not exceeding)
3	0	and	+ 1.2°
6	1	and	+ 2.8°
9	3	and	+ 4.4°

TABLE 23

Number of rabbits	Number of rabbits with a temperature rise of 0.6° or more (at least)		Summed temperature rise of the rabbits (exceeding)
3	1	and	+ 2.6°
6	4	and	+ 4.4°
9	4	and	+ 4.4°

2 Test for Vasodepressor Impurities

Use for the test a healthy, non-pregnant narcotized cat weighing 2 to 3.5 kg. The following solution is suitable to obtain narcosis.

Dissolve 0.2 kg of allylbarbital and 4.0 g of ethylurethane in 5 ml water for injection with mild heating. After cooling, fill up with water for injection to 10.0 ml.

Inject 2.5 ml per kg of body weight of this solution intraperitoneally into the cat. The cat soon becomes narcotized and can be tied out. Insert cannulae into the *carotic artery*, the *femoral vein* and into the trachea. Use artificial respiration if necessary. Register the blood pressure continuously (8.2 Vol. I, p. 165) and administer the injections into the *femoral vein*.

From the sample being tested, prepare solutions in accordance with the prescriptions of the individual monographs of the Pharmacopoeia. Use for comparison an isotonic sodium chloride solution containing 1.656 µg per ml histamine hydrochloride (equivalent to 1.000 µg of histamine base).

Test first the sensitivity of the cat for histamine. Inject for this aim into the *femoral vein* at 5 minute intervals a solution containing 0.05, then 0.10 and finally 0.15 µg histamine base per kg body weight. The solution should cause

continuously progressive depression in blood pressure. Then inject at 5 minute intervals a volume of the solution containing 0.1 μg histamine base per kg several times until no marked difference can be observed between the grade of the blood pressure depressions following each other. If this dose of histamine does not cause a blood pressure depression of at least 20 torr on the cat, the animal is not suited for the test. If the sensitivity of the cat for histamine diminishes after several injections during the course of the test, and the administered 0.1 μg histamine base does not cause any more a depression of at least 20 torr, the animal should be discarded as unsuitable for the test.

After checking the sensitivity of the cat to histamine, perform the test of the preparation for blood pressure depressive contaminating substances. Inject correspondingly 0.1 μg of histamine base per kg of body weight and the prescribed amount per kg of body weight of the preparation alternatively at 5 minute intervals. The amount of the injected fluid must be equal both for the comparative solution and the solution under test. If necessary, dilute the solution with isotonic sodium chloride solution. Inject each sample at least twice and, if necessary, even more frequently. The preparation meets the requirement if the average of the repeated blood pressure depressions does not exceed the average of the repeated blood pressure depressions caused by 0.1 μg of histamine base per kg of body weight.

3 Safety Test of Antibiotics

Inject into five mice weighing 18 to 25 g each the dose of the preparation prescribed in the individual monographs or its solution prepared with sterile isotonic sodium chloride solution, respectively. If the injection is meant for intravenous use, inject the preparation into a tail vein at a uniform rate of about five seconds. Observe the animals housed in the laboratory at 18 to 25° temperature for 48 hours after the injection.

The preparation meets the requirement if no animal dies within 48 hours. If one or more animals die during the observation period repeat the test again or several times. Perform the test at each repetition using 5 or more mice previously not used and weighing 19 to 21 g each. The antibiotic sample meets the requirement for safety if the total deaths observed within 48 hours do not exceed 10 per cent of all mice tested, including the first test.

4 Sterility Tests

4.1 Sample

4.11 Sampling

From the viewpoint of sterility testing, regard for a batch the total number of ampoules or flasks sterilized in one filling in one apparatus of a preparation manufactured in one process. Test of each batch of any preparation the amounts of ampoules or flasks directed in Tables 24 and 25, respectively.

The samples should be representative for the batch concerned. The samples of the sterilized preparations should be representative for each layer of the sterilizing area.

TABLE 24

Number of units in the batch	Number of units to be taken as sample	
	Steam sterilized	Prepared aseptically (without subsequent heat sterilization)
Up to 10	1	2
11– 100	2	4
101– 200	4	8
201– 500	6	12
501–1000	8	16
1001–2000	10	20
2001–3000	12	24
3001–4000	14	28
4001–5000	15	30
5001–and more	20	40

TABLE 25

Fluid-conten of the containetrs (ml)	To be inoculated on each medium (ml)
0.5	0.2
0.16–1.5	0.3
1.6 –3.0	0.5
3.1 –20	1.0
21–50	2×3.0
more than 50	2×5.0

4.12 Quantity of Inoculum

Inoculate the amounts specified in Table 25 of the fluid content of the single containers (ampoules, flasks). If the volume of the fluid to be inoculated is less than 1.0 ml, the volume of the nutrient medium (in normal test tube) should be 12 to 15 ml, if the volume of fluid to be inoculated is below 5.0 ml, the volume of medium should be 40 to 50 ml.

4.2 Media

For testing of bacterial contamination, *thioglycollate medium should be used.* (If there is no possibility for preparing thioglycollate medium it can be substituted by simultaneous application of 1 per cent glucose-bouillon medium and Holman's medium.) For testing of fungous contamination a fluid nutrient medium suitable for support the growth of fungi — *a fungus medium* — should be used.

4.21 Preparation of Nutrient Media and their Testing for Sensitivity

4.211 Fluid thiolı collate medium

l-cystine	0.5 g
Agar	0.75 g
Sodium chloride	2.5 g
Glucose	5.5 g
Yeast extract	5.0 g
Tryptic digest of casein*	15.0 g
Sodium thioglycollate**	0.5 g
(or thioglycollic acid	0.3 g)
0.1 per cent solution of Resazurin	1.0 ml
(or 1 per cent methylen blue solution	0.8 ml)
Distilled water	1000 ml

* Synonym: Trypcasein®

** Use only preparation produced specially for bacteriological purposes.

With the exception of the thioglycollate and the indicator-solution, dissolve all ingredients in water by warming. Add next the thioglycollate. Adjust the pH with sodium hydroxide solution in such a way that after sterilization it should be 7.1 to 7.2. Check the final pH, correct it if necessary. Filter through moistened filter paper, add the indicator solution, and distribute the medium in test tubes and sterilize it for 20 minutes at 121°. Preserve after cooling to 25° at room temperature, protect from light. If during storage the upper third of the fluid column has turned coloured (pink, or greenish-blue) heat the medium by immersing in a vigorously boiling water bath until the colour fades completely. After cooling it is suitable for use. This restoring process can be performed but once.

4.212 Glucose broth

Grind 500 g defatted beef meat or beef heart free from tendons and mix with 1000 ml of distilled water. Steep for a night in a refrigerator and then boil it and press through moistened cloth and filter the steep liquor through moistened filter paper, and complete with water to 1000 ml. Instead of meat, beef cream is also suitable for use. Dissolve from this 15 g in 1 l of distilled water and add 3.0 g of sodium chloride, 2.0 g of disodium hydrogen phosphate 10.0 g of tryptic digested casein and 10.0 g of glucose. Adjust the pH with the sodium hydroxide to 7.4 to 7.6. Boil and filter through moistened filter paper. Check the pH and correct if necessary so that after sterilization it should be 7.2 to 7.4. Distribute in test tubes and sterilize at 121° for 20 minutes.

4.213 Holman's medium

Grind defatted beef meat heart free from tendons three times in succession. Mix the ground meat with equal volume of water and steep in the refrigerator overnight. Next day heat slowly to boiling. Replace any loss of water and adjust the pH with sodium hydroxide solution so that after sterilization of the medium it should be 7.8 to 8.2. Press subsequently through a moistened cloth and filter the steep liquor through moistened filter paper. Dispense the boiled meat in test tubes in such quantities, that the height of the meat should be at least 4 cm, and fill enough broth in the test tubes as to form an approximately 1 cm high fluid layer above the meat. Sterilize the medium at 121° for 20 minutes. Heat the medium before use in a boiling water bath for 15 minutes and cool in cold water. After inoculation, the surface of the medium can be superposed with a 1 cm layer of sterile saft paraffin.

4.214 Fluid medium for fungi

Dissolve in 1000 ml of water 20 g of glucose and 15 g of tryptic digested casein. Boil, and adjust the pH so that after sterilization it should be 5.3 to 5.7. Filter through moistened filter paper. Dispense in test tubes; sterilize at 121° for 20 minutes.

4.22 Check of Sterility of the Nutrient Media

Confirm sterility of the media for bacteria by incubating in a thermostat at 30 to 32° for 24 hours and those for fungi by incubating at 22 to 25° for 48 hours.

4.23 Sensitivity of the Media

Establish the sensitivity of the media by inoculating into each of at least 3 media for bacteria 1 ml of the 1.10^{-4} dilution of a 18 to 24 hour broth culture of the strain SG. 511 of *Staphylococcus aureus*, of a *Streptococcus pyogenes*, and of a *Clostridium novyi* strain, respectively, into the media for fungi, however, 1 ml of the 1.10^{-3} dilution of a 72-hour culture of *Candida albicans*.

4.24 Incubation

Incubate the media inoculated with bacteria at 30° to 32° and the media inoculated with fungi at 22° to 25° for 48 hours in suitable thermostats.

4.25 Condition of Suitability

Only those media are suitable for use in sterility testing, of which in every test tube unobjectionable growth of bacteria and fungi could be observed, respectively, during the testing for sensitivity.

4.3 Incubation

4.31 Inoculated media for testing for bacterial contamination should be incubated in a thermostat at 30 to 32° for not less than 7 days.

4.32 The inoculated media for testing for fungal contamination should be incubated in a thermostat at 22° to 25° for not less than 7 days.

4.33 Simultaneously with testing specified in points *4.31* and *4.32*, non-inoculated media should be also tested in the same manner.

4.4 Evaluation of Results

4.41 Establish the result of testing — if there is no other prescription — only after an incubation of 7 days.

4.42 The preparation meets the requirement for sterility if in none of the inoculated media any growth of either bacteria or of fungi could be observed.

4.43 If in two or more samples of a series the growth of morphologically identical bacterium and/or fungus has developed in the smear, the preparation fails to meet the requirement.

4.44 If in only one sample of the inoculated media growth of bacteria and/or fungi occurs, the test should be repeated using the same number of samples.

4.45 If at repeated testing no growth of bacteria and/or fungi occurs in any of the inoculated media, the preparation meets the requirement.

4.46 If at repeated testing in one or several of the inoculated media growth of bacteria and/or fungi occurs, and these seem to be morphologically identical with the findings of the first test, the preparation fails to meet the requirement.

4.47 If the result of the repeated test is not convincing, in such a case, exceptionally, the testing can be repeated with a sample number and with the method directed by the controlling authority.

*4.5 Test of Parenteral Preparations (Injections, Infusion-Solutions, Solids for Injection), Sterile Solutions and Serobiological Preparations**

4.51 Perform testing of injections, parenteral infusions and other solutions sterilized by heat or produced aseptically (Vol. I, p. 220) without subsequent heat sterilization according to points *4.2*, *4.3* and *4.4*

4.52 When testing oily preparations, the inoculated media should be shaken daily during the time of incubation.

4.53 Dissolve solids for injection dispensed in "dry ampoules" in the solvent supplied by the manufacturer or in adequate amount of distilled water and perform the testing of the solutions so obtained according to points *4.2*, *4.3* and *4.4* with the addition that the inoculated media should be incubated in the thermostat for 10 days at the prescribed temperature.

4.54 If during testing according to point *4.53* growth of bacteria or fungi could be observed, test separately for sterility also the fluid used for dissolution according to point *4.51.*

4.55 Perform testing of vaccines according to points *4.2*, *4.3* and *4.4* with the addition that the inoculated media should be kept for 10 days at the prescribed temperature in the thermostat.

4.56 Dilute those preparations in which the active substance or any added preservative has a bacteriostatic or fungistatic effect — and in which this effect cannot be eliminated by any other means — to such an extent, at which their effect ceases to influence the test.

4.6 Test of Powders Intended for External Use

4.61 Apply 2.5 per cent of the total weight of bulk powders soluble in water (but not more than 5 g) as for a sample, and dissolve in 5 to 10 ml (if necessary, in an even larger volume) of sterile physiological sodium chloride solution and test according to points *4.2*, *4.3* and *4.4.*

4.62 If during the course of a test performed according to point *4.61* growth of bacteria or fungi occurs, check for the sterility separately also the fluid used for dissolution according to point *4.51.*

4.63 Transfer the whole amount of a powder insoluble in water if the content of one container is 100 mg or less, and approximately 100 mg if the content is more than 100 mg, in 12 to 15 ml of glucose broth, and incubate for 24 hours at 30 to 35° in the thermostat. After 24 hours shake the inoculated medium thoroughly and test according to points *4.2*, *4.3* and *4.4.*

4.64 From the bulk-powders, insoluble in water, apply for sampling 2.5 per cent of the total weight of the powder (but not more than 5 g) and dispense each g of the sample separately in 12 to 15 ml glucose-broth. Incubate the media for 24 hours at 30° to 32° in the thermostat.
After 24 hours, perform the test of the incubated media according to points *4.2*, *4.3* and *4.4.*

* Sterility testing of veterinary medicinal vaccines is controlled in Hungary by the Ministry for Agriculture.

4.7 Test of Antibiotics

4.71 For inactivation of penicillinase-sensitive antibiotics, use in the model-test a pre-determined quantity of penicillinase. Check sterility of penicillinase sample in advance.
Dispense the required amount of satisfactorily checked penicillinase in 12 to 15 ml of thioglycollate medium and incubate for 48 hours at 30° to 32°. For further work use only media which were found sterile.

4.72 In checking of preparations dispensed in single dose containers inoculate from each of 6 container (ampoule) 50 mg, from preparations dispensed in multiple dose containers or which are not distributed in ampoules or small containers, inoculate 6 times 50 mg portions into the media specified in point *4.71.*

4.73 Keep inoculated media for 2 hours at room-temperature and shake several times.

4.74 For checking of inactivation, inoculate in one of the media 1 ml of the 1.10^{-4} dilution of an 18-hour broth culture of *Staphylococcus aureus*, strain SG. 511.

4.75 Proceed with further work according to points *4.2*, *4.3* and *4.4.*
If in the test tube(s) inoculated according to point *4.84* no bacterial growth occurs, repeat testing with a larger quantity of penicillinase.

4.76 Antibiotic preparations, which cannot be inactivated specifically, dilute prior to testing to an extent at which, in the media inoculated with standard strains used for control, growth should be observable.*

4.8 Test for Surgical Dressings and Sutures

4.81 Draw samples from each batch of surgical dressings sterilized simultaneously in the same charge of one autoclave.
Cut out from the outer, middle and inner part of each packaged sample about 1 cm^2 portions, and incubate these portions in media according to point *4.2* such a way that they should be totally covered by the medium.
Perform testing according to points *4.2*, *4.3* and *4.4.*

4.82 Cut at least one whole filament (not less than 2 m long) of sutures produced from filaments of linen, silk or plastics into 2 cm long portions. Incubate in each portion of media specified in point *4.2* 10 to 15 pieces of the filaments. Take care, that they should be totally covered by the fluid medium.
Perform the test according to points *4.3* and *4.4* with the difference, that the media inoculated according to points *4.31* and *4.32* be incubated for 10 days at the indicated temperature in the thermostat.

4.83 Perform the test for sterility from each production batch of catgut put on the market either in stellate form, or in phials, at least with 10 pieces (phials). Test each filament of the sample separately. Cut filaments to be tested to about 2 cm long pieces and transfer without any further pretreatment to 10 test tubes filled with Thioglycollate Medium and to further 10 tubes filled with Fluid Medium for Fungi. In each unit of medium of about

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* Standard strains used for control can be procured form the Strain-Center of the Nationa Institute of Public Health.

12 to 15 ml place not more than 2 pieces of the sample. The filaments should be completely covered by the medium.

If in lack of Thioglycollate Medium, 1 per cent glucose broth and Holman's medium are used simultaneously, catgut cannot be tested only after adequate pretreatment.

Pretreatment: cut catgut filaments to be tested to 2 cm long pieces and keep them in the pretreating solution (1.0 g of sodium thiosulphate, 1.0 g of anhydrous sodium carbonate dissolved in 100 ml of water) for 24 hours in a thermostat at 30 to 32°.

After 24 hours, transfer the catgut portions in each of 5 test tubes, containing 1 per cent glucose broth, Holman's medium and fluid medium for fungi, respectively in such a way that into each medium 2 to 3 pieces of the sample should be placed and the filaments should be totally covered by the medium.

Perform the test according to points *4.3* and *4.4* but the media should be incubated for 14 days in the thermostat at the prescribed temperature.

4.9 Test of Transfusion and Infusion Assemblies

Check for any bacterial growth the plastic accessories sterilized by autoclaving by samples taken at random by the controlling authority.

Take five total sets from the accessories sterilized in one batch (in the same autoclave) for testing in such a way that they should be representative for all levels of the sterilizing area.

Perform the testing of the accessories for sterility in thiglycollate medium only.

Let pass through each set of accessories 40 ml of the medium at a slow flow rate and collect the medium in sterile flasks.

Perform the test according to points *4.3* and *4.4* but if the sets prove not to be sterile, the tests should be repeated with doubled number of sets, and simultaneously the conditions of the sterilizing process should be also examined.

5 Bacteriological Test of Gelatin

5.1 Germ Count

Draw samples from white gelatin according to the rules of asepsis and proceed strictly observing these rules.

Add to 1.0 g portion of gelatin powdered in a sterile mortar, 99 ml of sterile water in a sterile 200 ml flask. After the gelatin appears to be wetted, heat the flask in a water bath of 40 to 50° and shake it frequently. The sample of gelatin should be dissolved within 15 minutes. Perform the test by casting plates of solutions containing gelatin in 1 to 10, 1 to 100 and 1 to 1000 relations.

Transfer 1.0 ml portions of these dilutions in each of two series of 10.0 ml liquefied agar containing 0.5 per cent glucose cooled to 42° and after thorough mixing cast the plates. Incubate the plates at 37° in the thermostat and count the developed colonies after 24 and 48 hours. The germ count is the arithmetic mean of the number of colonies appearing during 48 hours of incubation. The germ count of gelatin must not exceed 10 000 bacteria per 1.0 g.

5.2 Test for Bacterium Coli

Spread 1.0 ml of a 1.0 per cent gelatin solution prepared according to *5.1* in each of 3 test tubes containing 0.5 per cent lactose broth, a suitable indicator and a fermentation tube (Durham's tube). Incubate the test tubes at 37° in a thermostat. Examine all 3 tubes after 24 and 48 hours. From the tubes where gas production occurs, spread one loopful on an Endo-plate. Incubate the plates also at 37°. Check the plates after 24 and 48 hour incubation. If metallescent pink colonies (characteristic for *Bacterium coli*) appear after 24 and 48 hours, stain the plates according to *Gram* and perform microscopic examination.

Qualify as *Bacterium coli* any organism fermenting lactose with gas and acid production, developing anaerobically on Endo-agar-plates forming pink coloured metallescent colonies, shaped of a rod, Gram negative and not sporulating bacteria.

In 10 mg — or less — gelatin no *Bacterium coli* should be detectable.

5.3 Test for Bacillus Anthracis

Inject 2.0 ml of a 1 per cent gelatin solution prepared according to *5.1* into the legs of each of 2 guinea pigs and mice subcutaneously. If the sample is contaminated with *Bacterium anthracis*, the inoculated animal dies within 1 to 4 days and the pathogenic organisms can be identified from the spleen, liver and blood of the perished animal both by culture and on stained preparation.

5.4 Test for Clostridium Tetani and for Clostridia Causing Gas Gangrene

Inject 2.0 ml of a 1 per cent gelatin solution prepared according to point *5.1* into the leg muscles of each of 2 guinea pigs. Observe the animals for 7 days for the developing of the characteristic features of tetanus and gasedema. If the animals perish in tetanus or gasedema, the pathogenic organisms can be cultivated from tissue portions dissected from the place of inoculation.

Gelatin must not contain any *Bacterium anthracis*, *Clostridium tetani* or any other pathogenic clostridia.

V TESTS AND ASSAYS OF CONTAINERS

1 Glass

1.1 Water-Soluble Alkali Content, Powdered Glass Test

Injections or *infusion solutions* must be filled into ampoules or glass containers releasing not more alkali than is equivalent to 0.06 mg of Na_2O per 2 g of glass (glass of the hydrolytic class I). For other solutions, use glass containers releasing not more alkali than is equivalent to 0.12 mg of Na_2O per 2 g of glass (glass of the hydrolytic class II).

Determine the hydrolytic class as follows: Wash carefully fragments of the glass container or a few ampoules with distilled water and dry. Comminute the dry glass in a steel mortar and pass through a stainless steel sieve. Perform the test with particles that pass through sieve No. IV but are retained on sieve No. V (particles between 0.3 and 0.8 mm). Remove steel particles fallen eventually in

the glass powder by using a magnet. Wash the powdered glass with alcohol, 96 per cent, until the last portion of the washing liquid is clear, dry at 110° (±2°) for 30 minutes, protected from dust, and preserve it in a glass container provided with a ground glass stopper. Boil for 30 minutes in two 100 ml conical flasks, about 60 ml each of 1 per cent hydrochloric acid. Wash and rinse the flasks with several portions of distilled water, until the last portion acquires a yellow colour on addition of one drop of I-methyl red solution. Use these two flasks as containers for the test.

Transfer 2.00 g of powdered glass into one of the conical flasks, add 50 ml of freshly boiled distilled water, cover with a beaker and immerse for one hour into a water-bath of 100°. Cool, add 2 drops of **I**-methyl red solution and titrate with 0.01 **N** hydrochloric acid. Perform a blank test in the other conical flask using the same reagents but omitting the glass powder. The difference of the two titrations is the "alkali release" expressed in mg of sodium oxide (Na_2O) per 2.00 g.

Each ml of 0.01 **N** hydrochloric acid is equivalent to 0.3099 mg (lg .49122) of sodium oxide (Na_2O).

1.2 Water-Soluble Alkali Content, Surface Test

1.21 Ampoules, Containers for Injections and Infusions

Dispense to *ampoules* and *glass containers*, carefully rinsed with freshly distilled water previously, the following quantities of hydrochloric acid.

Capacity of the tested glass container ml	Volume and normality of hydrochloric acid to be dispensed		Capacity of the tested glass contanier ml	Volume and normality of hydrochloric acid to be dispensed	
	ml	N		ml	N
1	0.23	0.001	100	0.48	0.01
2	0.36	0.001	125	0.58	0.01
5	0.65	0.001	150	0.66	0.01
10	0.10	0.01	200	0.80	0.01
20	0.16	0.01	250	0.90	0.01
25	0.20	0.01	300	1.02	0.01
30	0.23	0.01	500	1.45	0.01
50	0.31	0.01	1000	2.30	0.01

(Prepare the 0.001 **N** hydrochloric acid from 0.01 **N** hydrochloric acid by dilution immediately prior to test.)

Fill the ampoules or containers to the neck with freshly boiled and cooled water, containing each 100 ml 0.5 ml alcoholic methyl red solution, 0.2 per cent. Seal the ampoules by fusion, cover the other containers with aluminium foil, previously washed twice in aceton, heat in an autoclave at 120° for 30 minutes and cool. The liquid in the containers may acquire a transient colour, but yellow colour must not be observed. The pH of the solution must not exceed pH = 6.2. Perform the test with 100 containers each of 1 to 10 ml capacity, with 30 containers each of 20 to 100 ml capacity and with 10 containers each 125 to 1000 ml capacity.

1.22 Medicine bottles

Clean the bottles carefully, rinse three times with freshly boiled distilled water and fill to the half of their volume with freshly boiled distilled water, containing 2 drops of **I**-phenolphthalein solution in each 10 ml. Cover with a beaker or loosely seal with a stopper and heat in a water-bath for 30 minutes. The liquid must remain colourless.

2 Rubber Closures

Quality requirements and test methods for rubber closures for ampoules, containers of substances for injection and multiple dose containers

Rubber closures are made of compact, soft rubber, having a uniform colour, possibly free from colouring additives (pigments). They may have a faint, characteristic odour of rubber; any other odour is unacceptable. The closure must not be contaminated or sticky, but should have an intact, smooth surface devoid of lamellar fragments or bloom. No swelling, gap, slit, sponginess or blisters are permissible.

Qualitative test

(a) *Extractive and colouring matter.* Wash rubber closures carefully with distilled water using a brush. Transfer 10 g of the rubber closures into a 500 ml conical flask that has been thoroughly rinsed with distilled water, and contains 400 ml freshly boiled distilled water. Seal the flask with a cotton plug wrapped into cellophane and heat in an autoclave at 120° for 20 minutes. The water decanted from the rubber closures must be clear, colourless and tasteless and may have at most a faint odour characteristic of rubber. The closures must not adhere to each other and must not be sticky.

Perform the tests (b), (c), (d), (e) and (f) with this liquid.

(b) *Acidity, alkalinity.* Determine the pH of the liquid under (a) and that of a blank, performed with 200 ml of freshly distilled water. The two values may not deviate by more than one pH unit at the most.

(c) *Heavy metals (lead, iron).* To 10 ml of the liquid under (a) add 1 ml of R-ammonium chloride solution, 1 ml of R-ammonia solution and 3 drops of R-sodium sulphide solution. Any colour of test solution must not exceed even after 5 minutes that of a blank processed simultaneously.

(d) *Ammonia.* To 10 ml of the liquid under (a) add 3 drops of R-Nessler—Winkler's reagent. Any colour produced must not exceed that of matching fluid *S2*. Check the colour after 5 minutes.

Quantitative tests

(e) *Extractive substances.* Evaporate on a water-bath to dryness 100 ml of the liquid under (a) in several portions in a weighing vessel, provided with a ground stopper, in a place free of dust. The weight of the residue, dried at 105° to constant weight, must not exceed 0.002 g.

(f) *Readily oxydizable substances.* Transfer 20.00 ml of the liquid under (a) to a glass stoppered conical flask, add 20.00 ml of 0.01 **N** potassium permanganate solution, allow to stand for 15 minutes, add 0.1 g of potassium iodide and 2 ml of R-hydrochloric acid. Seal the flask loosely with the stopper, allow to stand for

5 minutes, add I-starch solution and titrate with 0.01 N sodium thiosulphate solution. Perform the same determination with the blank solution under (b). The difference of the ml's of the sodium thiosulphate consumed is equivalent to the consumed ml's of 0.01 N potassium permanganate.

A 20.00 ml portion of the solution to be tested must not consume more than 1.5 ml of 0.01 N potassium thiosulphate.

Mechanical test

(g) *Fragmentation test.* Seal a multiple-dose container, containing distilled water with the rubber closure and penetrate with a needle of 0.8 mm diameter ten times. No fragments must be detached.

(h) *Hermetic sealing.* The container sealed with the perforated closure must ensure hermetic sealing at 0.5 torr reduced pressure; no water droplets must be visible at the perforation sites.

(i) *Heat stability.* Place 5 to 10 rubber closures into a covered glass beaker containing distilled water and heat in an autoclave at 120° for two hours. The surface of the closures must not alter, they must not stick to each other, no wrinkles or cracks should occur.

Note. Rubber closures used for infusion solutions must be suitably pretreated to exclude pyrogens.

Rubber closures used for containers of oily injections should be made of oil resistant material. Oil-resistancy must be tested by suitable methods.

3 Cellophane

Medicaments, in direct contact with the wrapping material, are mostly sealed into lacquered or heat-moulded cellophane films.

Normal cellophane is a transparent almost colourless film prepared from regenerated cellulose with glycerin and water. To diminish vapour penetration the cellophane films may be coated with lacquer layers on one or both sides. Heat-moulded cellophane is possibly coated with a lacquer-layer on one side, and a polyethylene layer on the other.

Test methods for cellophane films

Identification

(a) *Normal cellophane film.* Becomes easily moistened and wavy when in contact with water, and has a sweetish tase.

(b) *Lacquered cellophane film.* The coated surface is hydrophobic and water droplets run off from this side, whereas the uncoated surface is wetted by water. Smoothed onto the external wall of a beaker, containing water of 80 to 90°, neither the lacquered nor the uncoated side adhere to it.

(c) *Heat-moulded cellophane.* Water droplets run off. The polyethylene coated side is less bright and smooth than the uncoated one. Smooth cellophane onto the external wall of a baker containing water of 80 to 90°. Only the polyethylene coated side adheres. Tear the foil, the distended polyethylene layer is visible between the torn cellophane films.

Qualitative physical test

(d) *Vapour penetration.* Pour a few millilitres of distilled water into a Petri dish and cover it hermetically with the cellophane film in the following manner. Place onto the opening of the Petri dish the cellophane film, fix it by a rubber ring, over the side of the dish, stretch it tight and seal with wax at the edges. Transfer the Petri dish into a desiccator, containing anhydrous calcium chloride, and place it into a thermostate of desired temperature. Check temperature with a thermometer in the desiccator. After the temperature was equilibrated — not less than within two hours, — determine the accurate weight of the dish ($G1$), replace it into the desiccator and in the thermostate, respectively. Allow it to stand for 6 hours then weigh the dish again ($G2$). Calculate the vapour penetration on the basis of weight loss as follows:

$$A = (G_1 - G_2) \frac{24 + 10^4}{F \times t},$$

where A = vapour penetration g/m² in 24 hours
$G_1 - G_2$ = decrease of weight in g
F = free area of the cellophane film in cm²
t = timelapse between the two weighings, in hours.

Perform the test at 25° and 35° (±0.5°). The arithmetical mean of at least two parallel tests is the result of the determination. When testing normal (uncoated) cellophane films, or cellophane films coated on both sides with the same material the opposite sides of the films should be in contact with the water vapour in each of two parallel tests.

If cellophane films coated only on one side are tested, place the sample in such a manner onto the dish that the direction of the vapour migration be the same as when used for pharmaceutical packings. For example, tablets (hygroscopic substances) should be packed in cellophane coated on one side, the coated side must be without. The tests must be performed in dishes having a free surface of at least 10 cm².

Vapour penetration for lacquered cellophane films must be not more than 70 g and for heat-moulded cellophane films not more than 50 g.

4 Collapsible Tubes

Metal tubes for ointments

4.1 Tin- and Tin-Plated Lead Tubes

Cut the *empty tubes* longitudinally and prepare from the sheets troughs of 2×3×3 cm or 2×2×4 cm. The inner side of the tube should form the inner side of the trough.

Pour 10 ml of R-acetic acid into the trough and place it for one minute on a waterbath. Cool, transfer the liquid into a test tube, alkalize with R-ammonia solution and add a few drops of R-sodium-sulphide solution. The liquid must not acquire a brown colour.

If *filled collapsible tubes* are tested, cut off the sealed end, cut the tube longitudinally along the axis and unfold. Remove the contents first with a horn spoon

and clean with dry cotton wool. Be careful not to damage the protective layer. Wash the sheet with cotton wetted with petroleum ether and perform the test as described above.

4.2 Lacquered Aluminium Collapsible Tubes

Prepare the empty or filled collapsible tubes as described in *4.1* without forming a trough and perform the following tests.

(a) *Intactness of the lacquer layer.* Moisten the lacquered surface with a small cotton plug soaked in mercury(II) chloride solution, 6.5 per cent. White efflorescence should not occur after a few minutes.

(b) *Quality of the lacquer layer.* Fold two or three times, the lacquer layer must remain intact.

Substances and Preparations

I CHEMICAL SUBSTANCES

(A) TESTS AND ASSAYS OF GASES

1 Sampling

Perform sampling of gases for the test as follows. Mount a 1000 ml glass-stoppered, dry volumetric flask with a two-bore cork. Insert a glass tube reaching nearly the bottom of the flask through one bore. Into the other bore fit another glass tube bent rectangularly. Connect the former tube to the pressure reductor of a gas flask, the latter to a 100 ml soap-film flow-meter. Adjust the rate of the glass flow to 100 ml per 10 seconds. After allowing the gas to pass for 10 minutes (about 6 litres), remove the cork without interrupting the gas flow and stopper the flask quickly with a glass stopper. Neglect the volume from themark up to the stopper.

1.1 Soap-Film Flow-Meter

Determine the volume or volume rate of flowing gases by means of a soapfilm flow-meter (Fig. 21). It is a glass tube of about 15 mm inner diameter and about 60 cm length, the volume of the tube between the etched marks is 100 ml. (If necessary, several marks or graduations may be etched.) Close the lower narrower end of the tube by a rubber cap. Fill as much of a synthetic detergent solution into the apparatus as is necessary to attain that the level of the solution should reach nearly the orifice of the side tube end serving for the introduction of gases. Press the rubber for a few seconds; this results in raising the level of the solution, and soap-bubbles are formed as a result of gas-flow. The rate of raising of soapfilms in the tube depends on the rate of gas-flow. Measure the time needed for the film to rise from the lower mark to the upper mark. By means of this simple apparatus, measure the passage time of 100 ml of a gas saturated with water vapour at atmospheric pressure, at a known temperature (at room temperature). From the data of measurements, the volume of gas passing through in unit time, ml per minute or litre per hour, can be calculated.

Unless the rate of gas flow changes (this should be checked by frequent measurements), the volume of gas passing through the tube in a certain period can be calculated.

Bubbles forming at the top of the tube can be eliminated by means of a strip of filter paper. The flow-meter is connected either to the outflow tube of the gas tube or to the side arm by means of a three-way stop cock.

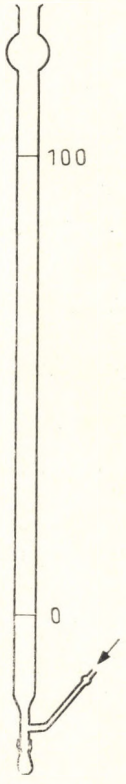


FIG. 21

2 Qualitative Tests

For qualitative tests, pour the reagent solutions into the gas-filled flask. Close the flask with a ground-glass stopper and shake it for a period specified in the special tests. Use 50-ml comparators (glass cylinders or colourless beakers) for reactions which result in a colour change or turbidity.

2.1 Acidity, Alkalinity

Add 0.15 ml of **I**-methyl red solution to 150 ml of freshly boiled and rapidly cooled distilled water. Fill equal volumes of the liquid into three comparators, and label them. Pipet into comparator *1* 0.2 ml, into comparator *2* 0.1 ml of 0.01 **N** hydrochloric acid. To the liquid in comparator *3* add no acid. Pour the liquid in comparator *2* into the volumetric flask filled with the gas; shake the liquid for 10 minutes and return it into the comparator. Any red colour of the liquid must not exceed that of the liquid in *1* and any yellow colour must not exceed that of the liquid in *3*.

2.2 Carbon Monoxide and Other Reducing Matter

Into a gas-filled volumetric flask fill 50 ml of a solution prepared by mixing 84 ml of water, 4 ml of R-silver nitrate solution, 10 ml of R-ammonia solution, and 2 ml of R-sodium hydroxide solution.

Shake the flask vigorously for 30 minutes: at room temperature, then for 5 minutes at about 50° (using a water-bath for warming of the reaction medium). The solution must be clear and colourless. Establish this by comparing the sample solution to 50 ml of the same solution which has not been shaken with the gas.

2.3 Carbon Dioxide

Into a gas-filled volumetric flask add 50 ml of clear R-barium hydroxide solution and shake the flask for 20 minutes. Any turbidity must not exceed that of a mixture prepared by adding 0.5 ml of a 0.2 per cent solution of sodium hydrogen carbonate, prepared with freshly boiled and cooled distilled water, to 50 ml of clear R-barium hydroxide solution.

2.4 Oxidizing Agents (Chlorine, Nitrogen Dioxide, etc.)

Into a gas-filled volumetric flask add 50 ml of a solution prepared by mixing 98 ml of water, 2 ml of **I**-starch solution, 4 drops of R-hydrochloric acid, and 0.5 g of potassium iodide. Shake the flask for 15 minutes. The original colour of the solution must not change. Compare this solution with 50 ml of a similar solution which has not been shaken with the gas.

2.5 Halogenides, Hydrogen Sulphide

Into a gas-filled volumetric flask pour 50 ml of a solution prepared by mixing 100 ml of water, 2 ml of R-silver nitrate and 10 drops of R-nitric acid. The colour of the solution must not change, and the solution must not show any opalescence. Establish this by comparing the sample solution with 50 ml of a blank solution, which has not been shaken with the gas.

3.1 Assay of Nitrous Oxide

The apparatus (Fig. 22) consists of a 100 ml *gas buret* (*B*) which is widened cylindrically. The upper and the lower parts of the cylindrical part join to tubes of about 8 mm in inner diameter. The upper tube is closed by a *three-way cock* (*C*). The tube is of a length of 10 to 12 cm, graduated into tenth-millilitres starting from the cock. The volume of the graduated tube should be not less than 5 ml. The lower tube of the buret is graduated also into tenth-millilitres. The cylindrical part should have such a volume that the graduation on the lower tube should start at 97 to 98 ml and terminate at 101—102 ml. The two stems of the *three-way cock* (*C*) are of 2 mm inner diameter and are bent rectangularly. One of the stems (*A*) is the air inlet, the other is sealed to an about 60-ml *reservoir* (*K*) (12 cm long and 2.5 cm in inner diameter) and this is connected to a 50 cm long *mirror manometer* made of a glass tube of 5 mm inner diameter and sealed at one end. The fourth stem of the four-way capillary tube can be closed by a *glass stopcock* (*D*). The lower stem of the gas buret is connected to an about 150 ml *levelling bottle* by means of an about 80 cm long, flexible, transparent plastic tube which is filled with mercury. The apparatus is mounted on a wooden stand so that the reservoir (*K*) could be immersed into a Dewar vessel of about 6 mm diameter and 25 cm of inner depth. After closing the cocks (*C*) and (*D*), the reservoir is immersed into a Dewar vessel filled with liquid air. Also the stem of the reservoir should be in liquid air. The equilibrium is attained in a few minutes and the pressure reduced to a few torrs. The gas buret (*B*) is to be filled completely with mercury. A pressure of about 50 torr is adjusted by means of the gas buret, levelling bottle and the stopcock of the gas buret (*C*). The pressure read from the manometer is recorded. The pressure must be constant for 1 to 2 minutes. (This is at the same time a check of the airtight sealing of the stopcocks as well.)

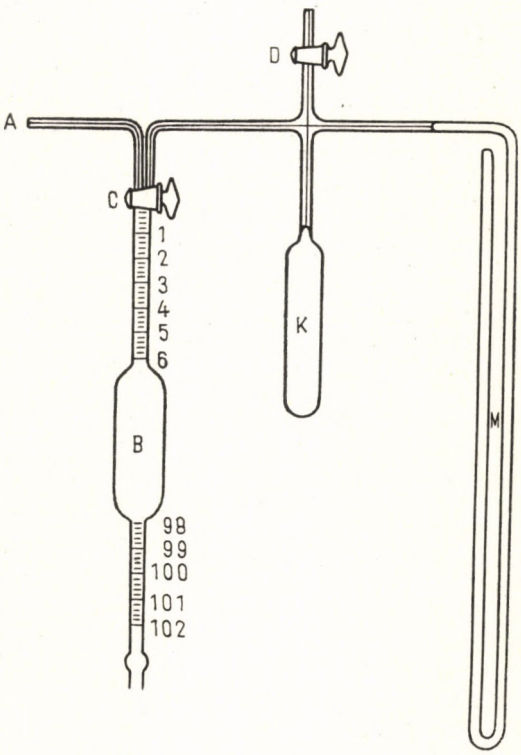


FIG. 22

Fill the gas buret with mercury up to the open end (*A*) of the outlet capillary tube. Connect the pressure reductor of a gas flask by a rubber hose and allow the gas to stream at slow rate. When the rubber hose is filled securely with gas under testing, connect it to outlet (*A*) of the apparatus and fill the gas buret with the gas to be tested. (The volume of the gas should slightly exceed 100 ml.) After closing stopcock (*C*) and

removing the rubber hose, adjust the volume of the gas to 100.0 ml with 0.1 ml accuracy. Then introduce the gas into the reservoir immersed into liquid air. Lift the mercury reservoir so that mercury should reach the hole of the stopcock of gas buret. Close stopcock (*C*) and attain equilibrium of pressure in a few minutes. After lowering the mercury reservoir, open stopcock (*C*) and transfer so much gas into the gas buret as necessary to obtain the same pressure which has been read earlier on the manometer. Equilibrate then pressure of the gas in the gas buret with the atmospheric pressure, and read the gas volume with 0.1 ml accuracy. If a 100 ml gas sample was used the volume of non-condensable gases (N_2, O_2) is obtained directly in volume per cent. Not more than ten measurements may be performed with the apparatus without evacuating the reservoir (*K*).

By removing the Dewar vessel and opening stopcock (*D*) dinitrogen oxide evaporates, and the apparatus is again ready for a new test.

Changes in the atmospheric pressure and in the temperature during the course of the experiments may be neglected.

3.2 Volumetric Absorption Assay of Gases (Oxygen, Carbon Dioxide)

Perform the determinations in an apparatus, illustrated on Fig. 23, which consists of a gas buret and a simple gas pipet. The gas buret is a tube, of about 6 to 7 ml inner diameter, widened in the middle, graduated into twentieth-millilitres both at the upper part (0 to 5 ml) and at the lower part (95 to 101 ml); it ends in a three-way, two-bore stopcock and in a ground stopper fixed with springs, respectively. Connect the tube end provided by a glass stopcock at the lower part of the gas buret to a levelling bottle by means of an about 80 cm long flexible, transparent plastic hose. Fill the levelling bottle with liquid (usually with distilled water saturated with the tested gas), and use it for the moving and measurement of the gas sample. The gas pipet is provided with stopcocks; the volume of its lower bulb is about 140 ml and the upper one is about 110 ml. Fill the gas pipet with an absorption liquid.

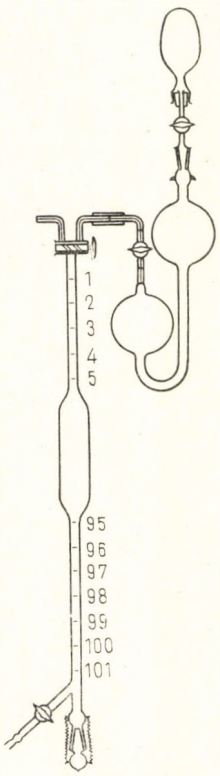


FIG. 23

Absorption liquid for the assay of carbon dioxide is 200 ml of a 38 w/v per cent potassium hydroxide solution.

Absorption liquid for the assay of oxygen

Dissolve 40 g of triacetyl-oxyhydroquinone in 200 ml of a 50 w/v per cent potassium hydroxide solution under nitrogen atmosphere. Transfer this solution into a gas pipet filled with nitrogen. Fill so much of the absorption liquid into the gas pipet as necessary to adjust the meniscus to the mark. Then close the stopcock on the gas inlet of the gas pipet and attach a tube, provided with a stopcock and glass bulb (see Fig. 23), to the ground joint of the feeding orifice. Fix the bulb by means of rubber rings. When the absorption liquid serves for measuring oxygen, fill the bulb with 300 ml of nitrogen.

Connect the tube ends of both the gas buret and the gas pipet with a 6 cm long vacuum rubber hose. Lubricate the stopcocks and ground joints with soft paraffin jelly.

Sampling of gas

Mount the reductor of a gas flask with a rubber hose, and allow gas to stream for 1 to 2 minutes. Open the two-bore cock of the gas buret towards the gas pipet. Equilibrate the pressure of the gas in the buret and the hose (N_2, residual gas*) with the atmospheric pressure by the aid of a levelling bottle, and then close the two-bore cock. Lift the levelling flask above the level of the sealing fluid of the gas buret and open the two-bore cock towards the sampling orifice. Fill both the gas buret and the sampling orifice bubblefree with the sealing liquid. Then connect the latter with the gas flask by a rubber hose (the gas stream should not be interrupted) and open the two-bore cock. Keep the level of sealing fluid in the levelling bottle constantly above that of the buret. (It is suitable to take 100 ml gas samples.) Close the two-bore cock. Measure the volume of gas sample after 10 minutes and check it with repeated measurements; record the readings.

Open the two-bore cock towards the gas pipet. Keep the stopcocks of the pipet closed. Adjust overpressure and reduced pressure, respectively, each for 5 minutes, by lifting and sinking the levelling bottle. The volume of the gas in the gas buret should not change, if there are no leaks in the apparatus. During the runs, the temperature of the laboratory should not change by more than 0.3°. This should be checked by means of a thermometer kept near the buret. If these conditions cannot be secured, the wide part of the gas buret should be thermally insulated. Introduce the gas sample into the gas pipet as follows: first maintain overpressure then open the cocks of the gas pipet. The level of the sealing fluid should reach the two-bore cock, and the latter must be closed.

Stir the absorption liquid in the gas pipet by gentle circular swinging (shaking must be omitted to avoid foam formation). After stirring for 5 to 6 minutes draw the gas into the buret and adjust the level of the absorption liquid to the mark. Close the gas inlet tube of the pipet and establish the volume of the sample. Repeat this procedure until two successive readings agree within the accuracy of the reading. Wait 3 minutes before each reading. Check the temperature as directed above. Atmospheric pressure, however, need not be controlled. Record the readings and calculate the volume per cent of the gas (O_2 or CO_2) absorbed.

3.3 Determination of Water Content of Gases

Pack two U-shaped drying tubes, of 10 mm inner diameter, with pumice coated by phosphor pentoxide or with anhydrous magnesium perchlorate. Connect the packed and sealed U-shaped tubes with each other, with the reductor of the gas flask and with a soap-film flow-meter by means of thick-walled glass tubes of about 1 to 2 mm inner diameter, and short rubber hoses. After opening the stopcocks of the U-shaped tubes, adjust the gas flow to 100 ml per 90 seconds by means of the metering valve.

After allowing the gas to flow for 10 to 15 minutes, close first the cock of the drying tube before the flow-meter, then the dispersing valve and finally the other cocks. Dismount the U-shaped tubes, wipe and place them into the box of a balance for 20 to 30 minutes. Prior to weighing, open one of the cocks for a short time. Weigh the U-shaped tubes with 0.1 mg accuracy.

* Residual gas must not contain the gas under determination, it should be removed prior to the assay either by introducing nitrogen into the apparatus or by performing an absorption procedure.

Then adjust againt the flow-rate to 100 ml per 90 seconds. Assemble the connected and opened U-shaped tubes. Allow the gas to pass through the system at a constant rate for a given period. Check the rate of gas flow frequently. Handle and measure the U-shaped tubes as directed above. Calculate the water content of the sample (mg water/normal litre of gas) from the weight increase of the tubes, from the volume of gas passed, from temperature (room temperature) and atmospheric pressure.

(B) ESSENTIAL OILS

(a) GENERAL NOTICES

Essential oils are liquids or solids with a pleasant odour obtained from balsams or odorous plant matter by distillation or solvent extraction, in some cases by pressing, or prepared synthetically. They are affected by air and light, and become viscous. Both the chemical composition and the physical constants alter in this case. Data given in this Pharmacopoeia refer to fresh volatile oils.

Essential oils must be stored separately in a cool place, in well-closed, and well-filled containers protected from light.

If an essential oil contains more water as permitted, it should be shaken with anhydrous sodium sulphate, the mixture allowed to stand for a while over the solid, then filtered through a dry filter paper. Unless otherwise directed in the monograph, neither synthetic essential oils nor residues obtained as a by-product on refining essential oils may be used.

(b) TESTS

1 Physical Indexes

Prior to the test, shake the essential oil with anhydrous sodium sulphate, if necessary, and filter it through a dry filter paper placed into a filter funnel and covered.

1.1 Specific Gravity

Measure the specific gravity of essential oils in a small glass-stoppered weighing bottle. The weighing bottle is about 6 mm of inner diameter, 20 mm height, the ground part is about 5 mm. Fill the tared, dry bottle completely with 20° water, and place it into a 20° water-bath for a few minutes, then stopper it bubble-free. Dry and weigh the bottle accurately. Perform the same procedure with the essential oil. Calculate the density (ϱ_{20}) from the specific gravity ($spgr_{20/20}$) by substracting the correction values given in the table below:

Specific gravity 20/20	Correction value to be substracted
0.700–0.900	0.001
0.900–1.200	0.002

By substracting 0.004 from the specific $gravity_{15/15}$, the approximate value for specific $gravity_{20/20}$ is obtained.

1.2 Solidifying Point

Introduce a thermometer into a thick-walled test-tube containing 2 to 3 ml of essential oil. The thermometer should be graduated into 0.1 degrees. Then immerse the test tube into an about 200 ml beaker. Fill the beaker with ice when anise oil, and with salted ice when caraway oil is tested. Thus the volatile oil is cooled 4 to 5 degrees under the expected solidifying-point. Lift the test tube out of the ice and stir the essential oil, by rubbing the inner walls of the test tube with the thermometer, until the rising mercury column starts to sink again. The observed highest temperature is the solidifying-point.

1.3 Optical Rotation

Determine the optical rotation of an essential oil at 20° in a 100 mm tube. Optical rotation of an essential oil is the angle of rotation measured directly. If, owing to the dark colour of the essential oil, a 100 ml sample cannot be used perform the measurement in a 50 mm or 20 mm tube, and multiply the readings by two or by five.

1.4 Refractive Index

Determine the refractive index of an essential oil by means of an Abbé refractometer at 20°.

2 Identification and Qualitative Tests

2.1 Macroscopical Tests

Into a colourless dry, test tube of 16 × 160 mm, fill 2 to 3 ml oil and test its consistency by shaking, while its colour and transparency by inspecting in incident light.

2.2 Odour

Onto an about 6 cm long and 1 cm wide filter paper strip transfer 1 to 2 drops of oil, then, on swinging the strip, test its odour from time to time. For comparison, test similarly essential oil of good quality.

2.3 Taste

Triturate in a porcelain mortar, 1 drop of the essential oil with 2 g of powdered sucrose. Shake the mixture with 500 ml of drink water, and taste the liquid. Be sure, prior to the test, that the sucrose and the drink water is odourless, and the apparatus is used scrupulously cleaned. For comparison, test similarly essential oil of known good quality.

2.4 Alcohol

To 10 to 20 drops of essential oil in a test tube add 10 ml of water, or add a few drops of essential oil to water. At the interface of the two liquids, no milky turbidity must form. For the detection of small amounts of alcohol, transfer 1 ml of essential oil into a test tube. Stopper the test tube with a small cotton plug; previously place in the middle of the latter 1 to 2 grains of magenta.

Immerse the test tube into boiling water for a few minutes. The cotton plug must not turn red.

For the determination of larger amounts of ethanol, shake 5.00 ml of essential oil with 5 ml of concentrated R-calcium chloride solution in a test tube graduated into tenth-millilitres. The volume of the essential oil must not decrease. Read the volume decrease with 0.1 ml accuracy, and multiply it by twenty to obtain the alcohol content of the essential oil expressed in volume per cent (per cent v/v).

2.5 Phthalic Esters

To 3 drops of the sample essential oil add 1 ml of 0.5 **N** propanolic potassium hydroxide solution, and heat to boiling. A clear solution must be produced, and no precipitate must separate even on cooling.

2.6 Heavy Metals

To 5 ml of essential oil add 10 ml of water acidified with 2 drops of R-hydrochloric acid, and shake the mixture vigorously. Filter the separated aqueous layer through a moistened filter paper. To the filtrate add 1 ml of R-ammonia solution and 5 drops of R-sodium sulphide solution. The liquid must not be coloured.

3 Quantitative Tests and Assays

3.1 Residue on Drying

Perform the test with about 2 g of essential oil, weighed with mg accuracy, according to *2.1*, Vol. I, p. 102. Evaporate the essential oil to the consistency of honey (2 to 3 hours), and dry the residue to constant weight at 105°. Express the residue on drying in weight per cent.

3.2 Acid Value

Dissolve in propanol a quantity of essential oil, as directed in the monograph, weighed to mg accuracy and test the solution according to **1**, Vol. I, p. 127. Use 0.1 **N** propanolic potassium hydroxide solution for titration.

Each ml of 0.1 **N** propanolic potassium hydroxide solution is equivalent to 5.610 mg (lg .74899) of KOH. Calculate the acid value according to **1**, Vol. I, p. 127.

3.3 Ester Value

To the solution neutralized at the determination of acid value add 10.00 ml of 0.5 **N** propanolic potassium hydroxide solution and test the liquid according to **2**, Vol. I, p. 128. Cool the solution after saponification, and titrate it after adding 20 ml of a saturated sodium chloride solution. Saponification-time is 15 minutes.

Each ml of 0.5 **N** propanolic potassium hydroxide solution is equivalent to 28.05 mg (lg .44796) of KOH.

Calculate the ester value according to **3**, Vol. I, p. 128.

If the ester value of an essential oil is to be expressed in weight per cent, use the following equation for the calculation:

$$B = \frac{(a—b) \times T \times e \times 0.1}{m} \text{ per cent}$$

where a = millilitres of 0.5 **N** hydrochloric acid consumed in the blank run
b = millilitres of 0.5 **N** hydrochloric acid consumed in the test
T = titer of 0.5 **N** hydrochloric acid
e = milligrams of the ester equivalent to 1 ml of 0.5 **N** standard solu tion
m = weight of the sample in grams
B = ester content in per cent by weight.

The following relations exist between the ester value (A) and the ester content expressed in per cent by weight (B):

$$B = \frac{A \times e \times 0.1}{28.05} \; ; \; A = \frac{B \times 28.05}{e \times 0.1}$$

If an essential oil contains aldehydes or phenols, the latter should be removed prior to the determination of the ester value. (Remove aldehydes by shaking the sample with a 30 per cent sodium hydrogen sulphite solution and phenols by shaking with a 3 to 5 per cent sodium hydroxide solution [*3.5*, Vol. I, p. 192 and *3.7*, p. 193].) Dry the separated essential oil over anhydrous sodium sulphate and filter through a dry filter paper.

3.4 Determination of Alcohols

Transfer 5 ml of essential oil into a 100 ml acetylation flask (Fig. 24) and add 5 ml of R-acetic anhydride, 1 g of anhydrous R-sodium acetate and some pumice. Attach the condenser to the flask and heat the mixture over a small flame (or on a sand-bath) and keep it boiling for 1 hour. Then cool the flask, add 20 ml of water, shake and replacing the condenser tube heat it on a water-bath under shaking for 15 minutes. Transfer the cooled mixture into a 100 ml separatory funnel and remove the aqueous phase. To the acylized essential oil in the funnel add 20 ml of saturated sodium chloride solution and shake. Repeat the procedure, using 20 ml of saturated sodium chloride solution containing 1 per cent of sodium hydrogen carbonate and then saturated sodium chloride solution, until the washing liquid shows a neutral reaction when tested with litmus paper. Finally, shake the acylated essential oil with 10 ml of water, transfer it into a small, dry flask, shake with 1 g of anhydrous sodium sulphate and filter through a dry cotton tuft. Neutralize a certain quantity of the filtrate, as directed in the paragraph *3.2* and saponify it according to *3.3*.

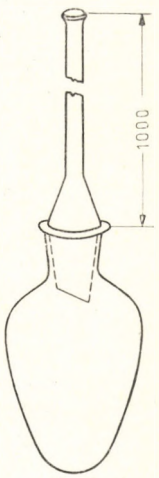


FIG. 24

Calculate the contents of free alcohol, ester alcohol and total alcohol of essential oils, expressed in per cent by weight as follows:

Calculate first the millilitres of 0.5 **N** hydrochloric acid referred to 1 g of original (c) and 1 g of acylated volatile oil (d), respectively, by the formula:

$$c = \frac{(a - b) \times T}{m}, \text{ and } d = \frac{(a - b') \times T}{m'}$$

where a = millilitres of 0.5 **N** hydrochloric acid consumed in the blank test
b = millilitres of 0.5 **N** hydrochloric acid consumed in the titration of the original oil

h' = millilitres of 0.5 N hydrochloric acid consumed in the titration of the acetylated oil

T = titer of 0.5 **N** hydrochloric acid

m = weight of the original oil in grams

m' = weight of the acylated oil in grams

c = millilitres of 0.5 **N** propanolic potassium hydroxide solution or equivalent 0.5 **N** hydrochloric acid, consumed for the saponification of esters present in 1 g of the original oil

d = millilitres of 0.5 **N** propanolic potassium hydroxide solution or equivalent 0.5 **N** hydrochloric acid consumed for the saponification of esters present in 1 g of acylated oil.

Substitute the calculated values of c and d into the following equations:

$$\text{Free alcohol} = \frac{M \times (d - c)}{20 - 0.42 \times d} \text{ per cent by weight}$$

$$\text{Ester alcohol} = \frac{M \times c}{20} \text{ per cent by weight}$$

$$\text{Total alcohol} = \frac{M \times d \times (1 - 0.021 \times c)}{20 - 0.42 \times 4} \text{ per cent by weight}$$

where M = molecular weight

0.42 = one-hundredth of the weight difference between 1 g mole of free alcohol and 1 g mole of acetic ester (C_2H_2O = 42.04).

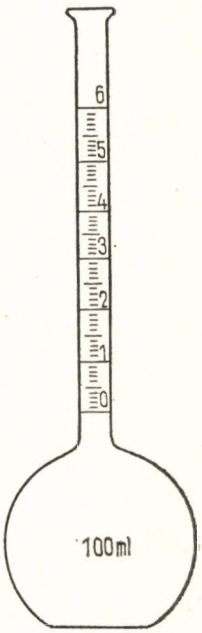


FIG. 25

3.5 Determination of Aldehydes

Transfer a certain quantity (10.00 or 5.00 ml, as directed in the monograph) of essential oil of known temperature into a "Cassia" flask (see Fig. 25) and add an identical quantity of a 30 per cent sodium hydrogen sulphite solution. Shake the flask and place it into a hot water-bath until the precipitate is dissolved. Then add 5 ml of a 30 per cent sodium hydrogen sulphite solution to the mixture and repeat the procedure until no further precipitate is formed. Dilute the liquid with a 30 per cent sodium hydrogen sulphite solution to the zero mark, and heat it on a water-bath until two layers are formed. Collect oil droplets adhering to the walls of the flask by swinging and knocking the flask. Cool the flask to room temperature and dilute the liquid with a 30 per cent sodium hydrogen sulphite solution to facilitate the reading of the volume of the separated essential oil. Subtract the volume of the separated essential oil from the volume of essential oil weighed. Multiply the difference by ten when 10.00 ml or by twenty when 5.00 ml of essential oil is tested, to obtain the aldehyde content (expressed in volume per cent) of essential oil. By measuring the density of both the aldehyde and the essential oil, the aldehyde content of the essential oil in per cent by weight can be calculated.

3.6 Determination of Ketones (Carvone, Pulegone)

Test essential oils containing carvone and pulegone by adding 70 to 75 ml of a 40 per cent sodium sulphite ($Na_2SO_3 \cdot 7H_2O$) solution to 5.00 ml of an essential oil in a special "Cassia" flask (Fig. 25). Shake the mixture, add a few drops of I-phenolphthalein solution and heat the liquid on the water-bath. If the liquid turns pink, add R-acetic acid until a faint pink colour is obtained. If the liquid is turning pink on the addition of 2 to 3 ml of a 40 per cent sodium sulphite solution, add again some R-acetic acid. Repeat this procedure until the heated liquid does not change its colour on the addition of a further portion of sodium sulphite solution. Dilute the liquid to volume with water and proceed according to *3.5*. Subtract the volume of the separated essential oil from the volume of essential oil weighed (5.00 ml). Multiply the difference by twenty to obtain the ketone content of the essential oil in per cent by volume (v/v).

Camphor, fenchone and menthone can *not* be determined by the described methods.

3.7 Determination of Phenols (Eugenol, Thymol, Carvacrol)

Transfer into a special "Cassia" flask (Fig. 25) 5.00 ml of essential oil and add about 80 ml of sodium hydroxide solution (3 or 5 per cent, as directed in the monograph). Shake the mixture vigorously. Then dilute the mixture with a sodium hydroxide solution of the same concentration so as to facilitate reading of the volume of the separated essential oil. In some cases the separation of the essential oil requires several hours. Collect oil droplets adhering to the walls of the flask by swinging and knocking the flask.

Subtract the volume of the separated essential oil from the volume of the essential oil weighed (5.00 ml). Multiply the difference by twenty to obtain the phenol content of the essential oil in per cent by volume.

3.8 Determination of Cineol (Eucalyptol)

Test 3.00 ml of eucalyptus oil according to *3.7*. Instead of sodium hydroxide solution, however, use a 50 per cent of aqueous resorcinol solution and shake the mixture for 15 minutes prior to making up to volume with resorcinol solution.

Subtract the volume of the separated essential oil from the volume of the essential oil weighed (3.00 ml). Multiply the difference by 33.3 to obtain the cineol (eucalyptol) content of the essential oil in per cent by volume (v/v).

(C) FATS, WAXES AND MATERIALS OF SIMILAR CONSISTENCY

(a) GENERAL NOTICES

Fixed oils should be preserved in well-closed, well-filled containers protected from light, in a cool place. Special storage instructions of fixed oils, fats and waxes are described in the monographs.

If the oils, melted fats, waxes and materials of similar consistency contain sediments, they should be filtered through a dry filter paper. If the liquid fats, waxes and materials of similar consistency are turbid owing to moisture, they should be shaken with anhydrous sodium sulphate (5 to 100 g of anhydrous sodium sulphate should be used per one kilogram), allowed to sediment and filter through a dry filter paper.

1 Physical indexes

1.1 Specific Gravity

1.11 Materials of Oily Consistency

Measure their specific gravity according to *6.1* Vol. I, p. 74.

1.12 Materials of Ointment Consistency

For the measurement of specific gravity, use a 20 ml cylindrical medicine glass or a thick-rimmed conical flask, the rim of the latter should be smoothly ground. Weigh the dry bottle together with a fitting glass disc, with mg accuracy, then fill it with 20° water so that the water level should be above the rim. Keep the bottle in a 20.0° water-bath for 15 minutes and stopper it by means of the glass disc, bubble-free. Weigh again the bottle. Then fill the bottle, heated above the melting point of the sample, bubble-free with the material (filter if necessary), so that the level of the sample should be above the rim. Immerse the bottle into a 20.0° water-bath and add dropwise some more melted material to fill the bottle completely.

After 30 minutes (in the case of lard, after 1 hour), remove the excess of the material by a clean knife, and weigh the bottle, together with the glass disc, with mg accuracy.

1.13 Materials of Waxy Consistency

Melt the sample, filter, if necessary, and allow to cool at room temperature. The solid should not contain air bubbles. Cut out a cube of about 1 cm edge length, and smooth its faces with a hot knife. Prepare a 20° mixture of ethanol and water in which the cube floats. Finally, measure the specific gravity of the mixture according to *6.1*, Vol. I, p. 74. The obtained value is the specific gravity of the sample.

2 Qualitative Tests

2.1 Paper Chromatography of Fats

Fats are saponified and the produced fatty acids are subjected to chromatography. The fatty acid composition is characteristic to most of the fats. Perform the identification of fats by the detection of the characteristic fatty acids and further by the measurement of their spot area. Fat impurities can be detected similarly.

2.11 Preparation of the Test and Reference Solutions

In 150 to 250 ml flasks weigh 1 g portions each of the test and the reference fat. Into each flask add 25 ml of alcohol 96 per cent, and 1 g of potassium hydroxide. Attach a reflux condenser or a 1 m long glass tube to the flask and boil the liquid for 1 hour. Then transfer the mixture into 100 to 150 ml separatory funnels and rinse with water to make the total volumes to about 50 ml. After cooling add 15 ml of petroleum ether and shake vigorously for 1 minute. Allow the petrol-

eum ether layer to segregate. Separate the lower phase (soap-solution), add fresh 15 ml petroleum ether portions and repeat extraction twice. Dilute the lower (soap-solution) layer with 20 ml of water and 20 ml of 10 per cent hydrochloric acid, checking the acid reaction of the solution with I-methyl orange indicator. Heat the liquid on water-bath until fatty acids collect on the surface to form a distinct layer. Separate the fatty acids from the aqueous solution in a separatory funnel. To the residual fatty acids add 10 ml of petroleum ether. (In the case of Castor oil, in addition to the 10 ml petroleum ether add also 1 ml benzene.) Wash this solution with two successive portions of 20 ml of lukewarm water and filter the petroleum etheral solution into a vessel tared with 1 mg accuracy, through anhydrous sodium sulphate spread over a cotton plug. Expel petroleum ether on the water-bath, cool the residue in a desiccator, and then weigh the residue with mg accuracy. Dissolve the residual fatty acids in enough benzene to obtain a 1 per cent benzene solution of the fatty acid for the chromatographic test and preserve the substance in a dark and cool place in a well-closed container.

2.12 Impregnation of the Chromatographic Paper

Saturate Schleicher-Schüll No. 2043/b chromatographic paper with a mixture of liquid paraffin and benzene (1 + 19 volumes), and suspend the paper in open air to allow benzene to evaporate (about 10 minutes).

2.13 Procedure

Place 30 μl (= 300 μg fatty acid) portions of the test and the reference solutions (*2.11*) on the impregnated chromatographic paper (*2.12*). Perform ascending chromatography, according to *5.13* of the paragraph *Chromatographic Test*, in a chamber saturated for 1 hour with the vapours of a solvent mixture of concentrated acetic acid-acetonitrile-water (75 + 20 + 5 volumes). The mobile phase should ascend to 25 to 30 cm height (8 to 16 hours).

Dry the chromatographic paper at 100°, then soak it for 30 minutes in a 0.5 per cent copper(II) acetate solution. Remove the excess of copper(II) acetate by washing for an hour, and immerse the paper into a 0.1 per cent solution of rubianic acid in dilute alcohol.

Fatty acids appear as dark-green spots on the chromatogram.

2.14 Evaluation of the Chromatogram

The R_f values and the ratio of spot areas on the chromatogram of the tested sample and of the reference fat should be identical.

2.2 Rancidity

2.21 Identification of Epihydrine Aldehyde

In a 16 × 160 mm test tube mix 2 ml of oil with 2 ml of concentrated hydrochloric acid 36 per cent. Stopper the test tube with a cotton plug soaked in a R-etheral 0.1 per cent phloroglucinol solution and warmed by the warmth of the hand for 5 minutes. The cotton plug must remain colourless.

2.22 Determination of Peroxide Value

Peroxide value is the quantity of active oxygen in milligram equivalents present in 1000 g of the sample. Numerically it is equivalent to the number of milli-

litres of 0.001 **N** sodium thiosulphate solution consumed in the titration of iodine liberated when 1 g sample is tested.

Dissolve in a 200 ml conical flask a 4.50 to 5.5 g of sample weighed with 10 mg accuracy, in a mixture of 18.0 ml of glacial acetic acid and 12.0 ml of chloroform. Add a freshly prepared solution of 1 g of potassium iodide in 1 ml of water to the liquid and shake vigorously. After 1 minute add 30 ml of water and titrate the liberated iodine with 0.01 **N** sodium thiosulphate solution, using 2 ml of **I**-starch solution as indicator. Perform also a blank test.

Calculate the peroxide value by the formula:

$$P = \frac{10 \times T \ (a—b)}{m}$$

where T = titer of the 0.01 **N** sodium thiosulphate solution
a = millilitres of 0.01 **N** sodium thiosulphate solution consumed in the assay
b = millilitres of 0.01 **N** sodium thiosulphate solution consumed in the blank run
m = weight of the sample in grams.

(D) RADIOACTIVE ISOTOPES

(a) GENERAL NOTICES

The production, transport and application of radioactive isotopes and preparations are subject to special legal decrees and instructions. The processing, determination, measurement, quantitative and qualitative control of radioactive preparations require qualified specialists, and a suitable degree of instrumentation and laboratory conditions.

1 On Radioactive Isotopes in General

The isotopes of a certain chemical element possess an equal number of protons and a different number of neutrons. Nuclei of proton-neutron ratios unfavourable from the point of view of nuclear stability are radioactive. The symbol of the elements is used for denoting the isotopes; the atomic number is indicated in the left lower index and the mass number in the left upper index; e.g. ${}^{30}_{15}\mathrm{P}$, ${}^{31}_{15}\mathrm{P}$, ${}^{32}_{15}\mathrm{P}$ are the three isotopes of phosphorus (the first and the third ones are radioactive). Radio isotopes are stabilized by particle or photon emissions in a single step or in several steps. Radio isotopes decay with a *characteristic half-life*, emitting radiations of *characteristic type* and *energy*.

Half-life (T) is the time required for the decay of one-half of the nuclide. The half-life of a nuclide is a material constant of the isotope element concerned, which does not depend on common physical and chemical effects of the laboratory environment.

The most important *types of radiation* are: α-radiation (α-particles are helium atoms with double positive charges, i.e. helium nuclei); β-radiation (negative or

positive electrons); γ-radiation (electromagnetic radiation similar to X-rays but higher in energy). Generally, γ-radiation occurs not independently; it may be regarded as a phenomenon accompanying α- and β-decays or other nuclear transformation processes.

The energy of radioactive radiations is expressed in million electron volts (MeV):

$$1\ \text{MeV} = 1.6 \times 10^{-6}\ \text{erg}$$

The absolute activity of a radioactive preparation is characterized by the number of atoms decaying in unit of time. The *unit of activity* is the curie (*Ci*); one curie is 3.7×10^{10} disintegrations per second (it is independent of the type of radiation)

$$1\ Ci = 10^3 \text{m}Ci = 10^6\ \mu Ci = 3.7 \times 10^{10}\ \frac{\text{disintegrations}}{\text{seconds}}\ .$$

Specific activity of a radioactive preparation is its activity related to unit weight:

$$\frac{Ci}{\text{g}}\ ,\ \frac{\text{m}Ci}{\text{g}}\ ,\ \frac{\mu Ci}{\text{g}}\ ,\ \text{etc.}$$

The radiochemical concentration of a solution is its activity related to unit volume: $\frac{Ci}{\text{ml}}$, $\frac{\text{m}Ci}{\text{ml}}$, etc.

Radiochemical impurities are those compounds which are labelled with the same isotope as the basic substance (e.g. $^{51}CrCl_3$ in $Na_2{}^{51}CrO_4$). The radiochemical impurity is expressed in percentage of the total activity of the preparation.

Chemical impurities are those non-radioactive elements or compounds which are hazardous to the organism or influence the utilization of the preparation.

Radioisotopic impurities are other radioisotopes of similar or of different chemical elements present in the preparation (e.g. ^{199}Au impurity in a ^{198}Au preparation). Radio isotopic impurity is expressed in percentage of the basic isotope.

The *dosis constant* ($I\ \gamma$) of a γ-emitting isotope is the dose produced by an isotope of an activity of 1 m*Ci* in air at 1 cm distance within 1 hour. Its unit is $\text{r} \times \text{cm}^2/\text{m}Ci$ hour.

2 Fundamental Decay Law

The decay of radioactive nuclides follows the statistical laws. The decay rate $\left(\frac{dN}{dt}\right)$ is proportional to the number of atoms present. The *proportionality constant,* the so called decay constant (λ) is characteristic of the particular radioactive species. The decay of a radioactive substance follows an exponential law

$$N = N_0 e^{-\lambda t}\ ,$$

where N = the number of unchanged atoms at time t and,
N_0 = the number of unchanged atoms existing when $t = 0$.

A similar equation describes the decrease of activity of a radioactive substance:

$$A = A_0 e^{-\lambda t},$$

where A = the activity at time t and A_0 is the activity when $t = 0$.

The following relation exists between the decay constant (λ) and the half-life (T):

$$\lambda = \frac{0.693}{T}$$

3 Measurement of Radioactivity

The detection and measurement of radioactive emissions of alpha, beta, and gamma rays are based on their interaction (ionization, excitation, photochemical and thermal effect, etc.) with the medium through which they pass. The most often used devices are the *ionization chambers*, the *Geiger-Müller counting tubes*, and the *scintillometers*, which are coupled to electronic instruments. Geiger-Müller tubes are used mainly for the detection of beta radiation; scintillometers, however, using different scintillators, are convenient to detect every type of radiation.

The radioactive samples are placed, in the majority of the cases, outside the measuring device, which latter, depending on the geometrical arrangement, records only a fraction of the total radiation emitted. Signals appearing in the measuring devices are called impulses or counts.

Due to the statistical nature of radioactive decay, the counting rate in any experiment with radio-isotopes shows random fluctuations about the mean value.

The standard deviation or standard error is normally used as a measure of the statistical error in a number of counts. It is equal to the square root of the mean number of counts which whould be obtained from any observations made over the same time interval. In radio isotope technique the mean number (N) of counts is not normally known but the standard deviation is approximately equal to the square root of the number of counts recorded, provided that the latter is not small (thus the result of counting may be written as: $N \pm \sqrt{N}$).

4 Background Activity and Resolving Time

Radiation detectors will record some counts even when no radioactive material is near to them. Stray radioactive atoms are always present in the air, and cosmic rays are entering the atmosphere from space; these give rise to the *natural* or *background effect* and counting rate, respectively. This *background counting rate* should be always subtracted from the measured counting rate. When scintillometers are used, the black current of the crystal and the photomultiplier is also added to the background activity. The background counting rate should always be subtracted from the total counts.

If two ionising particles pass consecutively through a Geiger–Müller counter or any other form of detector within a certain time interval, they will be counted as one particle. This time limit is known as the *resolving time* (or coincidence loss)

of the system. A typical Geiger–Müller counter has a resolving time of the order of 100 μsec, this being determined mainly by the speed of travel of the positive ions to the cathode. A scintillation counter has a much smaller resolving time and can therefore count at a much higher speed.

The activity of the samples should be chosen in a way, that both samples should show counts of about 10 000 each per minute in the counting tube.

A number of counts which are not recorded because the resolving time is not zero, may be calculated in the following way.

If the resolving time is τ sec and there are N counts/min., the total time per minute during which the system is inactive is $N\tau$. Thus the time for which the equipment is sensitive during any one minute period of the count is $(1 - N\tau)$ min. The corrected counting rate N_0 can be expressed by the formula:

$$N_0 = \frac{N}{1 - N\tau}$$

The correction is negligible at low counting rates.

(b) TESTS AND ASSAYS

1 Identification and Qualitative Tests

The following measurements are performed singly or in combination, for the identification and purity test of a radioactive preparation, further for the detection or quantitative and qualitative determination of radioactive impurities.

1.1 Determination of Half-Life

Geiger–Müller tubes or scintillation counters are used for the determination of half-life.

The specific activity of the preparation is measured in successive measurements. The preparation is diluted so as to obtain at the beginning of the measurement 5000 to 10 000 counts per minute when a Geiger–Müller tube, or 50 000 to 100 000 counts per minute when a scintillation counter is used. Perform the measurements in constant geometry through at least five half-lives. Perform within each half-life not less than 5 measurements in equal intervals. Construct from the data of the measurements a plot to determine the half-life. The obtained value for the half-life should not deviate from the values in literature by more than ± 5 per cent when isotopes of half-life of less than 2 weeks or by more than ± 8 per cent when isotopes of longer half-life are assayed.

1.2 Determination of Half-Thickness for β-radiation

Beta radiation consists of particles of energies varying from zero up to a certain maximum value which is characteristic of the nuclide concerned. An approximate estimation of a maximum beta ray energy may be made by the "half-thickness" method. For this aim the thickness of the absorber required to reduce the counting rate to one-half of its value when no absorber was present is measured. Aluminium absorbers are usually used for beta absorption because they have little effect on gamma radiation. The maximum energy of beta radiation may be estimated from a graph of energy plotted against half-thickness. Half-thickness

is expressed in mg/cm^2 or g/cm^2. The half-thickness and the maximum energy values, respectively, should not deviate from the value in literature by more than ± 5 per cent.

1.3 Combined Measurement of Half-Life and Absorption

The absorption measurements, described in *1.2*, performed in different times render possible to detect isotope contaminants of long half-lives in radioactive preparations of nuclids of short half-lives.

1.4 Energy Spectra of Gamma Radiation

The energy of gamma radiation is measured by means of scintillation counters equipped with a NaJ (TI) crystal of at least 10 to 12 per cent resolution. The gamma spectrum of a particular isotope is compared to the following "standard" energy peaks: 0.320 MeV (^{51}Cr), 0.662 MeV (^{137}Cs—^{137m}Ba), 1.17 MeV and 1.33 MeV (both ^{60}Co).

1.5 Non-Radioactive Quality Tests

These tests are specified in the single monographs of this Pharmacopoeia.

2 Quantitative Tests

2.1 Activity Measurement

2.11 Activity Measurement by Ionization Chamber

Determine the activity of radioactive preparations emitting gamma radiation by an ionization chamber and compare the activity with Ra, being in equilibrium with its decay products, or with ^{60}Co or ^{137}Cs—^{137m}Ba standards. Calculate the activity of the preparation by the formula

$$A = A_0 \times \frac{I_{\gamma 0}}{I_\gamma} \times \frac{N}{N_0},$$

where A_0 and A are the activities of the standard and the sample, respectively; $I_{\gamma 0}$ and I_γ are the γ dose constants of the standard and the sample, respectively; N_0 and N are the background corrected ionization chamber effect induced by the standard and the sample, respectively.

2.12 Activity Measurement by 4 π-proportional Counter

A 4 π-proportional counter can be used directly for the absolute measurement of activity. Transfer a radioactive sample onto a thin film expanded by means of a holding ring and place into a counter. Prepare the film very thin so that its radiation absorbing capacity be negligible. The obtained counts give directly the number of decay.

2.13 Activity Measurement by the Coincidence Technique

The absolute activity of isotopes with mixed types of radiations (e.g. $\beta + \gamma$) may be measured by the coincidence technique. Place the sample between a β and a γ counter. Denote counts per minutes by A and B, respectively. Feed A

and B impulses into a coincidence unit and record time-coincident pulses in the counters. Denote the number of coincidence signals per minute by C. The actual disintegrations per minute can be calculated by the formula

$$D = \frac{A \times B}{B}$$

2.2 Non-Radioactive Quantitative Tests

These tests are specified in the monographs of this Pharmacopoeia.

(c) NOTES

On the *label* of the glass vessel containing a radioactive preparation the following data must be indicated: the international disc-shaped symbol for radioactive isotopes; the chemical symbol and mass number of the particular isotope; the chemical compound; the activity fixed for the time indicated in the *invoice of delivery*, and the serial number.

An invoice of delivery should be attached to every preparation and should contain the following informations:

1. The manufacturer's name and address
2. Symbol of the isotope and denomination of the preparation
3. Total and specific activity of the preparation at a given time (year, month, day, hour)
4. Total quantity of the preparation (ml, g, number of pieces)
5. Preserving agent (if added)
6. Serial batch number
7. Mode of package
8. Date of expiration
9. Warning to radiation hazards
10. Notes.

II VEGETABLE AND ANIMAL DRUGS

(A) GENERAL NOTICES

A *vegetable or animal drug* is that part of a medical plant or of an animal which is declared as such in the Pharmacopoeia. The vegetable drug is of characteristic colour and odour.

No vegetable drugs must be used other than those described, nor drugs that are visibly deteriorated, mouldy, affected by fungal diseases, sprayed with protective agents, nibbled by insects. As a rule, in the drug no foreign matter must be present, no impurity or substance other than the vegetable part described (dead or living insect, larva, feather, sand, grit etc.).

If any extraneous matter is noticed in the article, it should be removed and the article submitted to a thorough examination.

Subterranean plant parts (roots and rhizomes), barks, shoot drugs (herbs) and leaves are usually marketed in comminuted or powdered state.

Comminuted drugs, seeds, fruits and flowers should be freed of dust by sifting through the sieve No. V after being procured. Stored vegetable drugs should again be sifted out at least every twelve months.

If the drug is to be dispensed in cut state as tea or the ingredient of a tea-mixture, do not add the parts finer than those prescribed; such finer parts should be sifted out. If the drugs are meant to be extracted in the comminuted state by means of a solvent, the finer parts should not be removed.

When preparing powders, the drug must be powdered completely, without anything being thrown away. Powder should be prepared only of vegetable drugs complying completely with the prescriptions of the Pharmacopoeia.

(B) TESTS AND ASSAYS

1 Macroscopical Tests

Examine the vegetable drug first macroscopically. In most cases, this informatory examination permits to verify the identity and adequacy of the drug, in fact, often its contamination or falsification too. The macroscopic test means the ascertainment of external morphological properties characteristic of the drug, visible to the naked eye or through a hand magnifier. It helps to determine the quality of fractures and section surfaces, the consistency, the colour, the scent, often the taste etc. of the drug.

These tests include those simple chemical reactions permitting to examine the changes caused by reagents dropped on the drug, on its fracture or section surface or into an extract made of the drug.

These tests all together amount to the *macroscopic identification* and the *qualitative test* of the drug.

The macroscopical *quantitative tests* are aimed at determining the quantity of any *foreign matter*, i.e. any matter other than the described drug or the plant it is supplied by, that can be mistaken for the drug, derives from another plant, that is of toxical (spray) or contaminating effect (dead or living insects, larvae, feather, grit etc.), on one hand, and that of other parts of the plant, on the other hand. *Other parts of the plant* are those parts of the very plant supplying the drug which, however, do not make up the drug as described in the Pharmacopoeia (e.g. root in a leaf drug).

In case of a comminuted drug, this test includes also the quantitative determination of the powder sifted out of the drug.

Execution of the test

Weigh with an accuracy of 1 g a 0.5–1 kg portion of a good average sample of the drug marketed in large pieces. Examine carefully according to the viewpoints described above. Separate any heterogeneous or extraneous parts, weigh to the nearest 1 g and indicate in percentage of the examined drug.

Weigh with an accuracy of 0.1 g a 50 g portion a good average sample of the drug smaller than 10 mm and larger than 0.75 mm. Sift out any fines through the sieve No. V; weigh to the nearest 0.01 g and put it aside for an eventual microscopical or other test (microscopical test, determination of ash and test for plant protecting spray). Indicate the quantity of the sifted powder in percentage of the examined drug.

Spread the drug that remained on the sieve in a thin layer on a glossy paper of suitable size. Separate with the naked eye or eventually with the help of a hand magnifier any pieces deriving from the proper plant, but from other parts than those described, as well as pieces deriving from a foreign plant and impurities (earth lumps, dead or living insects, etc.). Weigh each kind of contaminant separately to the nearest 0.01 g and indicate their weight in percentage of the examined sample. Pieces deriving from foreign plants should be submitted to a more thorough (microscopical) test.

The quantity of powder that can be sifted out through the sieve No. IV, or if needed, No. V from the drug smaller than 10 mm and larger than 0.75 mm must not exceed 5 per cent in flower, leaf and shoot drug (herba), and 2 per cent in bark, root and rhizome drug and 1 per cent in fruit and seed drug respectively.

The ash contents of the powder sifted out of the drug must not exceed the double of the value indicated in the single drug monographs.

The comminuted drug must contain not more than 10 per cent of pieces larger in size than prescribed by the monographs and not more than 3 per cent of fines which can be sifted out through sieve No. V.

If a drug with no alkaloid content is examined, the test should be also extended to detect whether the extraneous parts do not contain any alkaloids or other toxic substances. In drugs bearing no alkaloids the contamination by alkaloid-containing drugs should be checked as follows: boil 1 g of the comminuted and powdered drug in a test tube with 10 ml of water and 3 drops of R-hydrochloric acid. The cooled and filtered fluid must not change if a few drops of R-Mayer solution are added.

The drug procured in powdered condition should be controlled with microscopical test — in case of a drug powder with no alkaloid contents — also with the excluding reaction described above.

2 Microscopical Tests

The microscopical test is a conclusive supplement of the macroscopical test. A microscopical preparation (slice) made of powdered, cut or entire drug should be used for this purpose. The microscopical preparation may be a powdered preparation, a maceration, a clarified preparation, a stained preparation, a microsublimate, a section etc. The section may be a surface section, a cross-section, a radial and tangential longitudinal section.

2.1 Preparation of Vegetable Drugs for Microscopy

2.11 Flower, Leaf and Shoot Drug (herba)

Select possibly intact flowers or leaf and stem parts for testing, and macerate them in water. Pick out characteristic pieces of the macerated plant parts and place them on a glass slide. Decompose the sample with a needle and examine through a 10-fold magnifier.

Examine similarly also stem, flower and leaf fractions of shoot drugs. Cut out the leaf apex and the parts along the midrib, as well as the midrib itself of the leaf or the leaf fractions, and prepare cross- or surface sections.

2.12 Bark, Root, Rhizome

Powder pieces of bark, root or rhizome and examine after due preparation (powdered preparation).

Boil for 3 to 5 minutes some characteristic pieces of the drug in 5 per cent potassium hydroxide solution, wash with water and decompose with a needle on a glass slide. Examine the preparation usually in aqueous glycerine (1 + 1). In order to make sections, boil 1 to 2 cm long and 0.5 to 1 cm broad pieces of the drug for 1 to 3 minutes in water. Apply, if necessary, 5 per cent potassium hydroxide solution instead of water.

2.13 Seed and Fruit

Microscopical test is generally required for the identification of entire seeds and fruits. Sections of soft seeds, particularly those with fixed oil and protein contents can be made without any preliminary preparation.

Medium hard and dry drugs should be softened by maceration in water for 15 to 20 minutes, according to the nature of the sample.

Hard seeds or fruits. Boil the sample for a few minutes in water in a conical flask, or enwrapped in gauze, steam in the vapour space of the conical flask for 1/4 to 1/2 hour or longer, if needed. Prepare the section of the sample softened in this way. Perform superficial test of the *seed coat and fruit wall* with 2 to 3 seeds or fruits boiled for 1 to 2 minutes in a test tube in 5 per cent potassium hydroxide solution. Drugs of darker colour and harder coat should be boiled longer. After elution in water, place the seed on a glass slide. Detache the coat or husk with a needle and then examine in aqueous glycerine (1 + 1). Squash the kernel and examine separately on the glass slide.

2.14 Preparation of Sections

Prepare *a superficial section* generally with a cut parallel to the longitudinal axis of the drug; from leaves by pulling off the epidermis carefully. In the latter case, wind the leaf around the index, press with the thumb and the middle-finger against the index and incise carefully; catch the incised part with pincers, bend backwards and pull off carefully.

Prepare *cross-sections* always with a cut at right angle to the longitudinal axis.

Prepare *longitudinal sections* parallel with the longitudinal axis, either in radial direction (*radial section*) or chordwise, parallel with the tangent (*tangential section*). With the preparation of a cross- or longitudinal section proceed as follows: cut out, with the help of a plunger key or a jackknife, the proper piece of the prepared drug piece to be tested, place between a piece of elderpith cut in two and clamp in a hand fastener or a microtome. Thick pieces can be cut without elderpith. Prepare sections with a plane-concave razor; take care that the edge of the razor's plane placed on the cut surface should include an angle of 15 to 20° with the contact line of both elderpith pieces. When cutting, pull the razor from left to right or push from right to left. Moisten, if necessary, the surface to be cut, with aqueous glycerine (1 + 1). Take off the sections with wet brushes and collect them in a few ml of water in a porcelain dish. Use for testing the proper sections taken out of the dish.

Embedd the small and spherical or too flat seeds and fruits in hard paraffin of 50 to 54° melting point. Prepare a $1 \times 1 \times 1.5$ cm prism of the paraffin and

melt a small hole by means of a hot needle or a thin glass stick. Press into this hole the material to be cut preferably dry, or softened in steam.

2.15 Clarified Preparation

The section is clarified in order to test the histological structure. For this purpose, boil cautiously the sections in a porcelain dish in 2 per cent potassium hydroxide solution. Reject the alkaline fluid after it has cooled down, and wash carefully the sections sticking to the bottom of the dish with water.

Sections can also be clarified by dipping them for 5 to 10 minutes into 75 per cent aqueous chloral hydrate solution and eventually boiling them. In this case the sections can be tested in chloral hydrate solution too.

Of leaf drugs cut out generally pieces of 3 to 6 mm for *clarification* or *digestion* purposes.

Put the pieces of leaf in a porcelain dish and boil with 10 ml of water; add 5 ml of 5 per cent potassium hydroxide solution. Heat the fluid again until boiling. Stop the heating and add 2 ml of 3 per cent hydrogen peroxide and heat the fluid again carefully until it boils. Reject then the alkaline fluid and wash out carefully the pieces of leaf transferring the mwith water on a glass or porcelain filter. Flush the pieces of leaf from the filter with 96 per cent alcohol into a porcelain crucible, 4 cm in diameter. Boil the content of the pot carefully so as to expell any gas bubbles from the tissues and to extract the chlorophyll that is not yet digested. Reject after sedimentation the alcohol and examine the leaf pieces with the microscope in alcoholic glycerine (1 + 1).

Perform the microscopical test of *powdered drugs* as follows: Place 0.01 to 0.02 g of the sample on a glass slide, add 1 to 2 drops of the fluid complying with the purpose of the test and cover with a covering glass slide. Powdered drugs should be clarified as described above, with the difference that the drug powder is not filtered but washed out several times with water by sedimentation.

When examining the cell content, place the section or powdered preparation into alcoholic glycerine (1 + 1) dropped on the glass slide, then cover; press down the glass cover with the point of the needle and blot the superfluous fluid pressed out at the edges with a stripe of filter paper. Examine the preparation with small and then with large magnification. If any reagent or dye solution is needed, it should be dropped to one edge of the glass cover and sucked through with a stripe of filter paper on the opposite side.

Tissues should be tested mostly in clarified preparations, because the observation is disturbed by cell contents, particularly by the starch. Test the clarified sample in alcoholic glycerine (1 + 1), or if clarified with chloral hydrate, in 75 per cent chloral hydrate solution.

2.2 Detection of Cell and Tissue Contents

Starch. Examine the preparation in 0.01 **N** iodine solution. The starch turns blue. The blue colour disappears upon heating or from the effect of an alkali, but reappears at cooling or if acidified.

Fixed oil. Macerate the section in R-alkannin solution or Soudan-III solution for 1/4 to 1/2 hour, wash with water and examine in alcoholic glycerine (1 + 1). The fixed oil drops are stained red.

Mucilage. Examine the wetted drug powder or section in toluidine blue solution. In a few minutes the mucilage turns violaceous red. Diluted China ink solu-

tion (1 + 10) may also be used for the detection of mucilage. In this case, mucilage can be seen in a black field as a colourless mass in a short wile.

Tannin. Examine the section in R-iron(III) chloride solution: the tannin turns bluish black or greenish black.

Aleuron. Examine the section or powder preparation in 0.01 N iodine solution. The aleuron granules turn yellow.

Inulin. Place the drug powder or the section on a glass slide, dropp concentrated alcohol on it, and examine in pure glycerin. The inulin separates out in form of sphero-crystals.

Oxymethyl-anthraquinone. Examine the section or drug powder in 5 per cent potassium hydroxide solution. The cells containing oxymethyl-anthraquinone are stained red.

Calcium oxalate. Calcium oxalate crystals are dissolved in R-sulphuric acid without effervescence and gypsum crystals are separated out instead. In polarized light calcium oxalate crystals are birefractive.

Lignin. Add 1 to 2 drops of R-hydrochloric phloroglucine solution on a section or on the drug powder placed on a glass slide. Cover the preparation with a glass cover; add 1 to 2 drops of alcoholic glycerine (1 + 1) to one side of the glass cover, and suck the fluid through by a stripe of filter paper on the other side. The lignified elements turn cherry-red. If the section is tested in aniline sulphate solution, the lignified elements turn yellow. If tested in toluidine-blue solution they turn bluish green. It is equally convenient to test these preparations in 75 per cent chloral hydrate solution as described above.

Suberin. Examine the section or the drug powder as described for fixed oil: the suberized parts turn pale red or red.

2.3 Microscopy of Clarified Leaves

2.31 Test of Vein Islets

Vein islet is that smallest area seen on the top-view of the leaf-blade (*lamina*), which is bordered by the last branches (*capillaries*) of the vascular system (nervation). *Vein islet number* is the number of vein islets per 1 mm^2 of leaf area. *Vein islet branch* is a vein extension incurving on the area of vein islet, ending blindly and composed of one or several rows of tracheids; it is either not ramified, or is ramified regularly, sometimes even repeatedly.

Number of tracheids bordering the vein islet: in regard to the structure of the bundle bordering the vein islet, it is important to know how many tracheids constitute the veins in width. *Bundle sheath:* a layer of parenchymatous cells next to the veins or their branches bordering the vein islet. The *vein termination* may be *simple* if the vein is closed at the last section of the vein islet branch in maintaining its original width and structure. The vein termination may consist of a *pair of tracheids:* two smaller and somewhat broader tracheids at the end of the vein branch. It may also be formed of a *tracheid funnel*, i.e. of one or more tracheids, expanding in the shape of a funnel or a fan. And finally, at the termination there may be a group of tracheids, when it is composed of several adjacent, irregularly arranged tracheids.

Perform the vein islet test of clarified leaf with a magnification leaving comfortable place in the visual field for 1 mm^2 area. Determine the area of 1 mm^2 by means of a stage-micrometer and a reticulate or trombocite objective of adequate scale.

Calculate the vein islet number as follows. Count every complete vein islet on the 4 adjacent areas of 1 mm^2 each, that are in the bisector between the midrib and the leaf edge, as well as all the vein islets — situated not entirely on the above area — along the bottom and the right side of these areas. The quarter of the total sum of vein islets counted in this way will be the vein islet number of the leaf in question.

2.32 Calcium Oxalate

In the cells, calcium oxalate can be seen in the form of single crystals, bundles composed of crystal needles, so-called raphides, clubshaped crystals and sand composed of small crystals. Related to the veins, the crystals are situated in three different ways: 1. along the veins only; 2. inside the vein islets only; 3. on both places.

2.33 Structure of the Stoma (stoma types)

(a) *Ranunculaceae type.* There are no subsidiary cells next to the terminal cells.

(b) *Cruciferae type.* There are mostly 3 and seldom 4 subsidiary cells next to the terminal cells. One of them is generally smaller than the rest.

(c) *Rubiaceae type.* There are two subsidiary cells developing around the terminal cell and situated parallel with the stoma.

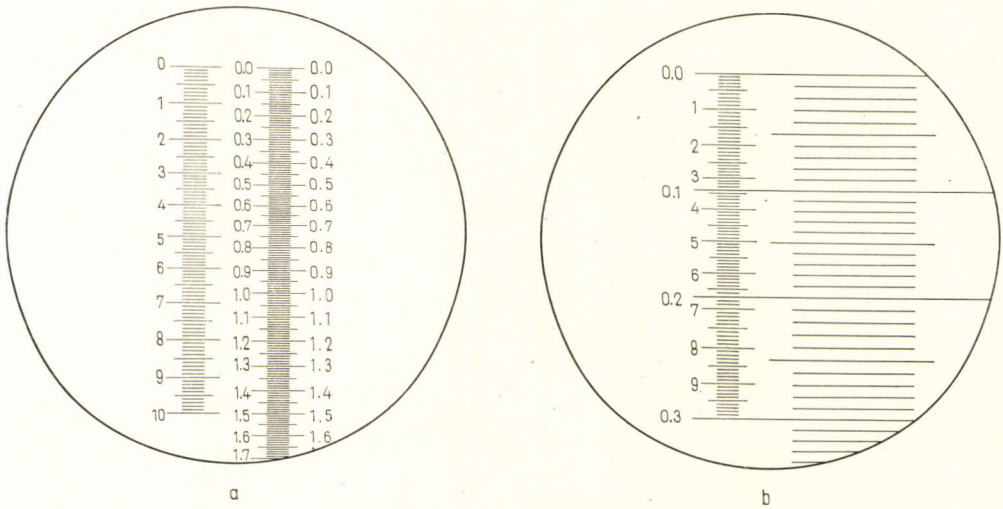
(d) *Labiatae type.* Two subsidiary cells are developing around the terminal cell, with their longitudinal axis at right angles to the longitudinal direction of the stoma.

(e) Stomata of other structure than those described above.

2.4 Microscopic Measurement

The ocular micrometer used for microscopic measurement is a round glass-plate placed into the eyepiece, with a scale of 100 graduation (Figs 26*a* and 26*b*; left side scale). When measuring, place the ocular micrometer into the eyepiece. For this purpose, unscrew the upper lens of the microscope eyepiece, place the ocular micrometer on the diaphragm at mid-height of the tube, and screw back the upper lens. The side which should be turned upwards is usually marked with a letter "O" on the frame of the micrometer, because in reversed position the scale becomes blurred or invisible. There are also eyepieces with built-in ocular micrometer. The upper lens of such eyepieces can be moved vertically in order to obtain a sharp sight of the scale. The scale value of the ocular micrometer depends on the objective, i.e. on the magnification applied. Therefore at different magnifications every spacing of the scale on the ocular micrometer represents a different distance. The scale value of the ocular micrometer belonging to the different magnifications can be determined once and for all by means of the micrometer glass slide. In the middle of the glass slide there is a scale where 1 mm is graduated to 100 parts. So, one spacing represents 0.01 mm or 10 μm. This scale can be seen in 60-fold magnification on the right side of Fig. 26*a*. The O point of the ocular micrometer to be seen on the left side of this figure coincides with the 0.0 graduation of the micrometer glass slide, while the point 1 of the ocular micrometer, i.e. the line 10, coincides with the mark 0.15 (the 15th line on the glass slide). On the glass slide a spacing represents 10 μm, 15 spacings thus amount to 150 μm. This distance is included by 10 spacings of the ocular micrometer, thus a spacing of the eyepiece micrometer represents 15 μm in case of 60-fold magnification.

The same micrometer glass slide scale can be seen with 300-fold magnification on the right side of Fig. 26*b*. On this figure the O point of the ocular micrometer equally coincides with the 0.0 mark, i.e. with the first line of the micrometer glass slide, while the point 10, i.e. the 100th line of the ocular micrometer coincides with the mark 0.30, i.e. with the 30th line of the glass slide. The 30 spacings on the scale of the micrometer glass slide, ranging from 0.0 to 0.30, represent 300 μm. This distance is included by 100 spacings of the ocular micrometer, so a spacing of the ocular micrometer represents 3 μm in case of 300-fold magnification.



a b

FIG. 26

The scale value of the ocular micrometer depends also on the length of the microscope tube, so if a microscope has an extension tube, every measurement must be performed with the same tube length (usually 160 mm).

2.5 Microsublimation

Prepare a 1 to 2 mm layer of 0.1 to 0.2 g of the powdered drug in the middle of the glass slide, on an asbestos wire mesh. Place another glass slide over the glass slide, so that one of its ends is resting on the asbestos plate and the other on a 4 to 5 mm thick asbestos band or glass plate, leaving a 2 mm sublimation space between the two slides. Then heat the asbestos plate with the small flame of a micro-burner. The steam coming from the water content of the drug is the first to condensate on the glass slide placed crosswise, but it disappears soon. A permanent opacity indicating the process of microsublimation appears shortly on the receiver glass slide. Observe the sublimation through a hand magnifier, and change the receiver glass slide from time to time. Examine the sublimate at first through the microscope, with small magnification, without glass cover. Then test the sublimate on the same slide with chemical reactions. Examine, if necessary, any changes occurring during the reaction with a more potent magnification. Cover in such case the preparation first with a glass covering slide.

3 Determination of Water Content

3.1 Determination of Loss on Drying

Reduce to coarse powder (IV) an about 20 g portion of a fair average sample of the drug and place into a tightly closing vessel. Weigh about 2 grammes of the drug powder or the coarsely powdered drug to the nearest 1 mg into a drying vessel (with glass lid) 45 mm in height, 70 mm in diameter, or into a covered aluminium vessel of the same size both tared previously with an accuracy of 1 mg. Place the vessel uncovered into a drying oven and heat for 1 hour at 100°. After drying, let the vessel cool down in a calcium oxide desiccator, close with the lid or the cover, and weigh again to the nearest 1 mg. Repeat drying at 100° for 30 minutes until the difference of the two last measurements does not exceed 2 mg. Indicate the water content (loss on drying) in weight per cent.

Note. Oily seeds and drugs with volatile oil content should be dried once for 3 hours.

3.2 Determination of Water Content by Distillation

The water content of drugs bearing essential oil can also be determined with the distillation method.

Weigh as much coarsely powdered (IV) air dry drug for the test with an accuracy of 0.01 g, as is required for the volume of the read water to make 2 to 3 ml. Perform the test according to *5.2*, Vol. I, p. 105.

4 Determination of Ash and Sand Content

4.1 Determination of Ash Content

Weigh accurately about 2.5 g of the drug powder dried according to *3.1* into a platinum, nickel or stainless steel dish. Moisten the sample with a few ml of concentrated alcohol, place the dish on a wire triangle and ignite the alcohol. Heat after combustion the dish cautiously at first on a small and then on stronger flame until the ash turns white and no coal lumps can be seen any more in it. If the drug is difficult to be ashed, interrupt heating and wet again the remains in the dish with a few ml of alcohol after cooling down; set aflame the alcohol once more and, after it is burnt, continue heating. Coal particles burning difficultly should be squashed with a glass-stick of flat end. Flush the glass-stick with alcohol into the dish, and procede as above.

Cool down after heating the dish in a calcium oxide desiccator, then weigh exactly to the nearest 0.1 mg. Indicate the ignition rest — *ash* — as percentage, by weight, of the drug dried previously according to *3.1.*

4.2 Determination of Sand (Acid Insoluble Ash) Content

If the drug was not incinerated in a platinum dish, transfer the accurately weighed ash to an accurately tared 50 ml beaker, add 20 ml of R-hydrochloric acid and cover with a watch-glass. Heat the beaker for 5 minutes on a water bath. After it has cooled down, collect the ash insoluble in hydrochloric acid on a piece of analytical filter paper of 4 cm diameter and wash with hot water until it becomes free of chloride. Dry the funnel together with the filter paper, then place the filter in an accurately weighed crucible, incinerate and ignite

cautiously. Cool the crucible in a desiccator and weigh accurately to the nearest 0.1. mg Express the ignition rest — *sand* (acid insoluble ash) — as percentage by weight of the drug dried previously according to *3.1.* If the drug was incinerated in a platinum dish, dissolve the ash with hydrochloric acid in the same dish, and incinerate the filter paper also in it.

5 Determination of Extracts

5.1 Determination of Extracts of Powdered Drugs

Weigh to the nearest 1 mg a 5.00 g sample of the prepowdered (V) air-dry drug, macerate for 24 hours in a 150 ml glass-stoppered conical flask, at room temperature, with 100 ml of water or of alcohol of a concentration prescribed in the single monographs. Filter the fluid into a 100 ml measuring flask (through a corrugated filter paper covered with a watch-glass); wash the flask and the filter with water or with the solvent prescribed for extraction and make up the measuring flask to the mark. Evaporate 20.00 ml of the filtrate (= 1.00 g drug), eventually in several portions, on a water bath into a predried glass-stoppered drying vessel weighed to the nearest 1 mg, and dried previously for two hours in a drying oven at 100°. Cool down in a calcium oxide desiccator. Cover the beaker and weigh to the nearest 1 mg. Express the extract content as percentage by weight of the dried drug.

5.2 Determination of Mucilaginous Extracts

If the extract content of a drug bearing mucilage is to be determined, proceed as follows. Wash 20 g of sea sand with hydrochloric acid and water, dry and ignite. Place the ignited sand, after cooling, with a small glass stick into an empty drying vessel, dry at 100°, and weigh them exactly together. Pour the fluid to be tested on the sea sand and mix it evenly with the sand using the glass stick. Evaporate the content of the beaker while mixing. Proceed further on as under *5.1.*

6 Determination of Bitter Value

Bitter value is the highest dilution of an aqueous extract related to 1 g drug dried at 100°, the 5 ml portion of which produces still the sensation of a bitter taste within 30 seconds.

Pour on 1.000 g of coarsely powdered (IV) drug filled in a 250 ml flask, at first, 5 ml of water and then, after 10 minutes, 100 ml of hot water. Place a funnel on the flask and stir its content by concentrical swingings every five minutes for 1 hour. Filter the fluid from the settled drug through a small piece cotton plug into a 100 ml measuring flask. Wash the drug with water by moving the flask concentrically and transfer it with the wash liquor through the filter into the measuring flask. Make up the volume of the filtrate with water to 100 ml. Prepare different dilutions of the extract; start testing with the highest dilution, trying to find the one, the 5 ml fraction of which produces in the mouth a bitter taste within not more than 30 seconds (by moving it mainly near the root of the tongue). After rinsing the mouth with drinking water, resume the tasting after 10 minutes.

Determine the individual sensitivity of the mouth with proper dilution of the aqueous solution of quinine hydrochloride (1 + 10 000). The quotient of the normal bitter value (1 + 150 000 solution of quinine hydrochloride) and of the noticed dilution is the bitterness factor of the testing person. Calculate the normal bitter value related to the drug by multiplying with the factor the bitter value observed in the sample. The bitter value is related to the drug dried at 100°.

7 Determination of Swelling Value

Swelling value is the swelling of 1 g air-dry drug in water or in the different liquids specified by the monographs, observed at room temperature after 4 hours, related to dried drug, expressed in ml.

Moisten the prescribed quantity of air-dry drug comminuted to the fineness specified by the monographs with the specified moistening liquor (acetone, alcohol) in a 25 ml glass-stoppered volumetric cylinder graduated to 0.2 ml, where the graduation ranging from 0 to 25 ml is 10 to 12 cm in length. Shake up the fluid thoroughly with 25 ml of water or with the liquid specified for the different drugs. Agitate the mixture every 10 minutes for an hour and then maintain for 4 hours at room temperature. Read off the volume of the drug in ml together with any adhering mucilage. Calculate the mean value of at least two simultaneous tests related to 1 g of drug dried as under *3.1.*

8 Determination of Hemolytic Index (HI)

8.1 Reagents

Buffer solution (pH = 7.4). In a volumetric flask, dissolve 1.743 g of potassium hydrogen-phosphate (KH_2PO_4), 9.596 g of disodium hydrogen-phosphate ($Na_2HPO_4 \cdot 2H_2O$) and 9.00 g of sodium chloride to 1000 ml in water.

2 per cent blood suspension. Agitate in a widemouthed glass-stoppered flask (containing a few glass-pearls) fresh bovine blood until the separation of fibrin is completed. Filter the blood then through a double layer of gauze in a vessel placed into a 20° water bath, then dilute a 10.00 ml portion in a volumetric flask with 20° buffer solution to 500 ml. Prepare blood suspension freshly on the day of the test.

R-saponin solution, 0.02 per cent. Dissolve 0.0200 g of saponin in a volumetric flask with the buffer solution to 100 ml. Prepare the solution freshly on the day of the test.

8.2 Preparation of Extract

Wash the powdered (IV) and air-dry drug in a quantity specified by the different monographs, and weigh accurately into a 250 ml flask with 100 g of the buffer solution. Heat the content of the flask covered by a small glass funnel on hot water bath for 30 minutes, while the content of the flask is agitated four times by concentric swinging. Filter the hot extract into a 100 ml volumetric flask through a 0.15 g piece of cotton plug, placed between two layers of gauze (about 7 × 7 cm).

8.3 Informative Test of Extract

Transfer 0.2, 0.4, 0.6, 0.8, 1.00 and 1.2 ml of drug extract into 6 test tubes, make up the content of the test tubes with buffer solution to 5.0 ml, and add 5 ml of blood suspension to every test tube:

Test tube	1	2	3	4	5	6
Drug extract (ml)	0.2	0.4	0.6	0.8	1.0	1,2
Buffer solution (ml)	4.8	4.6	4.4	4.2	4.0	3,8
Blood suspension (ml)	5.0	5.0	5.0	5.0	5.0	5.0

Seal the mouth of the test tube with the dry fingertip, washed in soap and water and dried with alcohol, homogenize its content by turning the mouth of the test tube downwards. Strong agitation should be avoided in order to prevent foaming. Observe the results of the informative test in 2 hours. The limit is indicated by the test tube the content of which is still transparent, but that of the next one in the order of decreasing drug amount is already opalescent.

8.4 Determination of Apparent Hemolytic Index (hi)

Since, after the informative test of 2 hours, it may be expected that a hemolysis may be produced also by an amount of drug extract inferior to that determined as the limit, prepare a series of 12 test tubes of the same drug extract. The 12th member of the test tube series will be the limit dilution of the informative test, thus transfer a gradually decreasing quantity of the extract into the following tubes, each dose being inferior to the previous one by 0.05 ml. (The first test tube will therefore contain 0.55 ml less drug extract than the 12th.) Make up the content of each test tube with buffer solution to 5.0 ml and then mix with 5.0 ml of blood suspension. Continue the test in the way described at the informative test with the difference that the limit dilution (h) should be determined in 6 hours.

Calculate the apparent hemolytic index (hi) of the drug from the value H with the help of the following formula:

$$hi = \frac{10}{p},$$

where p = drug content of the extract filled into the test tube, expressed in g (related to the drug dried at 100°).

In order to obtain the hemolytic index (HI) of the drug, the apparent hemolytic index (hi) must be multiplied with the factor (F) of the blood employed:

$$HI = hi \times F$$

Determine the blood factor (F) as follows: In the way described for the drug extract prepare 0.02 per cent R-saponin solution with the buffer solution and dispense into 7 test tubes, starting from 1 ml and increasing the doses by 0.5 ml, and complete with buffer solution to 5.0 ml; dispense finally 5.0 ml of blood

suspension into each test tube. Perform the informative test and the subsequent detailed test in the way described for the drug extract. The apparent hemolytic index (*hi*) of R-saponin is thus established. The blood factor can be obtained by dividing the hemolytical index of standard saponin (25 000) with the apparent hemolytical index of the R-saponin examined (*hi*).

9 Determination of Tannines

Reagents

Folin's solution

Boil 10.00 g of sodium tungstate for 3 hours with 8.00 ml of 85 per cent phosphoric acid and 75 ml of distilled water in a flask mounted with a reflux condenser; decant the clarified solution from the sediment after 12 hour's standing.

Sodium carbonate solution, 50 per cent

Dissolve in a volumetric flask, 50.00 g of crystalline sodium carbonate ($Na_2CO_3 \cdot 7H_2O$) with water to 100 ml.

Pyrogallol solution

Dissolve 0.0500 g of freshly sublimated pyrogallol of analytical purity (m.p. 133°) in a volumetric flask with water to 100 ml, and dilute a 5.00 ml portion of the solution in a volumetric flask with water to 100 ml.

Plotting of pyrogallol concentration curve

Mix 0.50, 1.00, 2.00 and 3.00 ml portions of the diluted pyrogallol solution (containing 25 μg per ml) each with 1.00 ml of Folin's solution and 15.0 ml of 50 per cent sodium carbonate solution, and fill up with water to 20.0 ml. Determine the light absorption of the solution in 120 seconds with the colour filter S75 of the Pulfrich photometer, in a 1 cm cell against water.

Procedure

Boil the quantity of the air-dry powdered (IV) drug specified by the different monographs, two times with 40 ml water each for 15 minutes in a conical flask mounted with a reflux condenser, and filter the extracts through a filter paper of 5 cm diameter into a 100 ml volumetric flask. Wash the flask and the filter with water and fill up to the mark (solution I). Dilute a 2.00 ml portion of solution I in a volumetric flask with water to 25 ml (solution II).

Mix a 2.00 ml portion of solution II with 1.00 ml Folin's solution and 15.0 ml 50 per cent sodium carbonate solution; determine the light absorption of the solution in 120 seconds with the S75 colour filter of the Pulfrich photometer in a 1 cm cell against water (E_1).

Dilute a 4.00 portion of solution I with water to 10.00 ml and agitate strongly for 60 minutes in a small flask with 0.1000 g of hide powder, then filter through a filter paper of 5 cm diameter. Dilute a 5.00 ml portion of the filtrate in a volumetric flask with water to 25 ml (solution III).

Mix a 2.00 ml portion of solution III with 1.00 ml Folin's solution, 2.0 ml water and 15.0 ml 50 per cent sodium carbonate solution; determine in 120 seconds the light absorption of the solution with the S75 colour filter of the Pulfrich photometer in a 1 cm cell against water (E_2).

Calculate the extinction value (E) of polyphenol (tanning agent) bound by the hide powder from the difference between the extinction of total polyphenol (E_1) and the extinction of polyphenol not bound by hide powder (E_2):

$$E = E_1 - E_2$$

Calculate the amount of pyrogallol corresponding to the E value from the concentration curve plotted with pyrogallol and express the tanning agent content of the drug in percentage of pyrogallol related to the drug dried at 100°.

10 Determination of Essential Oil

Determine the essential oil content of a drug with the apparatus shown in *Fig. 27*. The apparatus is composed of a glass distilling flask (*a*) of 1 litre volume and of a distilling adapter to be joined with a glass grinding. The distilling adapter is composed of an ascending glass tube (*b*), of a vertical bulb condenser (*c*) and of a volumetric tube (*d*), with 0.01 ml graduation per 1 ml. Above the volumetric tube there is a glass-stoppered aperture (*e*) and at its bottom a drain tap (*f*).

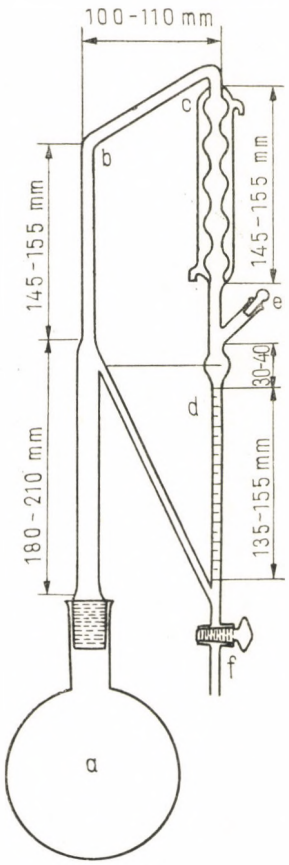


FIG. 27

The upper glass-stopper has a bore-hole in order to compensate the pressure. The volumetric tube is also connected with the ascending part of the adapter; latter must be carefully cleaned with chromic acid in sulphuric acid prior to being used. Place the amount of airdry drug specified by the monographs for determination, in whole pieces or comminuted as specified, into the distilling flask with 400 ml of water, add a few grains of pumice and mount the adapter. Through the aperture (*e*) let water into the volumetric tube (*d*) until it flows back into the distilling flask. Then heat the content of the flask by steadily moving the flame, and distil for 3 to 4 hours after ebullition. From time to time, move the flask in order to wash down the drug particles sticking to its wall. Essential oil is accumulated in the upper widening part of the volumetric tube and gets separated from the aqueous phase which flows back into the distilling flask. After the prescribed time is over, stop cooling; when the steam is already appearing in the lower part of the condenser, also interrupt distillation and resume cooling. Observe the volume of essential oil in the volumetric tube in 5 minutes. Then continue distillation for 1 hour and observe again the volume of oil as above. Regard the operation as finished if the volume of essential oil has not increased by more than 5 per cent. Otherwise the distillation must be continued. Place the volumetric tube (*d*) containing the essential oil into a water bath of 20° and observe the volume of essential oil in 30 minutes. Calculate the volume of essential oil related to the air-dry drug applied, and convert it for the drug dried at 100°, taking the water contents into consideration.

With certain drugs (*Absinthii* herba, *Anisi vulgaris* fructus, *Caryophylli* flos, *Chamomillae* flos, *Cinnamomi* cortex

Foeniculi fructus, *Valerianae* rhizoma et radix) perform the distillation by adding decaline. In this case, fill the distilling flask with water only, and — through the aperture (*e*) — add 0.2 ml of decaline with the help of a pipette the point of which penetrates deeply into the distilling tube. Heat the content of the flask to ebullition and distil for 30 minutes. Observe after 5 minutes the volume of decaline in the volumetric tube. Place now the drug sample into the distilling flask, and continue the procedure as described above. Deduct the volume of decaline observed previously from the decaline solution of the essential oil. The difference will be equal to the essential oil content of the air-dry drug, expressed in percentage, volume per weight — v/w — related to drug dried at 100°.

11 Determination of Alkaloids

Determine the alkaloid content of vegetable drugs according to *8.3*, Vol. I, p. 121.

12 Informative Test

Examine the vegetable drugs macroscopically or by the aid of a hand magnifier. Use a microscope if necessary for identification and check, respectively, according to the *General Indications* and *Macroscopic tests* (Vol. I, p. 202).

If a special instruction is indicated for a preexamination or for identification by a chemical reaction in the monograph of a particular drug, perform also these tests in the course of the informative test.

Identify essential oil-bearing drugs by their typical scent. Saponin-bearing drugs may be identified by the foaming of the aqueous extract of the powdered sample if no other test is specified by the Pharmacopoeia. Drugs containing alkaloids must always be identified by the method described in the respective monograph.

In special cases indicated in the single monographs, tests for the detection of contamination with alkaloid-containing drugs of non-alkaloidal drugs must be performed.

III PHARMACEUTICAL PREPARATIONS

(A) GENERAL PART

(a) GENERAL NOTICES

Pharmaceutical preparations are dosage forms suitable for direct therapeutic use prepared from substances of chemical, vegetable, animal or biological origin or galenical preparations and usually contain additions.

The definition, description and the general rules of preparation and control of galenicals and dosage forms are laid down in the respective chapters of the Pharmacopoeia.

Detailed instructions concerning preparation and quality control of the individual preparations are described in the monographs of the individual preparations (Vol. III). The general rules are directive also for the production and quality of preparations not official in the Pharmacopoeia. The prescriptions of the *Formulae Normales* must be taken into consideration when dispensing preparations on medical prescriptions not included in the Pharmacopoeia.

The mode of large-scale production of pharmaceutical (galenical) preparations may differ from the pharmacopoeial prescription, but the quality of the preparation itself must be identical with that prepared according to the prescriptions of the Pharmacopoeia.

Instructions of the Institute commissioned by the Ministry of Health should be followed concerning the manufacture, compositions, etc. of registered pharmaceutical preparations. Additives (vehicles, preservatives, solvents, etc.) may only be used if these are physiologically harmless in the applied concentration or amount and do not change the therapeutic effect of the drug unfavourably. In certain cases additives are used also to alter a certain therapeutic effect. The quantity of additives must not be more than absolutely necessary. Additives have to be chosen with regard to the mode of administration and the dose. In the pharmaceutical compounding, the quantity of the additives must be deduced from the weight of the prescribed vehicle and the name and quantity of the additive must be denoted by the dispensing pharmacist on the prescription.

For medicaments dispensed in a pharmacy only additives official in the Pharmacopoeia may be used. For the employment of additives, the prescriptions of the individual pharmacopoeial monographs and the Formulae Normales must be taken into consideration. Employment of additives in registered preparations must follow the instructions of the Institute commissioned by the Ministry of Health, and in case of veterinary preparations, that of the Institute commissioned by the Ministry of Agriculture, respectively.

When water is prescribed in the individual monographs, distilled water (*aqua destillata*) is to be used. The employment of demineralized water (*aqua demineralisata*) is regulated by the individual monographs or by special instructions. Demineralized water must not be used for the preparation of injections, infusions and ophthalmic solutions. If the concentration of alcohol is not stated, alcohol, 90 volume per volume per cent (*spiritus concentratus*) is to be used.

Only substances of a quality prescribed in the individual monographs must be used for their preparations. Contaminations deriving from the tools employed in the course of manufacture should be avoided.

Storage of the preparations is regulated by the individual monographs. Preparations deteriorating on storage must be prepared extemporaneously. Storage of galenicals and medicaments compounded on medical order is regulated by the Pharmacopoeia and the Formulae Normales, respectively. The quality of preparations deteriorating in the course of storage should be checked at intervals prescribed by the individual monographs.

The amounts of the main ingredients of galenical preparations are specified in the individual monographs by limiting values.

The tolerable deviation of the content of active ingredients in preparations compounded on medical order in the pharmacy is determined by their quantity, quality, the mode of manufacture and the accuray of the test method.

Directions of the chapter *Chemical Tests and Assays* (Vol. I, p. 89) are valid for the control of galenical preparations as well.

Instructions on the tests of dosage forms (Vol. I, pp. 226 to 253) are also valid for the control of the individual preparations.

Designation and putting into circulation of registered pharmaceutical preparations are regulated by special decrees.

Utensils and instruments used for the preparation of pharmaceuticals in the pharmacy are specified by National Standards.

Drugs of quantities smaller than 0.05 g have to be weighed with mg accuracy or dilutions must be prepared in order to ensure dosage accuracy. Dilutions are prepared with water, lactose or other, therapeutically indifferent, substances.

Only powder-dilutions (*triturations*) admitted by special regulations in the individual monographs, prepared with lactose (*lactosum*) in the ratio of 1 + 9 in quantities not exceeding 10 g, are permitted to be held on stock.

The label on the container of the powder-trituration must contain the name of the drug, the designation of potency, the ratio of dilution (1 + 9) and the maximum doses referred to the active ingredient. Triturations must be preserved according to the individual prescriptions concerning the active ingredient.

For the preparation of dosage forms in the pharmacy other than powder-triturations or solutions (dilutions) official in the Pharmacopoeia or listed in special decrees may also be used. These solutions (dilutions) or triturations must be prepared with official solvents or vehicles, that also are ingredients of the ordered medicament. Such solutions (dilutions) or triturations may only be prepared and stored in quantities not exceeding the short term requirement of the pharmacy, with consideration to the provisions of the Pharmacopoeia and of other rules.

(b) BALANCES AND WEIGHTS USED FOR COMPOUNDING AND DISPENSING IN THE PHARMACY

1 Control of Balances and Weights

For compounding purposes certified and standardized weight-series, so-called pharmacy-weights (individually designated each by a certification mark), certified counter balances of 1 kg capacity and standardized hand-scales are to be used.

Other standardized balances, equivalent in accuracy and sensitivity with the above mentioned ones, may also be used for compounding.

Weight-beam balances must not be used.

1.1 Control of Counter Balances

Mount counter balance onto a vibration-proof basement, with the pillar in vertical position, allowing free swinging of the suspended parts. The pointer should swing equal distances to the right and left from the scale centre and should rest there in equilibrium.

1.11 Checking of the Equilibrium

Determine the equilibrium position of the balance unloaded, and loaded alternately on both pans with 100 g and 1000 g, respectively. Observe the resting position of the pointer after swinging. If the resting position of the pointer does not coincide with the scale centre, no more weight should be necessary for adjustment than directed under *1.12*.

1.12 Checking of Sensitivity

Sensitivity of counter balances and hand scales is expressed for the sake of convenience generally by the *reciprocal of the sensitivity,* i.e. by the number of mg-s required to move the equilibrium point of the pointer from the scale centre by one scale unit (i.e. 2 mm).

Check the sensitivity (a) unloaded, (b) loaded with one tenth of the maximum load, (c) with the maximum load. First determine the equilibrium position according to *1.* (unloaded and uniformly loaded with weights in both pans). According to the sensitivity of the balance place the weights indicated in Table 26 into one of the pans and determine the equilibrium again. Repeat this procedure twice.

The equilibrium of the balance carrying the weight directed in Table 26 — unloaded and loaded by the same weights in both pans, respectively — must deviate from that of the unloaded balance by at least one scale-unit (2 mm).

1.2 Control of Hand Scales

Determine the equilibrium and sensitivity of the suspended hand scales in the same way as directed for counter balances, estimating the distance (in mm) of the pointer from the counter.

Sensitivity data of hand scales — The mg weights for the checking of sensitivity may be made from thin, uniform wire.

TABLE 26

Maximum permissible load in g	Weight-surplus required to produce 1 scale unit (2 mm) deviation in cg		
	unloaded	loaded with one tenth of the maximum load	at maximum load
	not more than		
100	5	10	30

TABLE 27

Maximum permissible load in g	Sensitivity surplus load, g		
	unloaded	loaded with one tenth of the maximum load	at maximum load
	not more than		
1	2	2	2
5	2	2	3
20	3	3	10
100	5	10	50

1.3 Control of Weight-series

Check the individual weights by comparing them on an analytical balance with respective analytical weights. The permissible maximum deviation of the weight is shown in Table 28.

TABLE 28

Designation	Maximum permissible deviation in mg		Designation	Maximum permissible deviation in mg	
	New	Used		New	Used
	weights			weights	
200 g	100	200	50 cg	1	2
100 g	50	100	20 cg	1	2
50 g	40	80	10 cg	1	2
20 g	20	40	5 cg	0.5	1
10 g	15	30	2 cg	0.5	1
5 g	10	20	1 cg	0.5	1
2 g	6	12			
1 g	4	8			

Clean the balances, except the knife-edges, and weights with cotton woo wetted with alcohol.

(c) GENERAL RULES FOR COMPOUNDING INCOMPATIBLE INGREDIENTS

The pharmacist is authorized to compound the preparation in another way than prescribed by the physician, if the components of the prescribed medicament undergo undesired physical or chemical interaction, or the uniform dosage of the medicament is impaired, its applicability is limited or perilous. Only such alterations may be performed that do not lead to unfavourable therapeutical effect with consideration to the site and mode of administration.

The properties of the substances and the references under "Incompatibility" of the individual monographs should also be taken into consideration.

The listed incompatibilities occur in general in certain dosage-forms and ratios.

Chemical changes due to incompatibility are not detailed in the Pharmacopoeia, but certain physical changes are listed. Acidic or alkaline medium allude to acids or bases and to substances undergoing acidic or alkaline hydrolisis. *Oxydation* or *reduction* are mentioned only if the substance is specially liable to oxydation or reduction.

The Pharmacopoeia lists in its monographs in general only incompatibilities for substances used for compounding in the pharmacy and such, that are likely to occur during compounding. Alterations occurring during storage are not included under "Incompatibility". Data given on incompatibility are valid only for the interaction of two substances and therefore in cases when three or more substances are likely to interact, these data are valid with limitations.

General rules for compounding incompatible substances:

1 Solutions

1.1 Neutralize acid or alkaline substances, if necessary, i.e. adjust to a suitable pH value.

1.2 If a substance is not soluble at the given concentration in the prescribed solvent, and if a soluble form of the drug is available, dissolve the latter, provided that the usual dose of the two forms of the drug is identical. If the usual dose of the two forms differ, the difference in molecular weight (percentage, etc.) should be taken into consideration.

1.3 If a suitable solution can be prepared from the prescribed drug with the aid of an additive not influencing the intended therapeutic effect, this may be used only in the minimum necessary quantity. Subtract the quantity of the additive from the prescribed vehicle.

1.4 If the prescribed drug cannot be dissolved according to *1.1*, *1.2* or *1.3* or if the dissolution is followed by precipitation or separation of one of the active ingredients a suspension or emulsion should be prepared according to rules.

1.5 If a solution cannot be prepared according to *1.1* to *1.4*, proceed as directed under *2.2*.

2 Other Dosage Forms

2.1 Change unsuitable vehicles.

2.2 Dispense the ingredient causing the incompatibility as a separate, possibly identical dosage form with regard to the dose. A direction for use must be enclosed.

Alterations should be noted on the medical prescription, and in case of 2.2 the presciber should also be notified.

(d) ASEPTIC COMPOUNDING AND DISPENSING

When compounding under aseptic conditions the following rules should be considered:

1 Aseptic compounding must be only performed in carefully cleaned, washed and disinfected suitable rooms, serving only for this purpose. The surface of the work-sites should be meticulously cleaned and disinfected prior to starting of work or at least once a day if the work is carried out continuously.

The bacterium count of the atmosphere of the work rooms should be suitably reduced by ultraviolet irradiation or spraying with germicide aerosols, etc. before work is started.

Aseptic compounding must be carried out after thorough disinfection, in suitable work clothes.

All ingredients, utensils, containers, etc. used in aseptic compounding must be sterilized by a method corresponding to their properties. Weigh or measure the sterilized materials with sterile utensils, mix them or dissolve them in previously sterilized substances and solvents, resp., so as to avoid possible contamination. Substances, which cannot be sterilized by heat or any other method may be used without sterilization.

Solutions may be rendered free of microorganisms by suitable filtration. Medicaments prepared under aseptic conditions should be stored and dispensed in well closed or air-tight containers which prevent contamination. According to the directions of the individual monographs, in certain cases bactericides may be added to ensure a suitably low bacterial count.

Other details of aseptic manufacture of medicaments are regulated by special instructions.

2 In cases when aseptic conditions detailed in part *1* are not feasible, medicaments of short storage, compounded in the pharmacy on medical prescription, should be prepared according to the following rules:

Compounding should be carried out in a suitable place with separate atmosphere (aseptic manipulator), the surfaces and the air space of which should be carefully disinfected. The hands should be washed with soap and thoroughly disinfected.

All materials and utensils used for compounding must be sterilized with regard to their properties, according to the individual monographs. All possibility of contamination must be excluded throughout operations.

Note. Preparations made under aseptic conditions may only be labelled as sterile if — on checking — they meet the requirements of the monograph *Sterilization.*

e) STERILIZATION

According to the Pharmacopoeia preparations, etc. may only be regarded as sterile if they were sterilized by a method described in the Pharmacopoeia or were prepared and stored by a method ensuring sterility, further if no living microorganism could be grown in them under either aerobic or anaerobic conditions (see *4*, Vol. I, p. 170).

The chosen test method depends on the properties of the material to be sterilized. Eventual heat-sensitivity must be taken into consideration, and sterility must be achieved without causing change in the therapeutic action. The following methods should be used for sterilization of medicaments, materials, utensils, etc.

1 Heat Sterilization

Medicaments, etc. to be sterilized are kept for a definite period at the prescribed temperature. The time of exposure begins when the whole system (medicament and container, etc.) acquired the prescribed temperature.

1.1 Flame Sterilization

The material to be sterilized is ignited in the colourless flame of a gas- or alcohol-burner or is drawn through the flame so as the flame comes into direct contact with the material 12 to 15 times.

1.2 Dry-heat Sterilization

The object to be sterilized is kept in a sterilizing oven or drying cabinet
1.21 at 155 to 165° for 2 hours, or if the nature of the material does not permit this temperature without damage:
1.22 at 135 to 145° for 3 hours.

1.3 Sterilization by Heating in an Autoclave

The material or utensil is sterilized for 20 minutes at 118 to 122° in saturated steam (at about 1 atm overpressure).

A heat-equilibration of aqueous solutions, filled into glass containers, is 10 minutes for volumes up to 100 ml and 20 minutes for volumes up to 1000 ml, beginning when the temperature of the steam in the autoclave has reached the prescribed degree.

Aqueous solutions filled into glass containers should be sterilized for the prescribed period prolonged with the time necessary for equilibration.

2 Other Sterilizing Methods

Methods of sterilization other than the prescribed ones (irradiation methods, gas sterilization, etc.) may be applied only with the permission of the Institute commissioned by the Ministry of Health.

3 Sterilization of Pharmaceuticals

Methods of sterilization for frequently used drugs are listed in Table XXIII, Vol. IV. Heat sterilization of preparations ready for use must be performed in that container (ampoule, etc.) in which they are dispensed. Not more than 1000 ml should be filled into one container.

Sterile solutions and other sterile preparations produced on industrial scale must be prepared in separate rooms — under aseptic conditions (Vol. I, p. 220).

Liquids are to be sterilized filled into carefully cleaned glass containers, provided with ground glass stoppers, intact rubber closures, or sealed ampoules. The closures must be covered with parchment paper or cellophane prior to sterilization.

Cotton wool, gauze and surgical dressings and sutures (not including catgut) are sterilized for 30 minutes as directed under *1.3*.

A free air space of about 20 to 30 per cent should be maintained in glass containers when sterilizing aqueous solutions. Alcoholic solutions, containing not more than 80 per cent of alcohol have to be sterilized according to *1.3* in sealed ampoules or glass containers provided with ground glass stoppers, permitting enough free air space.

Liquid paraffin, fixed oils, soft paraffin, wax are sterilized according to *1.2*.

Glycerin and solutions containing glycerin with a water content of not more than 20 per cent, are sterilized according to *1.3*.

Powdered medicaments and ointments are sterilized in carefully cleaned suitable glass containers, as prescribed in the respective monographs (Vols II and III).

4 Sterilization of Utensils, Containers, etc.

Utensils, containers, etc. have to be meticulously cleaned prior to sterilization.

Metal instruments are sterilized, according to *1.1*.

Glassware, ampoules, mortars and porcelain containers are sterilized according to *1.2* and *1.3*, respectively.

Rubber closures are sterilized according to *1.3* and have to be preserved under sterile conditions.

Filter-paper is sterilized in ready for use form singly, or moistened and placed into the funnel in a heat sterilizing oven at 130° for 3 hours.

Small glass or metal objects are sterilized according to *1.1.*

Uncoated collapsible tubes are sterilized according to *1.2* or *1.3;* in the latter case, they should be dried at a temperature not exceeding 140°.

Clothes (towels, coats, etc.) are sterilized according to *1.3.*

(f) PREPARATION OF ISOTONIC SOLUTIONS

Isotonic solutions have the same osmotic pressure than blood, tissue or lachrymal fluids. The pharmacopoeial standard method for the establishment and checking of osmotic pressure is the determination of freezing point-depression proportional to osmotic pressure. The freezing point-depression of isotonic, 0.9 w/v per cent sodium chloride solution is 0.52°. To render a solution isotonic, sodium chloride or another suitable inert substance (e.g. glucose, potassium nitrate, etc.) may be added in calculated quantities.

The quantity of sodium chloride, required to render solutions of commonly used drugs isotonic are presented graphically by the Pharmacopoeia (Vol. IV, Table XXI).

(g) CALCULATION OF CONCENTRATION OF INFUSION SOLUTIONS

The components of infusion solutions are expressed by the Pharmacopoeia in *millivalencies* related to one litre (mval/litre).

A solution has *1 mval/litre* concentration when one milligram-equivalent of the substance is dissolved in one litre. The designation of this constant is the term *milliequivalency* (*mEq*), which is frequently used. Mval-values are indicated in the individual monographs of the Pharmacopoeia with respect to the ion-components of the compounds.

The mval/litre value is calculated from the quantity of the components by equation (1):

$$\text{mval/litre} = \frac{a \times n}{mwt} \tag{1}$$

If the composition is prescribed in mval. units the quantity of the ingredients is to be calculated by equation (2):

$$x = \frac{mwt \times \text{mval}}{n}\,, \tag{2}$$

where x = the quantity of the ingredient in mg/litre
a = the quantity of the dissolved substance (mg/litre)
mwt = molecular weight of the substance concerned
n = valency of the ions.

For infusion solutions containing several components, the quantities and mval-values, respectively, are calculated individually by the above equations.

Non-dissociating (nonionic) components of infusion solutions (e.g. glucose) are expressed in *milliosmol* (*mosm*) per litre (*mosm/litre*).

A solution has *1 mosm/litre* concentration when one milligram-molecular weight (mmol) of the non-dissociating substance is dissolved in one litre.

In the case of dissociating substances the numerical value of the mosm/litre concentration is equal to the total of the mval/litre values of the dissociated ions. From the point of view of infusion therapy 1 mval/litre concentration is equivalent to 1 mosm/litre concentration.

The mval and mosm values of some substances used in infusion therapy are listed in Table 29.

(h) OPERATIONS CONNECTED WIT H THE EXTRACTION OF VEGETABLE DRUGS

1 Maceration

The vegetable drug (drug mixture) comminuted to a fineness indicated by the individual monographs, is macerated with recurrent agitation for six days at room temperature with the whole amount of the menstruum in a well-closed container, and expressed.

Extraction with water at elevated temperatures is described in the monograph *Decoctions and Infusions* (Vol. I, p. 230).

TABLE 29

Subtance	Mol weight	Ion components and mval/litre values of 1000 mg of substance (milligram equivalent weight per litre)						Quantity required for the preparation of a solution of 1 mval/litre concentration
		cation	mg	mval	anion	mg	mval	in mg
Ammonium chloratum	53.50	NH_4^+	337	18.69	Cl^-	663	18.69	53.5
Calc. chloratum cryst.	219.09	Ca^{++}	183	9.13	Cl^-	324	9.13	109.5
Kalium bicarbonicum	100.12	K^+	341	9.99	HCO_3^-	610	9.99	100.1
Kalium chloratum	74.56	K^+	525	13.41	Cl^-	476	13.41	74.5
Magnesium chloratum ($6H_2O$)	203.33	Mg^{++}	120	9.84	Cl^-	349	9.84	101.6
Magn. sulfuricum cryst.	246.50	Mg^{++}	98.7	8.11	SO_4^{--}	390	8.11	123.2
Natrium aceticum cryst.	136.09	Na^+	169	7.35	$CH_3CO_2^-$	434	7.35	136.0
Natrium bicarbonicum	84.01	Na^+	274	11.90	HCO_3^-	726	11.90	84.0
Natrium chloratum	58.45	Na^+	393	17.11	Cl^-	607	17.11	58.4

TABLE 29

Substance	Ion components and mval/litre values of 1000 mg of substance (milligram equivalent weight per litre)							Quantity required for the preparation of 1 of a solution mval/litre concentration
	mol weight	cation	mg	mval	anion	mg	mval	in mg
Natrium citricum	294.11	Na^+	235	10.19	$[C_6H_5O_7]^{3-}$	643	10.19	98.0
Natrium lacticum	112.06	Na^+	205	8.92	$[C_3H_5O_3]^-$	795	8.92	112.0
Natrium phosphoricum acidum (H_2O)	138.00	Na^+	167	7.25	$[HPO_4]^{2-}$	696	14.49	138.0
Natrium phosphoricum cryst.	358.16	Na^+	128	5.58	$[HPO_4]^{2-}$	268	5.58	179.0
Natrium sulfuricum cryst.	322.21	Na^+	143	6.21	$[SO_4]^{2-}$	298	6.21	161.1
Natrium sulfuricum siccatum	142.05	Na^+	324	14.08	$[SO_4]^{2-}$	676	14.08	71.0
Glucosum anhydricum ($C_6H_{12}O_6$)	180.15	(1000 mg anhydrous glucose/1 litre = = 5.55 mosm)						180.2
Glucosum ($C_6H_{12}O_6 \cdot H_2O$)	198.17	(1000 mg glucose/1 litre = = 5.05 mosm)						198.2

2 Percolation

The vegetable drug (drug mixture), comminuted to a fineness, indicated by the individual monographs is moistened with the menstruum and allowed to swell for 3 hours in a well-closed container. The swollen drug is packed uniformly into a percolator and enough menstruum is added to cover the drug. After one-day standing the flow of the percolate is adjusted to a rate of about 1/50th of the drug volume per minute. The menstruum must cover the drug continually during extraction. Percolation is continued until the point indicated by the individual monographs or by the monograph *Extracts* (Vol. I, p. 231). On completing the percolation the menstruum is drained off, the marc is expressed and processed as prescribed under *Extracts*.

3 Evaporation

Unless otherwise specified, liquids have to be evaporated on a water-bath or in a still. "Evaporation under reduced pressure" means evaporation under a pressure not exceeding 50 torr for alcoholic liquids and 30 torr for aqueous liquids, respectively. The temperature of the liquid (measured with a thermometer immersed in the liquid) must generally not exceed 50° during evaporation.

(B) DOSAGE FORMS AND PHARMACEUTICAL PREPARATIONS

1 Adhesive Tapes (collemplastra)

Adhesive tapes adhere readily to the skin and serve to cover skin surfaces, wounds or to fix surgical dressings. The plaster material is spread onto unstarched, dense, smooth cotton or soft synthetic fabric. Adhesive tapes are marketed in rolls or in smaller pieces. Adhesive tapes contain, besides non-vulcanized rubber additives, such as resin, wax, wool fat, etc.

The label must indicate the length and width of the tape and the active ingredient content in per cents of the adhesive plaster material spread over the fabric.

1.1 Tests

The smoothed tape must not be shorter than the declared length by more than 2 per cent and its average width, determined at five evenly distributed sites, must be not less by more than 1.5 mm than the labelled one.

1.11 Fabric

Cut a size of known surface area (25, 50, 100, etc. cm^2) from the adhesive tape without removing the covering gauze. Determine its weight with mg accuracy (A) and extract in a separatory funnel with 10 ml portions of chloroform. Repeat extraction until a small amount of the chloroform leaves no residue on evaporation. Determine the combined weight of the dried fabric and the gauze (B), and the weight of the fabric separately with mg accuracy and calculate the weight of 100 cm^2 of the fabric and the weight of the adhesive plaster material spread over it.

Count the wefts and warps in one quadratic centimeter of the stretched fabric using a magnifying lens.

Calculate the amount (f) of adhesive tape material spread over 1 g of the adhesive tape, covered with gauze, using the following formula:

$$f = \frac{A - B}{A}$$

where A = weight of the adhesive tape and the gauze in grammes
B = weight of the tape fabric and the gauze without the adhesive plaster material in grammes

The tape fabric used for adhesive tapes must contain not less than 25 wefts and 25 warps for each cm^2. The weight of 100 cm^2 of the tape fabric must be not less than 1 g.

1.12 Adhesive Plaster Material

The thickness of the adhesive plaster material must be even and must adhere strongly to the fabric. It must not be unctuous and form filamens. No grains must be visible in the plaster material even under fourfold magnification. On unrolling the tape the plaster material must not come off or adhere to the other side of the tape fabric.

The thickness of the plaster layer is usually 0.1 to 0.3 mm. Each 100 cm^2 of tape fabric holds 1.2 to 2.0 g of adhesive plaster material.

1.13 Acid Value

Transfer about 1 g of the adhesive tape (eventually together with the covering gauze) weighed with mg accuracy, into a 100 ml conical flask. Add 5 ml of -chloroform and heat on a water-bath until the plaster material dissolves. Add 25 ml of R-alcohol neutralized against **I**-phenolphthalein, heat again and agitate, add 10 to 12 drops of **I**-phenolphthalein solution and titrate the alcoholic liquid with 0.1 **N** sodium hydroxide solution. Calculate the acid value by the following formula:

$$S = \frac{v \times T \times 5.61}{m \times f}$$

where v = 0.1 **N** sodium hydroxide solution, ml
T = titer of the 0.1 **N** sodium hydroxide
5.61 = amount of potassium hydroxide in mg equivalent to 1 ml of 0.1 **N** sodium hydroxide solution
m = weight of the adhesive tape in g
f = factor determined in test *1.11.*

The acid value must not exceed 40.

Note. If the adhesive tape contains salicylic acid, calculate the amount of potassium hydroxide in mg equivalent to the salicylic acid content of 1 g of adhesive tape and subtract from the apparent acid value.

1.14 Determination of Salicylic Acid

Reagents

Chloroform (Vol. II, monograph No. 130) Iron(III) chloride solution, 1 per cent [dissolve 1 g of iron(III) chloride (Vol. II, monograph No. 158) in 0.1 **N** hydrochloric acid to 100 ml].

Hydrochloric acid, **N** 0.01 (approximately).

Salicylic acid (Vol. II, monograph No. 21).

Transfer an accurately weighed portion of the adhesive tape (weighed together with the gauze layer), corresponding to about 0.02 g of salicylic acid into a 25 ml beaker, add 10 ml of chloroform, 15 ml of 0.01 **N** hydrochloric acid and heat on a water-bath until the chloroform has evaporated. Cool and filter the liquid through a cotton wool plug, moistened with water, into a 100 ml volumetric flask. Repeat the procedure five or more times until a 1 ml portion of the solution produces no violet colour on addition of 1 drop of R-iron(III) chloride solution. Combine the solutions and add enough 0.01 **N** hydrochloric acid to make 100 ml.

Transfer a 20.00 ml portion of the solution into a 100 ml volumetric flask, add 5.00 ml of iron(III) chloride solution, 1 per cent, and fill to volume with water. Allow to stand for 15 minutes and determine the extinction in a 1 cm cell in a Pulfrich photometer using a No. S 53 filter. Use as a blank 5.00 ml iron(III) chloride solution, 1 per cent, diluted with water to 100 ml.

Calculate the salicylic acid content using a calibration-graph. Dissolve 0.1000 g of salicylic acid in a 500 ml volumetric flask, in 50 ml of R-alcohol and fill with water to volume. Transfer, 10.00, 20.00, 30.00 and 40.00 ml portions of the stock solution into separate 100 ml volumetric flasks, add to each 5.00 ml of iron(III) chloride solution, 1 per cent, and proceed as described above. Plot the extinction values as a function of salicylic acid concentration, calculate the salicylic acid content of the preparation using the concentration plot. Express the percentual salicylic acid content of the plaster material. Multiply the weighing in with the *f* value determined under *1.11*.

1.15 Determination of Zinc Oxide

Transfer an accurately weighed portion of the adhesive tape (eventually together with the gauze layer) equivalent to about 0.04 g of zinc oxide, to a 200 ml glass stoppered conical flask. Add 10 ml of chloroform and agitate while gently heating. Add 5 ml of R-sulphuric acid, 10 ml of water and boil vigorously for 2 minutes. Cool, add 10 ml of chloroform, stopper the flask and agitate vigorously. Transfer the liquid quantitatively to another 200 ml conical flask, rinse the first flask and the fabric with three successive 10 ml portions of water and transfer the washing fluids to the second flask, leaving the tape fabric in the first one.

Add to the combined liquids 0.1 to 0.2 g of **I**-methylthymolblue solution and enough hexamethylene tetramine solution, 20 per cent, to produce a bright blue colour and titrate with 0.05 **M** Sodium Edetate solution to a yellow colour.

Each ml of 0.05 **M** Sodium Edetate solution is equivalent to 4.069 mg (lg .60949) of zinc oxide (ZnO).

1.2 Storage

Preserve adhesive tapes singly in metal or cardboard boxes or in paraffin paper bags, at room temperature.

Note. Desiccated, fragile adhesive tapes that are not adherent at body temperature must be discarded.

2 Aromatic Waters (aquae aromaticae)

Aromatic waters are clear, transparent or slightly opalescent aqueous or alcoholic liquids containing dissolved volatile oils or other aromatic substances. The taste and odour of aromatic waters is characteristic to the dissolved volatile oils or aromatic substances.

2.1 Preparation

Unless otherwise specified, triturate 1 g of the volatile oil or the aromatic substance with

10 g of talc (VI) (*talcum*) and transfer into a flask with small portions of
1000 g water (*aqua destillata*) of 40 to 50° temperature. Agitate the mixture vigorously for 15 minutes, allow to sediment, cool, and filter through a moistened filter paper, using talc as filtration aid.

Prepare aromatic water, official in the Pharmacopoeia, extemporaneously by dilution of the concentrates (*diluenda*), prescribed in the individual monographs, with freshly boiled and cooled water (1 + 9).

2.2 Storage

Aromatic waters have to be preserved in well closed, glass stoppered glass containers, protected from light, in a cool place. Aromatic waters should be kept on stock only in small quantities, depending on requirements.

Note. Aromatic waters which contain separated oil droplets or became mucous, cloudy or have lost the flavour should be discarded.

3 Capsules (capsulae medicinales)

Capsules are dosage forms for oral administration containing liquid or solid drugs. Capsules may have different shapes and are prepared of starch, gelatin or other suitable material.

3.1 Tests

3.11 Cachets (capsulae amylaceae)

Cachets are manufactured from aqueous starch paste by baking. They are round, odourless and tasteless capsules of different size, fitted with a suitable cover piece of the same material.

Only cachets the cover piece of which fits perfectly even when dry should be used.

Cachets must not break easily. One cachet submerged into 10 ml of water of 20° temperature, must become a tasteless and odourless paste within 10 minutes.

Shake one cachet with 10 ml of hot water, add 1 ml of water containing a few drops of R-hydrogen sulphide solution; no discolouration must be produced.

Wafers (*hostiae, nebulae*) must be of the same quality as cachets.

3.12 Gelatin Capsules (capsulae gelatinosae)

Hard or flexible capsules are cylindrical, spherical or ovoid. They may be colourless or coloured with a colouring substance authorized for medicaments and have a vitreous lustre. Gelatin capsules are manufactured of gelatin (*gelatina alba*) by additional water, eventually sugar and/or glycerin.

Flexible (soft) gelatin capsules are spherical or ovoid. The opening of such capsules may be sealed with a 10 per cent warm gelatin solution in water.

Hard gelatin capsules (*capsulae operculatae*) are cylindrical with hemispherical ends at one side and are open at the other. Two capsule-halves are combined to form a capsule shell.

Empty gelatin capsules are usually translucent or milky. They soon soften, swell in water and dissolve within 10 minutes when agitated in water at 36 to 40°.

Gelatin capsules are tested according to monograph *Gelatina alba* (Vol. II, monograph No. 162) [*g*].

Note. Dispense in gelatin capsules only medicaments which do not dissolve gelatin and do not react with it.

3.13 "Enteric Coated" Capsules (capsulae intestinosolventes)

Spherical or ovoid, colourless or light yellow, bright capsules of different size hardened with formaldehyde (*capsulae duratae*), or coated with certaine, shellac or other suitable material. The halves slid into each other are sealed with gelatin or collodion, with care that collodion does not cover the whole surface of the capsule.

When submerged into hydrochloric acid, 0.2 per cent, containing 1 per cent of pepsin, no dissolution must be noticed within two hours. Transferred into an aqueous solution containing sodium choleinate, 1 per cent, and sodium bicarbonate, 1 per cent, having a temperature of 40°, the capsules must dissolve within two hours.

3.2 Storage

Preserve capsules in closed containers, in a dry place.

4 Decoctions and Infusions (decocta et infusa)

Decoction (*decoctum*) and Infusion (*infusum*) are pharmaceutical preparations prepared from vegetable drugs, generally by aqueous extraction or by dilution of tinctures.

4.1 Preparation

The fineness of herbs, leaves and flowers comminuted for processing is generally sieve No. II, that of ligneous parts, roots and barks sieve No. IV.

Unless otherwise specified, moisten the comminuted drug (etc.) and allow to swell with water and macerate at room temperature for 10 minutes.

For decoctions extract the drug for 40 minutes, for infusions for 20 minutes in a steam bath. Decoctions are filtered when still warm, infusions after cooling and they are made up with water to the prescribed weight. If ipecacuanha-infusion is prescribed, dispense an aqueous dilution of Ipecacuanha tincture (*tinctura ipecacuanhae*) taking into consideration that 10 g of Ipecacuanha Tincture is equivalent to 1 g of ipecacuanha root.

If saponaria-decoction is prescribed, dispense an aqueous dilution of Saponaria tincture (*tinctura saponariae*), taking into consideration, that 2 g of Saponaria tincture is equivalent to 1 g of Saponaria Root (*saponariae radix*).

If an infusion of a drug containing cardiac glycosides (*digitalis folium, adonidis herba, convallariae herba*) is prescribed, proceed as follows: agitate repeatedly the comminuted drug (IV) with half of the amount of the prescribed quantity of water at room temperature, allow to stand for a quarter of an hour. Add the other half of the water hot, mix, filter after 15 minutes standing and make up with water to the prescribed weight.

If an infusion of a mucous drug (*althaeae radix, lini semen, etc.*) is prescribed, proceed as follows: rinse the drug (althaeae radix comminuted [III], lini semen

whole) with a small amount of water of room temperature, and unless otherwise specified, add for each part of drug 20 parts of water of room temperature. Allow to stand for half an hour under frequent stirring, filter and make up to the prescribed weight with water.

Other medicaments prescribed with the decoction or infusion are added to the filtered and cooled preparation.

The quantity of potent drugs must be indicated by the physician, in other cases, unless otherwise specified, 3 g of the drug is taken for each 100 g of filtrate.

Note. Prepare decoctions and infusions extemporaneously.

5 Emulsions (emulsiones)

Emulsions are liquid preparations, intended for internal or topical use, containing inmiscible liquid phases permanently dispersed in one another.

Only "oil in water" type (o/w) emulsions are suitable for internal administration.

Emulsions are prepared with the aid of emulsifiers and/or addition of thickening agents.

Emulsions of the o/w type generally are preserved with 1 per cent of preserving solution (*solution conservans*).

Correctly prepared emulsions do not separate on standing for one or two days; slightly separated (creamed) emulsions must be redispersible by one thorough shaking.

The composition of emulsions manufactured on large scale must not deviate from the declared one by more than ± 5 per cent.

6 Extracts (extracta)

Extracts are preparations prepared by extraction of drugs of vegetable origin and generally by evaporation. The consistency of extracts is either fluid, semi-liquid, pillular or dry (powdered).

The content of non-volatile matter in fluid extracts (*extracta fluida*) is between 15 and 50 per cent, that of semi-liquid extracts (*extracta subspissa*) between 50 and 70 per cent; in pilular extracts (*extracta spissa*) 70 to 85 per cent. Dry extracts contain not less than 94 per cent of non volatile matter, i.e. not more than 6 per cent of moisture.

6.1 Preparation

For the extraction of drugs only such solvents must be used which do not deteriorate the stability of the active ingredients.

Drugs are extracted by maceration (Vol. I, p. 224) or percolation (Vol. I, p. 225), according to the individual monographs.

Combine the extracts obtained with maceration or percolation and allow to stand in a closed vessel for 3 days on a cool place. Decant filter and evaporate at reduced pressure as directed under *Evaporation* (Vol. I, p. 226).

Continue percolation — unless othersiwe specified — until 5 ml of the percolate no longer exhibits the characteristics (colour, odour, taste) of the extract. With extraction of an alkaloid-containing drug, perform the following test:

to 5 ml of the percolate, collected at the tap of the percolator, add 1 drop of R-hydrochloric acid and evaporate on a water-bath to dryness. Dissolve the residue in 5 ml of water, filter and add 2 to 3 drops of 0.1 **N** iodine solution. Discontinue percolation when only a slight opalescence is produced. With preparation of fluid-extracts, collect the first portion of the percolate; evaporate the rest of the combined percolate under reduced pressure as directed under *Evaporation* (Vol. I, p. 226). Dissolve the residue in the first portion of the percolate, allow to sediment for several days in a cool place, decant and filter.

When prescribed by the individual monographs, hygroscopic extracts or such that have to be adjusted to a given content of active principle, are mixed with the required amount of lactose (V) (*lactosum*), previously dried at 105°.

6.2 Tests

The tests and assays for extracts are described by the individual monographs. Dry-extracts are assayed without previous drying but the content of the active principle is expressed with reference to the dried extract.

6.3 Storage

Preserve extracts in well closed or hermetically closed containers, protected from light, at room temperature (fluid and dry extracts) or in a cool place (semi-liquid and pilular extracts), respectively. Preserve dry extracts over a moisture adsorbing material.

7 Granulations (granulata)

Granulations are undivided, orally administered preparations, composed of grains of different shapes and surfaces, depending on the mode of their manufacture.

7.1 Preparation

Granulations are prepared from powders with the aid of additives and may be coated, if necessary.

Granulations have a fineness of sieve III if the usual single dose is not lower than 5 ml, and of sieve IV when the usual single dose is lower than 5 ml.

Granulations must contain enough diluent material to yield at least 3 ml for the usual single dose. Granulations must not contain potent drugs.

7.2 Tests

7.21 Particle Size (fineness)

Granulations are composed of particles of a fineness of sieve III or sieve IV, respectively. The quantity of smaller particles (powder) must not exceed 10 per cent of the total weight.

7.22 Distintegration

7.221 Plain Granulations

Transfer 1.00 g of the uncoated (plain) granulation into a 100 ml conical flask and add 50 ml of water of 35 to 39° temperature. Keep in a water-bath of 35 to

39° and swirl once in each 30 seconds. After 15 minutes, pour the contents of the flask onto a tared tray made of wire fabric (sieve V) (about 10 × 10 cm). Wash the granules remaining in the flask with three successive 10 ml portions of water onto the tray and dry at 105°. The weight increase of the tray must not exceed 0.10 g.

7.222 Coated Granulations

Perform the test with 1.00 g of granulation as described under *7.221* using instead of water simulated gastric fluid (*18.221*, Vol. I, p. 249). Coated granulation must disintegrate or dissolve within 30 minutes, unless otherwise specified.

7.223 Enteric Coated Granulations

Perform the test with 1.00 g of granulation as directed under *7.221*, using simulated gastric fluid (*18.221*, Vol. I, p. 249) instead of water. No disintegration or dissolution should be observed within three hours and any weight loss of the dried granulation must not exceed 10 per cent. Continue the test at 35 to 39° in simulated intestinal fluid (*18.231*, Vol. I, p. 250). Granulations must dissolve or disintegrate within one hour from the beginning of the test in simulated intestinal fluid.

7.23 Friability

Perform the test in an apparatus described in the monograph *Tablets* (*18.3*, Vol. I, p. 251).

Remove the powder by cautious sieving through sieve V, transfer 5.00 g of the granulation into the apparatus and let perform 100 revolutions. Remove the powder by cautious sieving through sieve V, and weigh the granulation remaining on the sieve with 0.01 g accuracy. The loss of weight must not exceed 1.00 g unless otherwise specified.

7.24 Active Ingredients

The content of active ingredients of granulations manufactured on large scale must not deviate by more than ±5 per cent from the one indicated on the label.

7.3 Storage

Preserve granulations in well-closed containers.

8 Injections (injectiones)

Injections are sterilized and/or aseptically prepared liquids or solids to be dissolved, intended for parenteral administration, sealed into ampoules or flasks. Included are infusion-solutions (*infusiones*), parenterally administered large volume sterile solutions. If the mode of application of the preparation is limited, this circumstance is mentioned in the monographs and must be indicated on the label.

8.1 Preparation

Injections have to be prepared by strictly following the rules laid down in the chapters *Aseptic compounding and dispensing* (Vol. I, p. 220) and *Sterilization*

(Vol. I, p. 221). If, during the preparation of an injection to be heat-sterilized, not all the conditions of aseptic compounding and dispensing can be fulfilled, the microbial content of the solution must be reduced prior to filling by filtration through a bacterium-proof filter.

Injection solutions are prepared with respect to the chapter on *Solutions* (Vol. I, p. 244), parenteral emulsions and suspensions according to the chapter *Emulsions* (Vol. I, p. 231) and *Suspensions* (Vol. I, p. 245), respectively. Microcrystalline injections contain the active ingredient, insoluble in the liquid carrier, in an easily redispersible form without formation of aggregates, during storage.

Injections have to be prepared of ingredients tested by all of the tests and assays directed in the respective monograph.

Aqueous solutions must be prepared with water for injection (*aqua destillata pro injectione*).

Oily solutions are prepared with oil for injection (*oleum pro injectione*).

Injection solutions are prepared by dissolving or diluting the weighed substance to the given volume (g/v per cent).

Suitable additives may be used to ensure the chemical and/or physical stability of the active ingredients.

To prevent the growth of microorganisms, bactericides may only be added if it is prescribed or permitted by the individual monographs or other prescriptions. Injections filled into multidose containers must contain bactericidal agents, except when the nature or concentration of the active ingredient excludes the growth of microorganisms.

The preservative and its concentration are prescribed by the individual monographs.

The quantity of added substances (including the preservatives) must not exceed the definitely necessary amount. For some preparations this is regulated by the individual monographs.

Other than the prescribed additives may be used — in case of industrial scale manufacture — only with the permission of the Institute commissioned by the Ministry of Health.

No chemical preservatives must be employed for preparations intended for intrathecal, intracysternal or peridural use and when the dose of an intravenous injection exceeds 15 ml.

Solutions for parenteral infusion must not contain chemical preservatives.

Stock solution may be used for the manufacture of injection solutions; these have to be prepared and stored in a similar manner as injection solutions.

Hydrochloric acid 0.1 **N**, prescribed in certain monographs, must be prepared extemporaneously. The pH value of injection solutions is usually between pH = 5 and pH = 7, but must not be less than pH = 3 and not more than pH = 8, unless otherwise specified by the individual monographs.

Injection solutions are generally isotonic (see [*f*] Vol. I, p. 223). Hypotonic solutions are usually rendered isotonic by addition of sodium chloride. The required amount of sodium chloride is prescribed by the individual monographs. For injections not official in the Pharmacopoeia, the required amount of sodium chloride is indicated by the graphs in Table XXI, Vol. IV.

To obtain solutions free of fibers, sintered glass filters G-4 and G-3 are used for the filtration of aqueous and oily solutions, respectively. Filtration is repeated until the liquid is clear and no fibers or suspended particles are visible by visual inspection in transmitted light with the naked eye.

The filtered solution must be dispensed immediately into suitable containers by volume, ensuring a free air space of about 20 to 30 per cent in the container.

Injections are filled into ampoules or containers that respond to the requirements of *1.1* and *1.21* (Vol. I, pp. 177—178). Ampoules and cylindrical multiple dose glass containers must be generally colourless and the latter have to be sealed by a closure in such a manner that they permit the withdrawal of the contents without removal or destruction of the closure. Containers of other materials may only be used with the permission of the National Institute commissioned by the Ministry of Health. The quality requirements for rubber closures are described by the chapter *Rubber closures* (*2*, Vol. I, p. 179). The containers must be thoroughly cleaned prior to use, and have to be rinsed with water for injection, covered with a suitable material not shedding fibers or particles, and are sterilized by dry heat.

Solutions, containing readily oxydizable substances are to be filled in an inert-gas atmosphere.

Light-sensitive solutions have to be protected under filling operations from direct sunlight or in artificial light. Ampoules and containers are transferred immediately after filling and sealing into a dark place.

While filling ampoules, the wetting of ampoule necks has to be avoided.

Each ampoule is generally filled with at least a volume-excess of the labelled size, listed in Table 30.

The ampoules have to be sealed by fusion immediately after filling; care must be taken to exclude the combustion products of the burner from the contents of the ampoule and no charring of the dissolved material in the neck of the ampoule must occur. Ampoules containing charred spots must be discarded.

The filled containers must be sealed hermetically, immediately after filling with clean and sterilized closures made of rubber or other suitable material. The sealing must be such that the unopened condition of the container may be easily controlled. Injection solutions must be sterilized immediately or not later than within one or two hours after filling. The method of sterilization is described by the individual monographs.

The still warm ampoules are submerged after sterilization into a warm methylene blue solution, 0.001 per cent. After cooling, the coloured ampoules are discarded.

Ampoules containing readily oxydizable substances are subjected to the same test using fluoresceine solution, 0.001 per cent, or other suitable dye as test bath. Injection solutions, filled into containers, are inspected individually. Cracked or not hermetically sealed containers have to be discarded.

Injections with noticeable change in colour or other properties must be rejected. Colour limits are prescribed by the individual monographs.

If the active substance is not stable in solution the drug is dispensed in so-called *powder-ampoules* or other suitable containers. Production of this type of dispensing form is carried out as de-

TABLE 30

Labelled size, ml	Content of ampoule, ml	
	mobile liquid	viscous liquid
0.5	0.6	0.65
1	1.1	1.3
2	2.2	2.3
5	5.3	5.5
10	10.5	10.7
20	20.6	21.0
50	51.5	52.0
100	103.0	105.0

scribed under *Aseptic compounding and dispensing* (Vol. I, p. 220). The container for a solid drug intended for parenteral use must permit the addition of the prescribed volume of the solvent and dissolution of the drug by agitation. The labelled quantity of the contents of the "powder-ampoules" must lie within the limits indicated by the individual monographs.

Injections, prepared in the pharmacy under aseptic conditions and not subjected to heat sterilization, must be prepared extemporaneously.

8.2 Tests

For the visual inspection of the contents of ampoules and containers, a larger number of these is slightly agitated: the solutions with the exception of emulsions and suspensions — must be clear and free of visible particulate matter. Their colour must not exceed that of the prescribed limit.

Emulsions, suspensions and microcrystalline suspensions must be homogeneous and uniform and must not contain aggregates after being thoroughly agitated for one minute. Emulsions and suspensions have to be heated to body temperature prior to the test. For determination of the volume of injection withdraw the contents of the ampoule into a controlled hypodermic syringe, expel air bubbles while holding the syringe vertically and read the volume of the liquid on the graduation of the syringe. This must be not less than the labelled volume.

The quality control of the ingredients of the injection is performed, after suitable preparation, according in essence to the prescriptions of the pertinent monographs.

Sterility is controlled according to the *Sterility Tests* (Vol. I, p. 170) and if prescribed in the individual monographs or the volume of the injection is exceeding 5 ml, test for pyrogens must be also performed, according to the rules indicated under *1*, Vol. I, p. 168.

8.21 Active Ingredients

The permissible deviation of the content of active ingredients from the prescribed values is dependent on the quantity of the substances, their properties, the mode of preparation. Injections manufactured on industrial scale, must contain the active ingredient, in general, within ± 3 per cent of the labelled amount. Preparations, tested by a biological assay, must contain the active ingredient within the limits prescribed by the individual monographs or by the monograph *Biological assays* (Vol. I, p. 154).

8.22 "Powder" Ampoules

The contents of the so-called "powder" ampoules must be homogeneous and must completely dissolve within 3 minutes on agitation with the prescribed quantity of the solvent of given composition. The solution is controlled similarly to injection solutions. Solid drugs intended for parenteral use must not contain more than 1 per cent of moisture (i.e. loss on drying), unless otherwise specified by the individual monographs. The moisture content (loss on drying) has to be determined with consideration to the quantity and properties of the solid drug sealed in the ampoule (container).

The permissible deviation of the content of the solid drug from the prescribed value is given in the individual monographs.

8.3 Storage

Preserve injections in containers ensuring the maintenance of sterility.

9 Ointments (unguenta)

Ointments are plastic gels intended for the treatment of the skin or mucous membranes. The consistency of ointments is dependent on the properties and proportion of the components.

Pastes (*pastae*) are ointments of harder consistency, containing a larger amount of powdered ingredients.

Ointments are composed of an ointment base or of a mixture of bases (the vehicle) in which the active ingredient(s) is (are) dispersed.

9.1 Preparation

Anhydrous ointment bases, containing several components are generally prepared by fusion and are filtered, if necessary. The fused mass is stirred until congealed and homogeneized the following day.

Fatty ointment bases may be dehydrated by stirring the fused mass with 20 g of anhydrous sodium sulphate for each kg of base, and filtered.

W/o type emulsion bases are prepared by dispersing the aqueous phase in small portions in the softened or fused base. Both phases are kept at about the same temperature. The emulsion is stirred until congelation and is re-homogeneized the following day.

O/w type emulsion bases are prepared by adding the aqueous phase to the fused fat phase under constant stirring. Both phases are kept at about the same temperature. The emulsion is stirred until congelation and any evaporated water is replaced. O/w type emulsion ointment bases and preparations containing such bases should be only filled into containers when they have reached room temperature.

Hydrogel ointment bases are prepared according to the properties of the individual components.

Preparing medicated ointments the properties of the active ingredients and of the ointment base must be taken into consideration.

Active ingredients must only be dissolved in an ointment base when completely soluble in it and when no separation occurs even on long period of storage.

Active ingredients insoluble in the ointment base, but forming stable solutions with water or with other indifferent solvents of required concentration, are dispersed as solutions. The quantity of the solvent is deduced from the amount of ointment base. When an emulsion type ointment base is prescribed in which the active ingredient has to be incorporated in the form of a solution, the base must be prepared extemporaneously, dissolving the ingredient in one of the phases. Ingredients which cannot be incorporated in form of solutions must be suspended in the ointment base. Such drugs are previously finely powdered (sieve VI) and either triturated with or levigated in the fused ointment base, or triturated with a small amount of an indifferent liquid similar to and incorporated in the ointment base. The quantity of the added material used for trituration is deduced from the quantity of the ointment base.

O/w type emulsion bases and hydrogels must be preserved with 1 to 2 per cent of preservative solution (*solutio conservans*) added to the aqueous phase.

If the ointment base is not specified, simple ointment (*unguentum simplex*) must be used. If an official prescription (Pharmacopoeia, Formulae Normales) prescribes a different base for a given drug, that base should be used. Unhomogeneous ointments must be homogenized, eventually after softening or melting. Evaporated water should be replaced, if necessary.

9.2 Tests

Ointments must be homogeneous. No coherent particles, droplets or knots must be observed on inspection at a fourfold magnification of the ointment spread in a thin layer over a microslide. Ointments must not be rancid.

9.21 Water Absorbing Capacity

The water absorbing capacity is the water content, expressed as per cent, weight per weight, of an ointment, prepared from the base tested as directed below.

Transfer about 10.0 g of the ointment base of 35 to 40° temperature to a tared, enamelled mortar with pestle weighed with 0.01 g accuracy. Add a quantity of water of the same temperature, corresponding to about 50 per cent of the expected water absorbing capacity indicated in the individual monographs, and emulsify. Add more water in small portions (0.5 to 1 ml), emulsifying each portion before a further amount of water is added, until no more water is absorbed. Decant excess water and blot the water droplets cautiously from the surface of the ointment and the mortar with filter paper. Determine the weight of the ointment and calculate the water absorbing capacity (W) by the following formula:

$$Wa = \frac{b - a}{b} \times 100$$

where Wa = water absorbing capacity (water content) in per cent
a = weight of the tested base in g
b = weight of the base saturated with water, in g.

9.22 Water Retaining Capacity

The water retaining capacity is the water content in per cent, weight per weight, of the ointment prepared according to *9.21* and treated as described below.

Keep the ointment, saturated with water according to *9.21*, in a well closed, completely filled container at room temperature for 24 hours. Transfer about 5.0 to 10.0 g of it to a tared glass plate, weighed with 0.01 g accuracy, and work it thoroughly and repeatedly with a spatula. Reject the separated water and blot water droplets with a filter paper. Repeat this procedure until no more water separates. Weigh and calculate the water retaining capacity (Wr) by the following formula:

$$Wr = \frac{100c - b(100 - V)}{c}$$

where Wr = water retaining capacity (water content) in per cent
b = weight of the ointment saturated with water prior to the test, in g
c = weight of the ointment after the test, in g
Wa = water absorbing capacity (water content) in per cent.

Note. The test may be carried out also by determining the water content of the ointment, prepared according to *9.22*, using the method described under *5.2*, Vol. I, p. 105. Express the water content in per cent.

The content of active ingredient of ointments manufactured on industrial scale must not deviate by more than ± 5 per cent from the declared value.

9.3 Storage

Preserve ointments at room temperature. Preserve emulsion type ointments and hydrogels and ointments prepared with polyoxethene bases in well-closed containers, other ointments in covered containers, protected from light.

10 Ophthalmic Ointments (oculenta)

Ophthalmic ointments are preparations of low bacterial content intended for the treatment of the eye.

10.1 Preparation

Ophthalmic ointments have to be prepared under aseptic conditions according to the chapters *Aseptic Compounding and Dispensing* (Vol. I, p. 220) and *Ointments* (Vol. I, p. 237), respectively. Special care must be taken to the dispersity and homogeneity of ophthalmic ointments.

If the physician does not prescribe the base for an ophthalmic ointment or prescribes soft paraffin, ophthalmic ointments have to be prepared with Simple Ophthalmic Ointment (*oculentum simplex*). Emulsion-type ophthalmic ointments must be prepared with water for injection (*aqua destillata pro injectione*).

Ophthalmic ointments are generally prepared extemporaneously and have to be dispensed into sterile collapsible tubes of less than 10 g capacity, provided with a tapering orifice.

10.2 Tests

Ophthalmic ointments must comply with the requirements specified under *Ointments* Vol. I, p. 237. The content of the active ingredient of ophthalmic ointments, prepared on large scale, must not deviate by more than ± 5 per cent from that declared.

10.21 Particle Size

Spread the ophthalmic ointment in a uniform, thin layer onto a microscopic slide. Measure 25 adjacent particles at four different sites of the preparation. None of the 100 particles must have greater linear dimensions than 20 μm.

10.3 Storage

Preserve ophthalmic ointments permitted to be held on stock in small quantities, according to requirements, protected from light, at room temperature.

11 Ophthalmic Solutions (oculoguttae)

Ophthalmic solutions are solutions of low bacterial content, intended for instillation into the eye.

11.1 Preparation

Ophthalmic solutions are generally prepared with preservative agents and, except in cases of incompatibility, are isotonic and in cases specially listed are isohydric.

Ophthalmic solutions have to be prepared in a separate, previously disinfected place. Utensils and containers used for dispensing ophthalmic solution must be sterilized or disinfected according to their character. In the course of preparation, care has to be taken to exclude any contaminations.

Ophthalmic solutions intended for single use (so-called "single-dose" ophthalmic solutions), compounded on medical order, must be prepared under aseptic conditions or be sterilized dependent on the properties of the individual components.

The mode of preparation and storage of the solvents for ophthalmic solutions are prescribed by the individual monographs. If the quantity of the ophthalmic solution is not more than 10 g the solvent may be measured volumetrically, using equal millilitres instead of grammes of the prescribed solvent.

Unless otherwise specified by the Pharmacopoeia or the Formulae Normales, ophthalmic solutions are prepared with solvent for ophthalmic solutions with phenomerbor (*solvens pro oculoguttis cum phenomerboro*) or, in case of incompatibility, with solvent for ophthalmic solutions with benzalkonium (*solvens pro oculoguttis cum benzalkonio*). Ophthalmic solutions prepared with these solvents are isotonic.

Ophthalmic solutions containing only
— ethylmorphine hydrochloride (*aethylmorphium hydrochloricum*),
— atropine sulphate (*atropinum sulfuricum*),
— ephedrine hydrochloride (*ephedrinum hydrochloricum*), and
— pilocarpine hydrochloride or sulphate (*pilocarpinum hydrochloricum or sulfuricum*)
are to be prepared with ophthalmic solution with phenomerbor (*solution ophthalmice cum phenomerboro*);

ophthalmic solutions containing only
— homatropine bromide (*homatropinum hydrobromicum*)
— methylhomatropine bromide (*methylhomatropinium bromatum*), and
— scopolamine bromide (*scopolaminum hydrobromicum*)
are to be prepared with ophthalmic solution with benzalkonium (*solutio ophthalmica cum benzalkonio*).

Ophthalmic solutions prepared with the above solvents are isotonic and isohydric.

If the prescribed drug itself is bactericidal or bacteriostatic, water for injection (*aqua destillata pro injectione*) is to be used as solvent. Similarly, ophthalmic solutions containing proteine silver or colloid silver are prepared with water for injection.

Oily ophthalmic solutions must only be prepared from drugs that are readily soluble in oil and which do not separate from the solution on standing even after a longer period; for this purpose, sterile castor oil (*oleum ricini sterilisatum*) is used as solvent.

Solutions having a freezing point depression of between 0.40° and 0.65° are considered as isotonic with lachrymal fluid. Ophthalmic solutions, prepared with water for injection, are rendered isotonic with addition of 0.7 per cent of sodium

chloride, or in presence of silver nitrate with 1.2 per cent potassium nitrate calculated for the total weight of the solution. Colloidal solutions (for example protein silver, colloid silver) must not be rendered isotonic.

Solutions having a pH value of about 7.2 are isohydric with lachrymal fluid. As there are only limited possibilities to render a solution isohydric, this should be reserved for cases prescribed by the Pharmacopoeia or the Formulae Normales.

To ophthalmic solutions containing adrenaline or resorcinol, 0.01 g of sodium pyrosulphite has to be added to each 10 g of the solution.

Ophthalmic solutions containing zinc sulphate are to be prepared in the pharmacy with dried zinc sulphate (*zincum sulfuricum siccatum*). To obtain the required quantity of the dried zinc sulphate the prescribed amount of crystallize zinc sulphate is multiplied with 0.6.

If the physician prescribes a viscous ophthalmic solution, the eye-drops have to be prepared with viscous solvent (*solvens viscosa*).

If the ophthalmic solutions contain particulate contaminations they have to be filtered through a No. G3 or G4 sintered glass filter, depending on the properties of the solution. Ophthalmic solutions have to be dispensed in containers provided with a dropper; the glass quality must meet the requirements specified under *1.1* and *1.22* of chapter *Glass*, Vol. I, p. 177. Collyria (*collyria*) are prepared similarly to ophthalmic solutions with solvent for ophthalmic solutions with phenomerbor (*solvens pro oculoguttis cum phenomerboro*), or in case of incompatibility, with solvent for ophthalmic solutions with benzalkonium (*solvens pro oculoguttis cum benzalkonio*).

Ophthalmic solutions and collyria have to be prepared, in the pharmacy on medical order, extemporaneously.

12 Pills (pilulae)

Pills have generally a weight of 0.1 to 0.3 g and are intended for internal use. Pills used in veterinary medicinal practice may be much larger (*boli*).

12.1 Preparation

Pills are prepared in the pharmacy by triturating and kneading the active component or a mixture of these with suitable additives to a homogeneous, plastic mass, that is rolled or pressed to a cylindrical string of uniform diameter, from which spheres of uniform weight are found. Pills keep their form immediately after preparation and solidify more or less during storage.

Small amounts of solid drugs are triturated with a suitable amount of lactose. Liquid medicaments, not miscible with the additives are incorporated emulsified or adsorbed on suitable agents. Pills containing powder of digitalis leaves are prepared by using 10 to 20 per cent of polyvidon (*polyvidonum*) and the inevitably necessary quantity of alcohol, 96 per cent.

Pills intended for longer storage are dried at a temperature corresponding to the nature of the material.

12.2 Coating of Pills

Pills, the components of which have to be protected from the air and moisture or those which should be insoluble in gastric fluid but *soluble in intestinal* fluid are coated with an enteric film of cellulose acetate phthalate.

Prior to coating, the carefully shaped pills are dried for a short time in a desiccator, at room temperature. After the dusting powder has been removed the pills are transferred to a wide-mouthed conical flask and moistened with enough cellulose acetate phthalate solution in acetone, 5 per cent, to wet the surface of the pills. The liquid is evenly distributed on the surface of the pills by swirling the flask. When the pills become tacky they are transferred into a dish. The solvent is allowed to evaporate. This procedure is repeated ten times.

12.3 Tests and Assays

Pills must have a smooth surface and be of equal size and must not change their shape on storage. They must not be soft or tacky. The cut surface of the pill must apear homogeneous on visual inspection.

12.31 Disintegration

Perform the test as directed under *Tablets* (*18.2*, Vol. I, p. 249). Uncoated pills must disintegrate or dissolve within 1 hour, coated pills within 2 hours.

12.32 Average Weight

Determine the average weight of pills as directed under *Tablets* (*18.4*, Vol. I, p. 251). The weight deviations must be not greater than those for tablets.

12.33 Assay

Determine the content of the active ingredient(s) as directed under *Tablets* (*18.52*, Vol. I, p. 252).

The content of active ingredient in pills, manufactured on industrial scale must not deviate by more than ± 5 per cent from the calculated or declared value.

12.4 Storage

Depending on the properties of the active ingredient, pills are preserved in well-closed containers.

13 Powders (pulveres)

Powders are intended for oral or topical administration either in dosed or in undivided form. The fineness (particle size) of powders is expressed by the sieves listed in Table 31. Dimension of other internationally used sieves is given in Table 32. For compounding in the pharmacy or preparation of galenicals the sieves listed in Table 31 are used. Sieves in Table 32 serve merely for information.

Simple powders are prepared by powdering the drug. Powdering may eventually be preceded by drying and comminution (cutting, breaking, etc.) of the vegetable drug.

Mixed powders are mixtures of simple powders, eventually also containing small amounts of liquids.

13.1 Preparation

The individual monographs indicate the fineness (sizing) of the ingredients by the Roman cipher put in parenthesis after the name of the drug. The complete

TABLE 31

Sieves

Number	Distance between the wires mm	Diameter of wire mm	Number of wires per cm	Fineness of powder
I	6.3	1.0	1.4	coarsely cut drug (*scissa*)
II	4.0	1.0	2.0	cut drug (*conscissa*)
III	2.0	0.8	3.57	finely cut drug (*minutim conscissa*)
IV	0.80	0.5	7.7	moderately fine powder (*pulvis grossus*)
V	0.32	0.2	19.25	fine powder (*pulvis semisubtilis*)
VI	0.16	0.1	38.4	very fine powder (*pulvis subtilis*)

Editors' Note to the English Text:

Sieves No. I. II and III are mostly used for the classification, according to particle size, of vegetable drugs which are cut to provide an appropriate material for the preparation of teas.

amount of drug, without any residue, has to be powdered. Drugs of vegetable origin containing no volatile oil are dried previously at a temperature not exceeding 60°. The greater part of the medicament should correspond to the prescribed fineness.

Drugs administered as powders are comminuted to a fineness corresponding to sieves V or VI, respectively, depending on their mode of application. Dusting powders should be of a fineness of at least VI.

The individual components of powder-mixtures have to be of equal fineness and should contain the components in homogeneous distribution.

Powders — if required — are sterilized as directed under *Sterilization* (Vol. I, p. 221).

Powders of more than 100 g have to be sieved as a step of preparation in all cases; lesser quantities only if the nature of the ingredients necessitates it. Powders have to be mixed after sieving.

Ingredients that are friable or contain much water of crystallization should be used in their siccated form in powder mixtures; deviation in the molecular weight or dose is taken into consideration.

Aromatic sucrose powder (*eleosaccharum*) contains 1 drop of volatile oil for 2 g of powdered sucrose (*saccharosum*).

13.2 Tests

Sieve the powdered medicament by passing through the prescribed sieve. The powder must completely pass the prescribed sieve and not more than 40 per cent of it must pass the sieve of the next higher number.

13.3 Storage

Preserve powders depending on their properties and the specification of the individual monographs in well closed containers.

TABLE 32

Sieves used internationally

German Industrial Standard (DIN)		USP XVII	
Number of sieves*	Nominal size of aperture mm	Number of sieves**	Nominal size of aperture mm
1	6.0	2	9.52
2	3.0	4	4.76
3	2.0	8	2.38
4	1.5	10	2.00
5	1.2	20	0.84
6	1.0	30	0.59
8	0.75	40	0.42
10	0.60	50	0.297
11	0.55	60	0.250
12	0.50	70	0.210
14	0.43	80	0.177
16	0.375	100	0.149
20	0.300	120	0.125
24	0.250	200	0.074
30	0.200		
40	0.150		
50	0.120		
60	0.100		
70	0.086		
80	0.075		
100	0.060		
130	0.042		

* The number of sieves indicates the number of meshes included in a length of 1 cm in each transverse direction parallel to the wires.
** The number of sieves according to the USP XVII indicates the number of meshes included in a length of 2.54 cm (1 in) in each transverse direction parallel to the wires.

14 Solutions (solutiones)

Solutions are clear liquids, free of sediment, prepared by dissolution of drugs and are intended for internal or topical use.

14.1 Preparation

Solutions may only be prepared of such drugs and concentrations that produce solution from which solids do not separate even on standing for longer periods, and in which the properties of the ingredients and the solution itself (colour, odour, etc.) do not alter. The ingredients must not alter with dissolution, except when this is intended by dissolution.

If an ingredient dissolves slowly at room temperature but is soluble by heating without decomposition or other alteration and does not separate on cooling, dis-

solution may be promoted by moderate heating at a temperature compatible with the properties of the material. Dissolution may be hastened in some cases by powdering the material. The order of succession and the mode of dissolution are dependent on the properties of the ingredients. Volatile substances or materials of strong odour are usually added last to the liquid. Solutions containing several components are usually prepared by dissolving the ingredients in the order of increasing quantities, with regard to their solubility. Stock solutions of known concentration may be used for the preparation.

The prepared solution is agitated and filtered, if necessary.

If the solvent is not specified, water (*aqua destillata*) is to be used.

If the concentration of the alcohol is not specified for the preparation of an alcoholic solution, alcohol, 90 per cent (*spiritus concentratus*) is to be used.

Solutions are prepared by dissolving or diluting the weighed substances to a given weight. (Weight by weight per cent.) Registered preparations may be prepared — with the permission of the Institute commissioned by the Ministry of Health — by dissolution of substances to a given volume (weight by volume per cent).

Components of sirups readily soluble in water or alcohol are added dissolved in a quantity of water, alcohol or a mixture of these equivalent to 5 per cent of the syrup, the prescribed quantity of which must be reduced by this amount. If official stock solutions (calcium bromide, calcium chloride, sodium bromide) are used in syrups, the quantity of water exceeding the 5 per cent should be also substracted from the amount of the syrup.

To ensure the stability of solutions and to enhance solubility of the active ingredient additives may be used. The amount of the additive must not surpass the inevitably necessary quantity. The preservative agent and its quantity is specified by the individual monographs.

The content of the active ingredients of solutions prepared on large scale must generally not deviate from the declared by more than ± 3 per cent.

15 Suspensions (suspensiones)

Suspensions are liquid preparations intended for internal or topical use in which the solid phase is uniformly dispersed in the liquid dispersion medium.

The finely powdered ingredients of the disperse phase have to be distributed uniformly throughout the liquid phase, the viscosity of which may be increased by suitable additives. The wettability of the solid ingredients may be increased by the addition of the just required amount of a surfactant.

If the dispersion medium is not specified a mixture of water and methyl cellulose mucilage (*mucilago methylcellulosi*) (7 + 3) has to be used. Aqueous-mucilaginous suspensions have to be preserved with 1 per cent of preserving solution (*solutio conservans*).

Properly prepared suspensions sediment slowly and are redispersible by a single, energetic agitation.

The composition of suspensions prepared on large scale must not deviate by more than ± 5 per cent from the declared.

16 Suppositories (suppositoria, globuli et bacilli urethrales)

Suppositories are preparations intended for application in body cavities, melting or dissolving in body fluids at body temperature.

Rectal suppositories (*suppositorium*) are conical, or cylindrical, with one conical end, or torpedo-shaped. The weight of rectal suppositories is usually about 2 to 3 g, depending on the quantity of the active ingredients. The weight of suppositories for babies or small children is 1 to 1.5 g.

Vaginal suppositories are globular (*globulus*) or have flattened ovular form (*ovulum*). Their weight is usually 1 to 4 g, that of vaginal cylinders 6 to 10 g.

Urethral suppositories (*bacillus urethralis*) are 4 to 6 cm long cylinders of 2 to 5 mm diameter, with one conical-shaped end.

The weight of suppositories, prepared on medical order, has to be recorded on the prescription.

As bases (*vehiculum*) for these preparations theobroma oil (*butyrum cacao*) solid fat (*adeps solidus*), polyoxethene mass (*massa polyoxaetheni*) or any other admitted material may be used. If the base has not been prescribed by the physician, theobroma oil or solid fat has to be used.

16.1 Preparation

Suppositories are prepared by moulding or pressing.

Insoluble or sparingly soluble ingredients are dispersed as fine powders (VI) in the base. Liquid ingredients, inmiscible with the base, are emulsified or dispersed in the molten base.

Ingredients that are readily soluble in the base and do not separate during storage, are dissolved in the molten base.

Active ingredients have to be incorporated possibly in the form of readily soluble compounds. If the prescribed, insoluble or slightly soluble in water compound has a readily soluble form, this must be used. If the usual dose of the insoluble and readily soluble forms are identical, the same quantity is used; otherwise the difference in molecular weight or percentual composition must be taken into account.

Dry extracts are to be dispersed in the base after liquefaction with just the required amount of water.

Small amounts of the active ingredient, insoluble in the base, are triturated with enough lactose (*lactosum*) to produce a powder mixture of 20 per cent by weight of the preparation and dispersed in this form in the base.

In the presence of ingredients which decrease the solidification point of the base, indifferent additives miscible with the base may only be used to such an extent that the preparation meets test requirements.

16.2 Tests

Suppositories must be homogeneous, uniform and of suitable consistency. They must not be rancid.

Unless otherwise specified, the preparations prepared with water-insoluble bases must liquefy within 15 minutes at 37°. Preparations, prepared with water-soluble bases must dissolve in water within 1 hour at 37°.

In doubtful cases the liquefaction or dissolution of *rectal suppositories* is to be tested in the apparatus shown in Figure 28. The apparatus consists of a water-

bath into which an inner tube is inserted and fixed with a stopper. The inner tube is restricted and is closed by a cork at the bottom. The apparatus is fitted with a thermometer. A 180 mm long tube, sealed at both ends, carrying a rim at its lower end, weighed with lead-shot, has a weight of 25.00 g. Water of 36 to 38° temperature is circulated in the outer tube.

Fill 20 ml of water of 37° into the inner tube, introduce the tested suppository with its point downwards and onto that, place the glass rod of 25.00 g of weight. The test begins at this moment. Melting or dissolution is considered as completed when the lower rimmed end of the glass rod reaches the narrowed part of the inner glass tube.

The liquefaction or dissolution of *vaginal or urethral suppositories* is tested as follows: transfer one vaginal suppository, or a 3 to 4 cm long piece of the urethral suppository, into a 200 ml conical flask. Add 50 ml of water of 37° temperature. Maintain the flask in a water-bath of the same temperature and swirl once every 30 seconds. The procedure is finished, when the total mass of the suppository has liquefied or dissolved.

The weight of the preparations prepared on large scale must not deviate from the average weight by more than ±5 per cent.

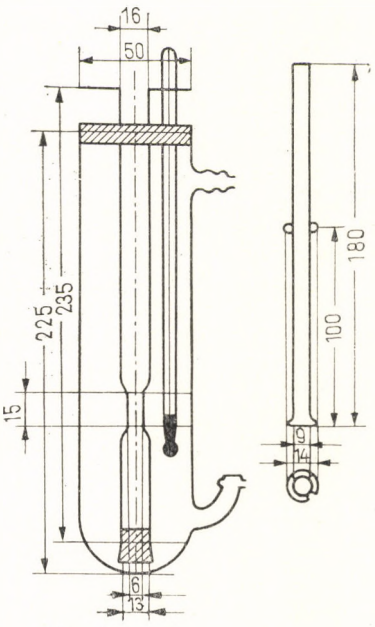


FIG. 28

The content of active ingredient of rectal, vaginal and urethral suppositories prepared on large scale must not deviate by more than ±5 per cent calculated for the average weight.

16.3 Storage

Preserve rectal, vaginal and urethral suppositories, singly wrapped, in well-closed containers, respectively, in a cool place.

17 Syrups (sirupi)

Syrups are concentrated sucrose solutions, in water, or drug solutions, containing eventually added flavouring substances.

17.1 Preparation

Sucrose (*saccharosum*) is dissolved in the manner specified by the individual monographs. After dissolution the syrup is clarified, adjusted with water to the prescribed weight and filtered. Some syrups are preserved with preserving solution (*solutio conservans*).

17.2 Storage

Preserve syrups in well-closed containers. Stocks should be stored in completely filled bottles, in a cool place.

18 Tablets (tablettae)

Tablets are dosage forms prepared by compression, containing prescribed amounts of one or several active ingredient(s).

Tablets are, depending on their route of administration and use oral, lingual, buccal, vaginal, are intended for implantation or for preparation of solutions. Tablets are flat or biconvex shaped discs, rarely oval or angular. The weight and size (diameter) of tablets are dependent on the amount of the ingredients and the mode of administration. Tablets prepared on large scale may be provided with a notch to facilitate dividing the tablet and/or the name of the preparation or other differentiating sign.

The surface of the tablets must be homogeneous, the edges intact. The surface of coated tablets are intact, even, smooth and polished. The surface of compression coated tablets is not polished.

Tablets and coated tablets must be suitable for storage and packaging.

18.1 Preparation

Active ingredients and additives are sized to specified fineness and thoroughly mixed to ensure homogeneity.

The powdered mass is granulated, if necessary, by a moist or dry method. If the amount of fine powder is disadvantageous for tableting, this is separated by sieving with sieve V and the granulate is compressed.

Oral tablets, generally, must not weigh less than 0.1 g.

A restricted number of extemporaneously prepared tablets, not intended for storage, may be prepared with the aid of simple granulation (*granulatum simplex*) as follows:

Ingredients

Lactosum	(V)	60 parts by weight
Saccharosum	(V)	16 parts by weight
Amylum solani	(V)	20 parts by weight
Talcum	(VI)	2 parts by weight
Magnesium stearinicum	(VI)	2 parts by weight

Mix the lactose, sucrose and potato starch and pass through sieve V. Granulate the powder with enough warm (60°) potato starch paste, 5 per cent, press the mass through sieve IV and dry. Pass the dry ganules first through sieve III, then through sieve IV, add the talc, the magnesium stearate and mix.

To the powdered (V) active ingredient add a suitable quantity of simple granulation, mix and compress.

Note. The amount of simple granulation used for tablets prepared on medical prescription in the pharmacy, has to be recorded on the prescription.

Unless otherwise specified by the individual monographs, the amount of talc must generally not exceed 3 per cent by weight of the tablet.

Implantation tablets must be prepared under aseptic conditions as directed under *Aseptic Compounding and Dispensing* (Vol. I, p. 220), using suitable additives. Implantation tablets must be packed and stored in individually sealed, sterile glass vials.

18.11 Coating

Tablets may be coated by wet or dry methods.

In certain cases the tablets may be coated as to render them insoluble in the gastric fluid, but permitting dissolution in the intestinal fluid (enteric coating).

In special cases the coating may also contain active ingredients.

The amount of talc must generally not be more than 10 per cent by weight of the coat.

For the colouring of oral tablets and their coatings only certified colouring agents, authorized for the dying of medicaments are permitted.

Small tablet batches may be enteric coated as described for *Pills* (Vol. I, p. 241).

18.2 Disintegration Tests

18.21 Disintegration of Uncoated Oral Tablets

18.211 Flask Test

Transfer three tablets to a 100 ml conical flask and add 30 ml of water of 35 to 39° temperature. Immerse the flask into a water-bath of the same temperature and swirl once every 30 seconds. Unless otherwise specified, the tablets mus-dissolve, disintegrate or soften to a degree that they disintegrate on slight prest sure within 15 minutes. In doubtful cases, or if the tablets do not dissolve, disintegrate or soften enough within 15 minutes, proceed as directed under.

18.212 Apparatus test

The apparatus consists of a basket into which a perforated, shafted plate is fitting. The sides of the basket and the shafted plate consist of a transparent, indifferent material. The bottom and the rear side of the basket are made of stainless steel sieve texture (IV). The basket, with the loosely fitting perforated, shafted plate, is kept by a mechanism in motion, and is making nine 2 millimeter swings, followed by one 10 millimeter swing within 10-second periods.

The apparatus is submerged into a 1000 ml beaker containing the test-liquid of 35 to 39°. The level of the test liquid must cover the upper edge of the basket when in resting (horizontal) position.

Unless otherwise specified, water is used as the test liquid.

Lift the basket out of the test liquid and place into it 3 to 18 tablets, depending on the size of the tablets. Place simultaneously only so many tablets into the basket that they form a single layer, without overlapping one another. Test altogether 18 tablets.

Place the perforated plate onto the tablets, immerse the basket into the test-liquid and start simultaneously the swinging motion of the apparatus.

Unless otherwise specified, 16 of the 18 tested tablets must disintegrate within 15 minutes.

18.22 Disintegration of Coated Tablets

18.221 Flask Test

Test the disintegration of coated tablets that are intended to dissolve in gastric fluid according to *18.211*, using *simulated gastric fluid* of the following composition as test liquid, at 35 to 39°.

2.0 g of sodium chloride (*natrium chloratum*), and
3.2 g of pepsin (*pepsinum*) in a mixture of
25.0 g diluted hydrochloric acid (*acidum hydrochloricum dilutum*) and enough water (*aqua destillata*) to yield 1000 ml of solution.

The pH of simulated gastric fluid is about pH = 1.2.

In doubtful cases, or if the tablets do not dissolve, disintegrate or soften to the required grade within one hour, perform the test as directed under *18.222*.

18.222 Apparatus Test

Perform the test according to *18.212* using simulated gastric fluid (*18.221*) as test liquid.

Unless otherwise specified, 16 of the 18 tested tablets must disintegrate within one hour.

18.23 Disintegration of Enteric Coated Tablets

18.231 Flask Test

Test the disintegration of enteric coated tablets as directed under *18.221*. The tablets must not show any sign of disintegration or dissolution within three hours in simulated gastric fluid and the loss of active ingredient of the dried enteric coated tablet must be not more than 10 per cent at the average.

Proceed with the test using simulated *intestinal fluid* of the following composition at 35 to 39°:

Dissolve
6.8 g of sodium acid phosphate (*natrium phosphoricum acidum*) in
250.0 g of water (*aqua destillata*). Add 19 ml of R-sodium hydroxide solution and
570.0 g of water (*aqua destillata*). Dissolve in the liquid
10.0 g of pancreatin (*pancreatinum*) and adjust the pH of the solution to pH = 7.4 to 7.5. Add enough water to make 1000 ml.

In doubtful cases, or if the tablets do not dissolve, disintegrate or soften to the required grade within one hour, in simulated intestinal fluid, perform the test according to *18.232*.

18.232 Apparatus Test

Perform the test according to *18.212* using first simulated gastric fluid (*18.221*). The tablets must not show any sign of disintegration or dissolution within three hours and the loss of active ingredient of the dried enteric coated tablet must be not more than 10 per cent at the average.

Proceed with the test using simulated intestinal fluid (*18.231*).

Unless otherwise specified, 16 of the 18 tested tablets must disintegrate within one hour from the beginning of the test in simulated intestinal fluid.

18.24 Disintegration of Tablets Intended for the Preparation of Solutions

Onto three tablets, placed into a small flask, pour enough water of room temperature to make a solution of similar concentration to that which is to be used. Swirl the contents of the flask once every 30 seconds. The tablets must completely dissolve within 5 minutes.

18.25 Disintegration of Other Tablets

Tablets intended for vaginal or buccal use are tested according to that prescribed for oral tablets. Unless otherwise specified, buccal tablets must disintegrate in not less than 5 minutes and not more than 30 minutes.

Vaginal tablets are tested in a solution of the following composition:
Dissolve in 30 ml of water (*aqua destillata*) of 40 to 50° temperature
3.0 g of calcium lactate (*calcium lacticum*) add
1.00 ml of lactic acid (*acidum lacticum*) and make up to
100 ml with water (*aqua destillata*).

Vaginal tablets must disintegrate in this solution within 10 minutes.

18.3 Friability Test

The test is performed in an apparatus consisting of a drum of 280 mm inner diameter and 40 mm depth, made of metal, or transparent plastic material that is protected from static electricity. One lid is removable. The inner surface of the apparatus is polished. A curved baffle plate, reaching from the centre of the drum to the superficies, is mounted in the drum. The drum rotates around the horizontal axis at 25 r.p.m. The tablets placed into the drum make rolling and sliding movements and fall freely with each revolution from a height of about 155 mm.

Place into the drum 10 tablets, carefully dusted with a soft hair brush and weighed with mg accuracy. Replace the lid of the apparatus and allow to make 100 revolutions. Remove the tablets from the apparatus, dust them cautiously with a brush and weigh them with mg accuracy. Express the weight loss as percentage of the weighed-in tablets. Perform three parallel tests with-each batch.

Unless otherwise specified the friability of the tablets, determined by the above method, must be not more than 3 per cent.

The tablets must not break or cap during the test.

The friability of coated and implantation tablets is not tested.

18.4 Average Weight

Determine the bulk weight of larger number, usually 50 tablets, with 0.01 g accuracy and divide the weight with the number of tablets. The limit of the weight deviation of the individual tablets from the calculated average weight — determined with mg accuracy — is listed below.

Average weight g		Permissible deviation per cent
up to	0.10	±15
0.10 to	0.25	±10
0.26 to	0.50	± 8
above	0.50	± 3

The weight of 90 per cent of the tablets must correspond to the average weight of the tablets within the admitted deviation range. The weight deviation of 10 per cent of the tablets must be not more than the double of the admitted.

The above admitted deviation limits refer also to the declared and established weight differences of the tablet.

The same method is used for the determination of the average weight of coated tablets. The established weight of coated tablets must not deviate from the average weight by more than ± 15 per cent.

18.5 Quantitative Tests and Assays

18.51 Preparation of Tablets for the Assays

When coated tablets are assayed, usually the coat must be removed. After the coating has been completely or partly removed, the average and individual weight of the tablets must be determined again to ensure a reliable basis for the calculation.

18.52 Assay of the Active Ingredient

Carefully powder 5 to 10 tablets. Weigh in the quantity of the powder, prescribed by the individual monographs. If necessary, remove the disturbing ingredients from the powder.

Perform the assay as described in the individual monographs.

Unless otherwise specified, the quantity of the active ingredient, calculated for the tablet of average weight, must not deviate by more than ± 5 per cent from the declared value.

18.53 Assay of Talc

If the tablet contains no substance insoluble in water, alcohol or chloroform, weigh accurately 0.5 g of 5 to 10 powdered tablets. Transfer the powder to an accurately tared paper filter, dried at 100° (**4**, Vol. I, p. 103). Wash the powder with small portions of warm R-alcohol, until 5 ml of the filtrate, evaporated on a watch glass, has no residue. Repeat the procedure with warm R-chloroform and finally with warm water. Proceed as directed under **4**, Vol. I, p. 103.

If one of the ingredients is removable with the solvents but is combustible, dry the filter with the washed powder, weigh, incinerate according to *3.1* Vol. I, p. 103, and ignite. In such cases the loss on ignition of talc and the residues on ignition of the other ingredients must be taken into consideration when calculating the quantity of talc.

18.6 Storage

Preserve tablets as directed by the individual monographs.

19 Teas (species)

Teas are mixtures of comminuted vegetable drugs. In certain cases teas may also contain salts.

19.1 Preparation

Prior to preparation of teas, the dust and powder is removed from the comminuted drugs by sieving through sieve IV. Prior to mixing smaller parts of drugs, containig volatile oils, are crushed. Smaller flowers are used without comminution.

Larger flowers are comminuted to sieve size I, herbs and leaves to sieve size II, roots, rhizomes, barks, ligneous parts, coriaceous leaves and fruits to sieve size III.

If salts have to be added to teas, the tea is uniformly moistened with an aqueous solution of the salt, dried and mixed.

19.2 Tests

Remove the powder with sieve V, from 50 g of the carefully mixed tea and weigh. The powder must not weigh more than 5 per cent of the tea. For teas containing powdered medicaments, subtract the calculated weight of these components from the quantity of powder removed by sieving. Spread out the tea remaining on the sieve, sort and weigh the components.

The quantity of the ingredients may deviate from the declared percentual composition as listed below:

Calculated percentual quantity of the ingredient	Permissible deviation, per cent
up to 10 per cent	40
11 to 25 per cent	20
26 to 50 per cent	10

19.3 Storage

Preserve teas in closed containers, those containing volatile oils in well-closed containers.

20 Tinctures (tincturae)

Tinctures are dilute alcoholic or ethereal-alcoholic extracts of vegetable or animal drugs. The odour and taste of tinctures are characteristic of the menstruum and the extracted drug.

20.1 Preparation

For the preparation of tinctures the drugs or drug mixtures have to be suitably dried and comminuted to a grade prescribed by the individual monographs.

Extract the drug, as directed in the individual monographs, by maceration (Vol. I, p. 224) or percolation (Vol. I, p. 225). Unless otherwise specified use alcohol, 70 per cent (*spiritus dilutus*) as the menstruum. Allow the tincture to sediment after maceration or percolation in a closed vessel, in a cool place, for at least 3 days, filter and make up with the menstruum to the prescribed weight.

In certain cases, the content of the active ingredient is to be adjusted to a prescribed concentration with the menstruum.

Control such tinctures each year. Record the date and the results of the control on the container.

20.2 Storage

Tinctures should be preserved in well-closing containers, at room temperature, protected from light.

IV SEROBIOLOGIC PREPARATIONS, DIAGNOSTICS AND BLOOD PREPARATIONS

(A) PREPARATIONS FOR HUMAN USE

1 General Notices

Serobiologic preparations for human and veterinary medicinal use are considered all microbial products and blood serum preparations which are suitable for the prophylaxis and seldom for the therapeutic treatment of human subjects or animals, and which are generally administered by the parenteral route to produce or enhance a state of specific immunity of the organism.

To this category belong also the so-called normal blood sera (non-specific serobiologic preparations) and diagnostic preparations of serobiologic origin.

Within the category of serobiologic preparations with specific activity there are some which are classified as serobiologic preparations with antigenic action. and a second group termed as serobiologic preparations with antibody activity,

2 Vaccines (vaccina)

Vaccines are preparations with antigenic effect containing living or dead microorganisms or antigens — metabolites of these — either in free or in adsorbed state. They also are termed as active serobiologic preparations, since — due to their activity — the recipient organism itself produces specific antibodies. Serobiologic products with antigenic action are suitable and generally used for prophylaxis, and rather rarely for therapeutic purposes. In the strictest sense of the term vaccines are preparations containing pathogenic microorganisms (i.e. their antigens) in some modified state in which they are harmless for the recipient or in which any unwanted side effects are markedly reduced.

The precipitated (adsorbed) vaccines are meant to reduce the frequency of administration of the preparation necessary to achieve the wanted antigenic effect.

The potency of vaccines is expressed in terms of their bacterium count per ml or by the number of bacteria used in the process of their production.

The potency of toxins is given in generally accepted units, or in the *International Units* (I.U.) of the corresponding antitoxins which neutralize the toxins in flocculation or binding tests. The potency of the Scarlet Fever Toxin is expressed in *Dermal Units* (DU), 1 DU being defined as the smallest quantity of the toxin which causes a reddening of 10 mm diameter when injected intradermally to susceptible subjects. The unit of diphtheria toxin is defined as the smallest quantity (*minimal lethal dose* = MLD) that, if injected subcutaneously, kills a guinea-pig weighing 240 to 260 g within 4 days.

The serobiologic preparations of antigenic effect, vaccines, are classified in 5 categories according to the method of their production. (Serobiologia preparations containing several kinds of antigens are also used.)

2.1 Vaccines Containing Avirulent Living Microorganisms (Smallpox, BCG, etc. Vaccines)

The microorganisms introduced by this type of preparations propagate in the recipient's organism, causing a mild infection, and stimulating the organism of the recipient to enhanced production of antibodies more effectively than the same microorganisms in dead state.

2.2 Vaccines Containing Dead Microorganisms (Typhoid, Cholera, Typhus, etc. Vaccines)

Pathogenic microorganisms are cultivated according to their individual nature, then killed, suspended and diluted with adequate vehicles to the necessary strength.

2.3 Vaccines Containing Extracts of Microorganisms (Adsorbed Typhoid, etc. Vaccines)

Microorganisms are digested by an adequate procedure and their antigenic ingredients extracted and adsorbed, generally to aluminium hydroxide.

2.4 Toxoids (Anatoxins, Diphtheria, Tetanus, etc. Toxoids)

Toxins of complete (intact) antigenic effect detoxicated by the combined action of formaldehyde and heat.

2.5 Toxins (Scarlet Fever Toxin, etc.)

Toxins may be used in a free state or adsorbed, or fixed by a specific blood serum.

According to their origin and composition, serobiologic preparations are either clear, transparent liquids or whitish, homogeneous suspensions and white or yellow-coloured, precipitous fluids respectively.

The active ingredients of serobiologic preparations are sensitive to both heat and light, and thus they must be preserved at 2 to 10° temperature, on a dry place, protected from light. Preparations, the colour or consistency of which has altered, or in which filaments, granules, knots, furs or moulds occur, are unsuitable for use.

3 Antisera (Sera)

These preparations contain specific antibodies, active against the antigens of individual pathogenic organisms, possibly in concentrated state. There are sera containing antibacterial, antiviral and antitoxic antibodies, and convalescent sera.

There are also combined sera containing both antibacterial and antitoxic antibodies. All sera of this type introduced into the recipient organism produce passive immunity, thus supporting the recipient organism to overcome disease. Antisera are also meant primarily for prevention, and only in some cases for therapy.

Antisera are produced most frequently in horses. It is, however, necessary to produce also antisera in other animals (cattle, sheep, pigs) in order to avoid anaphilaxis or serum disease occurring after repeated application of sera derived of the same animal species.

Purified sera contain large amounts of antibodies in a small volume.

The potency of sera is determined on the ground of their toxin-neutralizing, agglutinating, protective and virus-neutralizing effect. The potency is expressed in generally accepted units. The International Unit (I.U.) of Diphtheria Antitoxin is the quantity of the Diphtheria Antitoxin which is adequate to neutralize by binding 100 guinea-pig MLD. The Unit of the Scarlet Fever Antitoxin in Hungary is the quantity of the antitoxic serum which neutralizes 1000 DU of the Scarlet Toxin.

Antisera are classified according to their active ingredient content and method of production into 4 categories.

3.1 Antibacterial and Antiviral Sera (Anthrax, Erysipeloid, etc. Antisera)

Antisera acting by the destruction of pathogenic microorganisms within the recipient organism or by supporting latter in their destruction.

3.2 Antitoxic Sera (Diphtheria, Tetanus, etc. Antitoxins)

Sera acting by the binding of toxins circulating in the recipient organism.

3.3 Antibacterial and Antitoxic Sera (Dysentery, etc. Antisera)

Sera combining the effects of both antibacterial and antitoxic serum preparations.

3.4 Reconvalescent Sera (Scarlet Fever, etc. Antitoxic Antisera)

Sera containing the antibodies of human patients recovered from a viral or bacterial disease.

4 Non-Specific Sera

Non-Specific sera are the so-called normal blood sera prepared from the blood of healthy animals (horses, cattle, sheep, etc.) not treated with any antigens (non-immune sera), thus containing no specific antibodies. Hence these sera are not suitable for the treatment of specific diseases, however, they are applied in the treatment of some non-specific conditions (internal bleedings, haemophilia, etc.).

Fresh blood sera are clear, transparent, light or somewhat more intensive yellow-coloured, odourless liquids, which, however, may become later turbid due to separation of some labile proteins. Their potency rapidly decreases in the first period after their production, therefore they are issued only after 4 to 6 months of storage. The potency of the sera decreases also at high temperatures, hence they must be preserved at 2 to 10° temperature, on a dry place, protected from light. To compensate for any loss of potency during storage, the potency of immune sera when issued, must be by 20 per cent higher than the one labelled on the container.

As preservatives, blood sera may contain 0.5 per cent of phenol, 0.3 per cent of tricresol , 0.1 per cent of Quinosol or 0.01 per cent of Merthiolate. They must not contain living microorganisms. Any precipitation of proteins must not exceed the one causing not more than a slight opalescence. Sera turbid without having been shaken, those containing filaments or mold, those changed in colour or having a smell different from that of the preservative must be discarded.

5 Diagnostic Preparations

Diagnostic preparations (e.g. Tuberculin, Mallein, Schick Toxin, Dick Toxin as well as agglutinating, precipitating, haemolytic, etc. sera and others) are preparations used for the testing of susceptibility or resistance to infectious diseases, for determination of the blood group, for typing of microorganisms, etc., by microbiological or serological methods. They may contain, as active agents one or several antigens, antibodies or complement. According to the route of their production they may consist of suspended microorganisms of known antigenic properties, of cell extracts cellular substances or metabolites of these; organ extracts, and specific or non-specific blood sera of human or animal origin.

Their potency is characterized by their bacterium count or toxin content per ml, or by the highest dilution evoking the corresponding reaction.

Diagnostic preparations should be prepared and preserved as prescribed for vaccines and sera.

6 Registration and Control

Serobiologic preparations must be issued and preserved in hermetically closed vials or sealed ampoules. On the label of the outside packing and partly on that of the direct container the following data must be stated: the manufacturer's name and address; the name of the preparation; volume in ml and composition or potency, batch number, registration number, expiration date, direction for storage, etc.; for freeze-dried (lyophilized) preparations also the composition and quantity of the enclosed (or necessary) solvent. Direction for use, including indications and dosage, corresponding to valid legal regulations, must be attached to each package.

The following excess volume must be dispensed in each container:

up to 2.0 ml ... 0.1 ml
up to 5.0 ml ... 0.2 ml
above 5.0 ml ... 0.5 ml

Prior to issuing, each batch of serobiologic preparation must be checked by the manufacturer for quality, sterility and innocuity, and their potency must be determined. The method and process of production, as well as the quality of the end product is controlled by the National Institute of Public Health, too.

(B) VETERINARY PREPARATIONS

1 General Notices

Immune sera for veterinary use are produced from the blood of solipeds (horses, asses and mules) or by separating the serum from the corpuscular elements of the blood after coagulation and dispensing under sterile conditions in appropriate vessels. The sera thus prepared are issued after subjecting them to the official tests and assays. Similarly, blood sera may be prepared from ruminants, pigs, dogs and fowls. It is practical to separate serum from defibrinated blood by centrifugation. The sera prepared by the first route are pale yellow,

whereas those produced by the second one are, due to their haemoglobin content, somewhat reddish if freshly prepared, turning brown on prolonged standing.

Independently from the method of preparation all sera issued must be clear and transparent. During storage, some sedimentation may occur, whence they may become slightly opaque when shaken. Preparations being opaque without being shaken or those showing a pellicle on their surface are unsuitable for use and must be discarded.

Sera for veterinary use must not contain pathogenic microorganisms. Those, however, containing not more than 100 non-pathogenic bacteria per ml are not objectionable.

Serobiologic preparations for veterinary use may contain 0.5 per cent of phenol, 0.4 per cent of tricresol or 0.01 per cent of merthiolate as preservatives. For checking the phenol-, tricresol or merthiolate content each batch of the blood serum should be tested for innocuity by injecting 0.5 ml portions subcutaneously to 2 white mice weighing 15 g each. No symptoms suggesting intoxication must occur, or at most in a rather mild form e.g. some tremor in the very first hours if any.

Notes. (a) Serobiologic preparations other than blood sera must comply in their general properties with the requirements of the corresponding preparations for human use.

(b) If in a monograph alternative methods (*a* and *b*) are official for the determination of potency, it is sufficient to perform one of them.

2 Registration and Control

Serobiologic preparations for veterinary use must be dispensed in hermetically closed vessels or sealed ampoules.

The abbreviation AUV (for Ad Usum Veterinarium) must be indicated both on the outer labelling and on the vessel or ampoule.

Further, all data required by valid regulations concerning the manufacturer's address, the name, amount, composition and/or potency of the preparation, batch number, registration number, expiration date, directions concerning storage, etc. for freeze dried preparations also the kind and quantity of solvent must be similarly indicated.

To each package of any preparation an adequate direction for use must be enclosed including interpretation for dosage and route of application, in accordance with the val **d** regulations.

To preparations containing virulent microorganisms, directions must be attached also indicating the method of inactivation of any unused remainders.

(C) GENERAL CONTROL TESTS

1 Sterility Tests

Check sterility according to **4**, Vol. I, p. 170.

2 Safety Tests

2.1 Safety Test of the Active Ingredient

Inject subcutaneously into a guinea pig weighing 250 to 300 g 5.0 ml of the vaccine, toxin or toxoid, or 10.0 ml of the serum to be tested. Observe the animals generally for 10 days; when absorbed vaccine is tested for 30 days, and when diphtheria toxoid is tested, for 42 days. Check any local and general symptoms as well as the body weight of the animals weekly during the period of observation. The sample complies with the requirements if the preparation is tolerated by the animals without any considerable symptoms.

2.2 Safety Test of the Preservative Agent and Exclusion of Toxic Material of Bacterial Origin

Inject subcutaneously into 2 albino mice weighing 16 to 18 g each 0.5 ml of the sample. Observe the animals for 10 days. Any toxic symptoms occurring must be rather mild (e.g. for preparations preserved by the addition of phenol some tremor may occur in the first hour after injection).

3 Determination of Total Bacterium Count

Determine the bacterium count of serobiologic preparations using one of the following methods.

3.1 Nephelometric Method

Compare the opacity of the sample to that of a standard series of bacterium suspensions containing a known number of bacteria or to a series of glass suspensions of corresponding opacity.

3.2 Helber–Glynn Method

To 1.0 ml of the suspension sample add 9.0 ml of a gentian violet solution diluted just to the limit of transparency. Maintain the liquid at room temperature and place then 1 drop of the dilution in a Helber-Glynn counter cell. Determine the bacterial count by direct counting. Under the microscope, using an immersion lens system of not less than 600 to 700-fold magnification, count the bacteria in not fewer than 20 columns of the chamber and calculate the average count for a single column. The ground surface of a single column is 1/400 mm^2, its depth is 0.02 mm and its volume 1/20 000 mm^3.

3.3 Wright Method

Dilute the sample, if necessary, to contain not more than 500 million bacteria per ml. Mix 1.0 ml portions of the suspension to be tested with 1.0 ml defibrinated blood of known blood corpuscle count diluted 10-fold. Spread 1 drop of the mixture on a defatted object slide. Dry the preparation on air, fix it with a mixture of equal volumes of alcohol and ether for 0.5 to 1.0 minute. Place the slide, fixed surface downward, on glass rods in a Petri dish and stain with Mason's solution (cf. *Stain Solutions for Bacteriology* 1, Vol. I, p. 368) for 30 seconds. After drying, count both the red corpuscles and the bacteria under 600 to 700-fold magnification in 10 fields of sight each. Calculate the bacterium and red blood

corpuscle number of each 1.0 ml portion of the original suspension to be tested on the base of the ratio of the bacterium number to red blood corpuscle number and using the following equation:

$$\text{bacterium count} = D \times R \times \frac{Bc}{Rc}$$

where D = dilution of the suspension
R = red blood corpuscle count of the blood dilution used
Bc = total number of bacteria counted in 10 fields of sight
Rc = total number of red blood corpuscles counted in 10 fields of sight.

4 Chemical Tests

4.1 Test for Protein Content

Determine the protein content of an accurately measured sample containing presumably about 1 mg of nitrogen, according to **5**, Vol. I, p. 112.

5 Test for Pyrogens

Perform the test according to **1**, Vol. I, p. 168.

(D) BLOOD PREPARATIONS

Blood preparations are prepared from human whole-blood with or without separating the plasma or the serum from the cellular elements.

Donors may only be persons who were found by thorough examination to be free of infectious diseases, and whose freedom from syphilitic infection had been checked by adequate control methods. Those who have suffered from malaria or viral hepatitis must be also excluded from donorship. These conditions may be disregarded only if all possible pathogens are undoubtedly killed during the processing of the preparation. Examination of donors, withdrawal of blood, cleaning, assembling, stabilization, storage and use of the equipment, as well as recording of the process of preparations are regulated by special orders.

The method of processing of the blood, the tests to be performed during processing, storage, and in some cases immediately prior to use, are regulated by the Rules of Activity of the National Blood Transfusion Service. Data to be indicated on labels are regulated by the same Rules. Any additives used in blood processing, as anticoagulants, solvents and diluents must be pyrogenfree and non-toxic as ascertained by adequate biological safety tests.

The glucose to be added to blood preparations must comply with the requirements described by the monograph on *Glucosum pro Infusione*.

In the processing of blood preparations only citric acid and sodium citrate complying with the requirements of "pro infusione" preparations must be used.

V SURGICAL DRESSINGS AND SUTURES

Surgical dressings serve for absorbing blood or secretion and to cover certain parts of the body. Surgical sutures are used in sewing wounds.

Sterilization

Cotton wool: pack cotton wool into one layer of unsized, absorbent white paper the edges of which are well overlapping, and wrap this package into a sheet of paper leaving both ends open.

During sterilization no other cover should be around the packets.

Sterilize by heating in an autoclave at 120° for 30 minutes, according to *1.3*, Vol. I, p. 222. The time-interval of 30 minutes begins at the moment when the interior of the parcel reaches the temperature of 120°.

Sterilize gauze, gauze bandage, cellulose wadding, etc. as prescribed above but omitting the outer paper wrapping open at both ends, as prescribed for cotton wool.

Sterilize surgical sutures as prescribed in the individual monographs.

Packaging

Pack surgical dressings after sterilization besides the absorbent paper also into a parchment-like* white paper.

Sterile surgical dressings are packed into white paper carrying a label printed in *blue* colour and not sterilized surgical dressings, packed into white paper, carrying a label printed in *red* colour.

The label on the package of surgical dressings indicates the name, the dimensions (size), and the weight of the dressings, the date and mode of sterilization, the date of expiration of sterility, the manufacturer's name and address and other data prescribed in special regulations. The label on the package of the surgical sutures must also contain the batch number and the composition of the tubing fluid.

Non-sterilized surgical dressings must carry the inscription: "Not to be placed directly onto the wound."

Non-sterilized surgical sutures must be sterilized prior to using for sewing of wounds.

Storage

Preserve surgical sutures and dressings in a dry place, in well closed and easily cleaned cabinets, used specially for this purpose or smaller quantities in similar drawers.

Expiration date of sterilized surgical dressings

Sterilized cotton wool: 3 years; sterilized gauze and gauze bandage: 5 years, following sterilization.

Note. The manufacturer is responsible for the sterility, quality and quantity of surgical dressings, and for the conformity of the packing with regulations. The pharmacist is responsible for the intact character of each package and for the conformity of storage with regulations.

* Parchment-like paper meets the requirements prescribed in the Standards; for packaging surgical dressings use a parchment-like paper which has a weight of 40 to 42 g per square meter and is permeable to air with a velocity of 0.080 to 0.100 ml per second.

1 Absorbency Test of Surgical Dressings

Cut samples of 5×5 cm of spread-out cotton wool, several layers of gauze or cellulose waddings, using a steel shape. Adjust the weight of the sample to 1.00 g. Extend the sample onto a smooth surface, cover it with a glass or steel plate and place a weight of 1 kg for 5 minutes on the top of the plate. Weigh with centigram accuracy a basket, made of stainless steel wire of not less than 6 mm mesh, having a diameter of 8 cm provided with hooks for suspension. Place the basket into a glass cylinder of 1 liter capacity, having a diameter of 10 cm, and fill the cylinder with water of 20° temperature (Fig. 29).

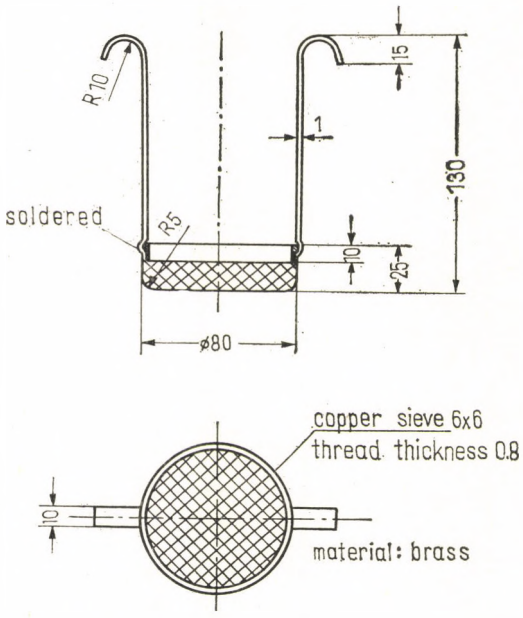


FIG. 29

Place the compressed sample with the aid of pincers onto the surface of the water and count the seconds that elapse until the upper surface of the sample just disappears under the water-level. The number of seconds is the *sinking time.*

Keep the sunk sample in the basket under water for 20 minutes, then lift the basket with the sample cautiously, without shaking, suspend it on a frame and let the excess water drain off for 5 minutes. Tilt the basket for 20 seconds at 45° to one of the corners of the sample to promote dripping.

Blot the water remaining in the basket or collected on the lower surface with filter paper, but take care lest it should come into contact with the sample. Weigh the wetted sample and the basket together with 0.1 g accuracy. Subtract the weight of the basket; the result, the water absorbed by 1 g of the sample is the *absorbency.*

2 Test for Neps in Cotton

Neps are pinhead-sized knots of filament, forming a close, intransparent mass. Use 10×10 cm (dm^2) part of a single layer veil for the test.

2.1 Preparation of Veil Sample

Place the sample of cotton wool onto a 10×10 cm plate (black felt-covered). Remove the excess layers of veil with the aid of pincers.

The weight of the properly prepared monolayer-veil is not less than 0.07 g and not more than 0.10 g. This can be determined following the test, if necessary.

2.2 Count of Neps

Perform the test by using a glass plate of 1 dm^2 surface, divided into 16 alternately transparent and opaque squares of 2.5×2.5 cm, distributed like a chessboard (Fig. 30).

Place the glass plate onto the sample and count the visible neps in each square and add up the figures. Revolve the glass plate and place it again onto the sample, count again the visible neps in each square and sum up the figures.

The total amount of the two counts is the nep content of the sample.

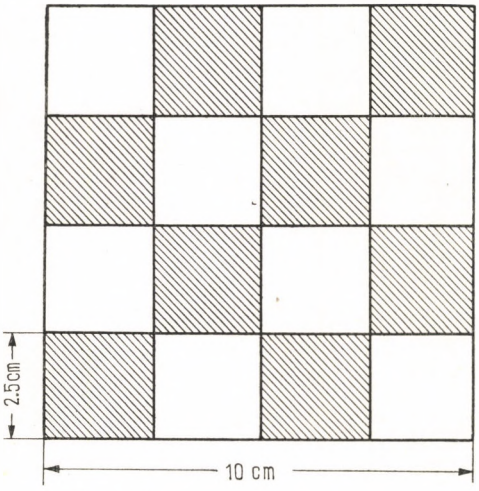


FIG. 30

3 Test for Synthetic Fibres

Synthetic fibre dissolves in a solution of zinc chloride in formic acid in contrast to cotton which does not. Formic cellulose, formed in the course of the test, is restored to cellulose by addition of ammonia solution.

Reagents

Zinc chloride solution in formic acid. (Dissolve 20 g of anhydrous zinc chloride in 80 g of formic acid, 85 per cent.)

Ammonia solution, 1 per cent.

Alcohol, 96 per cent.

Procedure

Weigh about 0.20 g of the sample with 0.1 mg accuracy in a weighing vessel, having a height of 7 cm and a diameter of 5 cm that is provided with a ground lid. Dry at 105° to constant weight, cool and weigh. Add 20 ml of zinc chloride solution in formic acid, cover and allow to stand for 2 1/2 hours at 40° in a thermostat.

Transfer the contents of the vessel onto a G1 glass filter, previously dried at 105° and weighed with 0.1 mg accuracy. Wash the residue with five subsequent 20 ml portions of water and two 20 ml portions of ammonia solution, 1 per cent, by suction. Rinse the filter with 20 ml of R-alcohol, 96 per cent, dry at 105° to constant weight, cool and weigh with 0.1 mg accuracy.

The synthetic fibre content is the difference between the weight of the sample dried at 105° and the weight of the residue dried at 105° after the synthetic fibre has been extracted.

4 Test for Fibre Length of Cotton

Perform the test by using the Johannsen–Zweigle apparatus, prescribed in the Hungarian Standard.

5 Tensile Strength of Sutures

Tensile strength is the load expressed in gram or kg which is necessary to break the test specimen under the given conditions.

Perform the test by using a deadweight type motor-driven apparatus with pendulum, checked by the Hungarian Board of Weights and Measures.

Breaking speed: 25.0 cm per minute,
clamped length: 20.0 cm.
The tensile strength is indicated in the individual monographs in kg weight.

Reagents

In order to facilitate retrieval of terms, the reagents (i.e. materials for reagents and reagent solutions as employed in tests) are presented in a combined index. Subsequent to the key-words of the basic materials, the key-words of the solutions prepared from these materials are given. The denotion applied before the name of certain basic materials refers to substances of particular grade of purity. The concentration grade of reagent solutions often employed in drug investigations is similarly denoted by *R*, placed before the name of solution. In that latter case, however, *R* refers, instead of quality, to the concentration grade. In the present Pharmacopoeia, the concentrations of reagent solutions are given generally in combined percentages, denoted however only as per cent, omitting the reference to w/v.

The reagent solutions should be always prepared from basic materials of the quality grade prescribed in the specifications. The symbol "AGR" after the name of reagent chemicals denotes an *Analytical Grade Reagent*. The basic materials should be weighed on a hand scales or on a counter balance. For the measurement of volume, volumetric cylinders and in certain cases volumetric flasks should be used.

Subsequent to the preparation of the reagent solutions, they should be filtered through a filter of adequate nature, in order to obtain a clear liquid.

The reagents must be stored in well-closing, glass-stoppered glass containers, protected from light. In certain cases, particular specifications are given in the Pharmacopoeia as regards the mode of proper storage.

Reagents, in general, are classified in the Pharmacopoeia into the following groups:

I *Reagent Substances and Solutions;*
II *Chromatographic Materials and Reagents;*
III *Chemical Reference Standards;*
IV *Limit Test Solutions (Limit Test Solutions for Contamination, Colorimetric Matching Fluids);*
V *Volumetric Solutions;*
VI *Indicators; Indicator Papers;*
VII *Buffer Solutions;*
VIII *Reagents and Stains for Use in the Clinical Laboratory.*

I REAGENT SUBSTANCES AND SOLUTIONS

Acetic Acid, Concentrated

Glacial Acetic Acid (cf. Vol. II, monograph No. 5)

Acetic Acid (about 1.0 **N**)

To about 50 ml of water in a 100 ml volumetric flask add 6.3 g of concentrated acetic acid, and dilute the mixture with water to 100 ml.

R-Acetic-Acid

(about 2.0 **N**)

To about 50 ml of water in a 100 ml volumetric flask add 12.5 g of concentrated acetic acid, and dilute the mixture with water to 100 ml.

Acetic Acid, 3 per cent

(about 0.5 **M**)

To 5 ml of R-acetic acid add 15 ml of water.

Acetic Acid, 20 per cent

(about 3.4 **M**)

Dilute 21 g of concentrated acetic acid in a volumetric flask with water to 100 ml.

Acetic Acid, 25 per cent

(about 4.2 **M**)

Dilute 26.3 g of concentrated acetic acid in a volumetric flask with water to 100 ml.

Acetic Acid, 30 per cent

(about 5 **M**)

Dilute 31.6 g of concentrated acetic acid in a volumetric flask with water to 100 ml.

R-Acetic Acid, Concentrated

Fill, to about 2/3rd of the volume, into the larger flask (of 250 to 300 ml) of the apparatus described in *Fig. 13,* Vol. I, p. 119, concentrated acetic acid, add some coarse pumice and distil, cautiously heating the flask. Discard the first 10 to 15 ml fraction. Continue distillation until only 20 to 25 ml residue remains in the distillation flask.

Acetic Acid, Dehydrated

(cf. Vol. I, p. 124)

Acetic Anhydride

AGR
$C_4H_6O_3$ (Mol. Wt. 102.09)

R-Acetic Anhydride

(cf. Vol. I, p. 132)

Acetone

(cf. Vol. II, monograph No. 3)

R-acetone

To 1000 ml of acetone in a 2 l flat-bottomed flask, equipped with a 1 meter long reflux condenser, add 25 g of powdered potassium permanganate. Vigorously boil the mixture on a water-bath for 4 to 5 hours. If the colour of potassium permanganate disappears during boiling add another portion (10 g) of potassium permanganate. Decant the cooled acetone and shake it with 100 g of freshly ignited potassium carbonate. Decant (filter) the treated acetone off the potassium carbonate and subject it to distillation. Use only the acetone fraction which distils between 56° and 57°.

Caution! Acetone is inflammable!

Acetonitrile

AGR
methyl cyanide
C_2H_3N (Mol. Wt. 41.05)

Acetylation Mixture

Dilute 25.0 ml of acetic anhydride in a volumetric flask with R-pyridine to 100 ml. Keep the solution in a well-closing container, protected from light. The solution is suitable for use for 2 days. Agitate prior to use.

Alcohol, Dehydrated

AGR
C_2H_5OH (Mol. Wt. 46.07)

Alcohol, 96 per cent

(cf. Vol. II, monograph No. 334)

Alcohol, 40 per cent

Prepare alcohol, 40 per cent by volume, according to Table VIII, Vol. IV.

Alcohol, 50 per cent

Prepare alcohol, 50 per cent by volume, according to Table VIII, Vol. IV.

Alcohol, 60 per cent

Prepare alcohol, 60 per cent by volume, according to Table VIII, Vol. IV.

Alcohol, Diluted

(70 per cent, v/v)
(cf. Vol. II, monograph No. 336)

Alcohol, 80 per cent

Prepare alcohol, 80 per cent by volume, according to Table VIII, Vol. IV.

Alcohol, Distilled from Potassium Hydroxide

To 96 per cent alcohol add 1 g (per 100 ml) of coarsely powdered potassium hydroxide. Allow the solution to stand for 1 to 2 days in well-stoppered flasks, under frequent shaking, then distil the alcohol on a water-bath.

R-Alcohol

Concentrated alcohol
(90 per cent v/v)
(cf. Vol. II, monograph No. 335)

R-Alcohol, Neutralized

R-alcohol neutralized in the presence of phenolphthalein or of an indicator specified in the respective paragraph, or alcohol distilled from potassium hydroxide.

Alumina

AGR
Al_2O_3 (Mol. Wt. 101.96)

Amino-Chlorobenzene-Disulphonamide

4-Amino-6-chlorobenzene-1,3-disulphonamide
$C_6H_8O_4N_3S_2Cl$ (Mol. Wt. 285.73)

Ammonia Solution, Concentrated

(11.8 to 15.7 **M**)
(cf. Vol. II, monograph No. 62)

Ammonia Solution, 1 per cent

(about 0.56 **M**)

Dilute 10 ml of 10 per cent ammonia solution in a volumetric flask with water to 100 ml.

Ammonia Solution, 10 per cent

(about 5.6 **M**)
(cf. Vol. III, monograph No. 453)

Ammonia Solution, 20 per cent

(about 11.2 **M**)

Measure an amount of concentrated R-ammonia solution equivalent to 20 g of NH_3 into a volumetric flask filled up with water to the half volume, and dilute it with water to 100 ml.

Ammonia Solution, 25 per cent

(about 13.3 **M**)

An ammonia solution of pharmacopoeal purity (Vol. II, monograph No. 62), which contains not less than 24.5 and not more than 25.5 per cent of NH_3.

R-Ammonia Solution

(about 2 **M**)

Dilute in a volumetric flask an amount of concentrated R-ammonia solution equivalent to 3.4 g of NH_3 with water to 100 ml.

R-Ammonia Solution, Concentrated

Insert in the neck of a 2 l, flat-bottomed flask, with the aid of a one-hole cork stopper, a glass pipe, 8–10 millimetres in inner diameter, bent twice rectangularly. The shorter stem of the glass pipe which intrudes into the flask, should be about 30 cm long, its horizontal section should be about 30 cm long, while the longer stem should be 60 to 70 cm long. Pour into the flask 1500 ml of concentrated ammonia solution, and add some coarse pumice powder. As receiver employ a 2 litre glass stoppered flask containing already 1 litre of water. Cool the condenser with ice-water. Cautiously heat the ammonia solution with a very small flame. Take care that the longer stem of the glass pipe be immersed always only just below the surface of the water in the receiver flask. Continue heating until the density of the liquid in the receiver decreases to about 0.91. Check the density with an areometer.

Ammonium Acetate

AGR
CH_3COONH_4 (Mol. Wt. 77.08)

Ammonium Acetate Solution, 20 per cent

Dissolve 20 g of ammonium acetate in a volumetric flask in water to 100 ml.

R-Ammonium Carbonate Solution

(about 1.0 **M**)

Dissolve 7.9 g of ammonium hydrogen carbonate in a volumetric flask in 50 ml of R-ammonia solution, and dilute the solution with water to 100 ml.

Ammonium Chloride

AGR

NH_4Cl (Mol. Wt. 53.50)

R-Ammonium Chloride Solution

(about 2 **M**)

Dissolve 10.7 g of ammonium chloride in a volumetric flask, in water to 100 ml.

R-Ammonium Chloride-Ammonia Solution

Dissolve 6.8 g of ammonium chloride in a volumetric flask, in 57 ml of concentrated ammonia solution, and complete the volume with water to 100 ml.

Ammonium Fluoride

AGR

NH_4F (Mol. Wt. 37.04)

Ammonium Hydrogen Carbonate

AGR

NH_4HCO_3 (Mol. Wt. 79.06)

Ammonium Molybdate

AGR

$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (Mol. Wt. 1235.95)

R-Ammonium Molybdate Solution

(about 0.08 **M**)

Dissolve 10.0 of ammonium molybdate in a volumetric flask, in water to 100 ml.

Ammonium Oxalate

AGR

$(NH_4)_2(COO)_2 \cdot H_2O$ (Mol. Wt. 142.12)

R-Ammonium Oxalate Solution

(about 0.25 **M**)

Dissolve 3.55 g of ammonium oxalate in a volumetric flask in water to 100 ml.

Ammonium Oxalate Solution, 2.5 per cent

(about 0.2 **M**)

Dissolve 2.50 g of ammonium oxalate in a volumetric flask in water to 100 ml.

Ammonium Persulphate

AGR
$(NH_4)_2S_2O_8$ (Mol. Wt. 228.20)

Ammonium Phosphate

AGR
Diammonium hydrogen phosphate.
$(NH_4)_2 \cdot HPO_4$ (Mol. Wt. 132.07)

R-Ammonium Phosphate Solution

(about 1.5 **M**)
Dissolve 20 g of ammonium phosphate in a volumetric flask in water to 100 ml.

Ammonium Sulphamate

AGR
$NH_4SO_3NH_2$ (Mol. Wt. 114.13)

Ammonium Thiocyanate

AGR
NH_4SCN (Mol. Wt. 228.13)

Amyl Acetate

AGR
2-Methyl-butanol-4-acetate
$C_7H_{14}O_2$ (Mol. Wt. 130.18)

Amyl Alcohol, Tertiary

AGR
2-Methyl-butanol-2-ol
$C_5H_{12}O$ (Mol. Wt. 88.15)

Amyl Nitrite

(cf. Vol. II, monograph No. 68)
$SbCl_3$ (Mol. Wt. 228.13)

Anilin

AGR
C_6H_7N (Mol. Wt. 93.12)

R-Anilin

Freshly distilled anilin (Boiling point: 184 to 186).

Anilin Sulphate

AGR
$C_{12}H_{14}N_2.H_2SO_4$ (Mol. Wt. 284.33)

Anilin Sulphate Solution

Dissolve 0.4 g of analin sulphate in the mixture of 1 ml R-sulphuric acid and 19 ml water.

Antimony(III) Chloride

AGR
$SbCl_3$ (Mol. Wt. 228.13)

R-Antimony (III) Cloride Solution

Dissowe 27.0g of Antimony (III) chloride in R-chloroform, under gentle heating, to 100 me. To the solution add 5 to 10 g of anhydrons sodium sulphate and allow the mixture to stand for 20 minutes, under frequent shaking. Use the supernatant liquid as reagent solution.

Prepare the reagent solution always freshly.

Arsenic Trioxide

(cf. Vol. II, monograph No. 7)

Ascorbic Acid

(cf. Vol. II, monograph No. 8)

Barium Chloride

AGR
$BaCl_2 \cdot 2H_2O$ (Mol. Wt. 244.31)

R-Barium Chloride Solution

(about 0.25 **M**)

Dissolve 6.11 g of barium chloride in a volumetric flask, in water to 100 ml.

Barium Hydroxide

(about 0.23 **M**)
$Ba(OH)_2 \cdot 8H_2O$ (Mol. Wt. 315.51)

R-Barium Hydroxide Solution

(about 0.23 **M**)

Agitate frequently for a day in a glass-stoppered flask, 8 g of barium hydroxide with 100 ml of previously boiled and cooled water. Use the clear supernatant after sedimentation.

Barium Nitrate

AGR
$Ba(NO_3)_2$ (Mol. Wt. 261.38)

R-Barium Nitrate Solution

(about 0.25 **M**)

Dissolve 6.53 g of barium nitrate in a volumetric flask to 100 ml.

Benzene

AGR
C_6H_6 (Mol. Wt. 78.11)
Caution: inflammable!

R-Benzene

Distilled benzene (boiling point from 79 to 81°).
Caution: inflammable!

Benzidine

AGR
$C_{12}H_{12}N_2$ (Mol. Wt. 184.23)

Benzidine Solution, 2 per cent, in Acetic Acid

Dissolve 0.2 g of benzidine in 10 ml of concentrated acetic acid.

Benzidine Solution, 1 per cent, in Alcohol

Dissolve 0.1 g of benzidine in 96 per cent alcohol.

R-Benzidine-Copper(II) Acetate Solution

To 1 volume of a 2 per cent solution of benzidine in acetic acid add 1 volume of R-copper(II) acetate solution. Prepare the solution always freshly.

Benzoyl Chloride

AGR
C_7H_5ClO (Mol. Wt. 140.57)

Benzyl Orange

AGR
Potassium-4'-benzylamino-azobenzene sulphonate
$C_{19}H_{16}O_3N_3SK$ (Mol. Wt. 405.50)

R-Bettendorf's Solution

Tin(II) chloride solution in hydrochloric acid

Dissolve in a volumetric flask, 50 g of tin(II) chloride in hydrochloric acid, 37 per cent, to 500 ml. Allow the solution to stand for a few days in a glass-stoppered flask, with the stopper bound firmly, in a warm place. Decant the clear supernatant liquid from the eventually formed sediment, and use the clear solution as the reagent.

Bismuth Carbonate, Basic

AGR
Informative formula: $(BiO)_2CO_3 \cdot 1/2\ H_2O$

Bismuth Nitrate, Basic

(cf. Vol. II, monograph No. 93)

Borax

(cf. Vol. II, monograph No. 239)

Borax Solution, Saturated

Dissolve 6 g of crystalline borax in 100 ml of water under gentle heating. Use the clear supernatant over the crystals formed on cooling.

Boric Acid

(cf. Vol. II, monograph No. 10)

Brillant Green

cf. Vol. II, monograph No. 371)

Bromine

AGR
Br_2 (Atomic Weight 79.909)
Keep in a cool place, in a well-closed, glass-stoppered flask, covered with a cap.

Bromine Water

(about 3.3 per cent bromine content)
A saturated aqueous solution of bromine.

Bromine-Hydrochloric Acid

A mixture of 1 volume of concentrated hydrochloric acid, 37 per cent, and 1 volume of bromine water.

Bromocresol Green

AGR
3,3',5,5'-Tetrabromo-m-cresol-sulphophthalein
$C_{21}H_{14}O_5Br_4S$ (Mol. Wt. 698.05)

Bromophenol Blue

AGR
3,3',5,5'-tetrabromophenol sulphophthalein
$C_{19}H_{10}O_5Br_4S$ (Mol. Wt. 670.00)

Bromothymol Blue

AGR
$C_{27}H_{27}O_5Br_2S$ (Mol. Wt. 624.39)

Butanol

(Butyl alcohol)
AGR
n-Butyl alcohol
$C_4H_{10}O$ (Mol. Wt. 74.12)

Cadmium(II) Chloride

AGR
$CdCl_2 \cdot 2\ 1/2\ H_2O$ (Mol. Wt. 228.36)

Cadmium(II) Chloride Solution in Isopropanol

Dissolve 13.5 g of cadmium(II) chloride in 40 ml of water under heating. Filter the solution. On cooling, dilute the liquid in a volumetric flask with isopropanol, to 100 ml.

Calcium Carbonate

(cf. Vol. II, monograph No. 101)

Calcium Chloride

(cf. Vol. II, monograph No. 102)

Calcium Chloride Solution, 3 per cent

(about 0.14 **M**)
Dissolve 3 g of crystalline calcium chloride in a volumetric flask, in water, to 100 ml.

R-Calcium Chloride Solution

(about 0.25 **M**)
Dissolve 5.48 g of crystalline calcium chloride in a volumetric flask in water to 100 ml.

R-Calcium Chloride Solution, Concentrated

(about 3.65 **M**)
Dissolve 80 g of crystalline calcium chloride in a volumetric flask, in water, to 100 ml.

Calcium Lactate

(cf. Vol. II, monograph No. 104)

Calcium Oxide

(cf. Vol. II, monograph No. 105)

Calcium Sulphate, Dried

(Gipsum, Ignited)
(cf. Vol. II, monograph No. 108)

Calcium Sulphate Solution

(Gypsum water)

Frequently shake for one day 0.5 g of dried calcium sulphate with 100 ml of water. Use the clear filterate as reagent.

(arbon Dioxide Gas

(cf. Vol. II, monograph No. 113)

Carbon Disulphide

AGR
CS_2 (Mol. Wt. 76.14)

Caution! Fire hazardous!

Store in perfectly closed, glass-stoppered, well-filled flasks, in a cool place, protected from light.

For analytical purposes, carbon disulphide freshly distilled from a flask, immersed in hot water, must be used (of boiling-point 46° to 47°).

Carbon Tetrachloride

(cf. Vol. II, monograph No. 114)

Carvone

AGR
(+)-p-6,8-Menthadiene-2-one
$C_{10}H_{14}O$ (Mol. Wt. 150.21)

R-Casein

Hammarsten casein

In order to check the preparation, dissolve 4 g of R-casein in a mixture of 3 ml of 1.0 N sodium hydroxide and 90 ml of water, and adjust the pH of the solution to 8.7. Not more than a slight opalescence must be perceptible in the solution.

Cerium(IV) Ammonium Sulphate

AGR
$Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ (Mol. Wt. 668.62)

Cerium(IV) Sulphate

AGR
$Ce(SO_4)_2 \cdot 4H_2O$ (Mol. Wt. 404.32)

Chloral Hydrate

(cf. Vol. II, monograph No. 125)

Chloral Hydrate Solution, 75 per cent

Dissolve 75 g of chloral hydrate in 25 g of water.

Chlorinated Lime

Active chlorine content not less than 25 per cent.

R-Chlorine Water

Transfer 10 g of powdered potassium permanganate into a 200 ml separatory flask. Insert into the neck of the flask a 50 ml, long-stem funnel equipped with a stop-cock (the lower third of the stem should have an inner diameter of 2 mm), by means of a one-bore cork stopper soaked in melted paraffin. The stem of the funnel should reach into the lower fourth of the flask. The stem of the funnel should be slightly longer than the combined length of the water columns in the trap flask and in the flask. Draw water into the funnel stem until the water level reaches the stop-cock. Connect the side pipe of the separatory flask to a trap flask filled with water to 1/3rd volume. Connect the outler pipe of the trap flask to a rectangularly bent glass pipe, the finely drawn end of which reaches the bottom of a 500 ml glass-stoppered flask filled with water of room temperature to 3/4th of its volume. The connecting glass pipes should closely fit to each other, in order to exclude possibly any contact between the developed gas and the rubber hoses holding together the pipe ends.

On assembling the apparatus, fill the funnel equipped with the stop-cock, with concentrated hydrochloric acid, 37 per cent, and by adequately adjusting the stop-cock, add the hydrochloric acid dropwise onto potassium permanganate. Proceed with the development of chlorine gas until the water is saturated with chlorine.

Note. Perform the preparation of chlorine gas and the saturation of chlorine water under a hood with proper ventilation or in a windy free place.

Chloroform

(cf. Vol. II, monograph No. 130)

R-Chloroform

Prepare R-chloroform from Chloroform of pharmacopoeial grade as follows.

In a large separatory funnel, shake chloroform thrice with equal volumes of water, separate chloroform from water and filter through a layer of anhydrous

sodium sulphate, spread over a small cotton plug into a flask into which previously freshly ignited potassium carbonate (100 g to each litre of chloroform) was placed. Seal the flask with a stopper, agitate its contents and allow to stand for 3 to 4 hours in a dark place. Filter and distil chloroform. Discard the first and last tenths of the distillate.

Refined R-chloroform can be used only on the day of preparation. However, on preserving it with one per cent (v/v) of light petroleum of a boiling point between 50 and 70°, refined chloroform can be used until no traces of phosgene and peroxide are detectable in it, when testes according to [*g*] and [*h*], Vol. II, monograph 130.

Chloroform-Isopropanol Mixture

A mixture of 3 volumes of R-chloroform and 1 volume of isopropanol.

Chlorogene

(cf. Vol. II, monograph No. 131)

R-Chlorogene Solution

Dissolve 5 g of chlorogene in a volumetric flask, in water to 100 ml. Prepare the solution freshly.

Chromosulphuric Acid

Triturate 100 g of powdered potassium dichromate in a large porcelain or glass mortar, with 900 g of concentrated sulphuric acid, added in small portions. Rinse the mixture into a glass-stoppered flask. The supernatant liquid over the undissolved salt is suitable for use until this liquid turns green.

Chromotropic Acid

(and sodium salt)
AGR
1,8-Dihydroxynaphthalene-3,6-disulphonic acid $C_{10}H_8O_8S_2 \cdot 2H_2O$ (Mol. Wt. 356.33) and,
Disodium 1,8-dihydroxy-naphthalene-3,6-disulphonate, respectively
$C_{10}H_6O_8S_2Na_2 \cdot 2H_2O$ (Mol. Wt. 400.31)

Chromotropic Acid Solution

Dissolve 50 mg of chromotropic acid (or of its disodium salt) in 100 ml of 70 per cent sulphuric acid.

The solution is suitable for use on the day of preparation.

Citric Acid

(cf. Vol. II, monograph No. 11)

Citric Acid Solution, 10 per cent

Dissolve in a volumetric flask 10.0 g of citric acid in water to 100 ml.

Cobalt(II) Chloride

AGR
$CoCl_2 \cdot 6H_2O$ (Mol. Wt. 237.95)

Cobalt(II) Chloride Solution, 5 per cent

Dissolve 0.5 g of cobalt(II) chloride in 10 ml of water.
Prepare the solution freshly.

Cobalt(II) Nitrate

AGR
$(Co(NO_3)_2 \cdot 6H_2O$ (Mol. Wt. 291.05)

R-Cobalt(II) Thiocyanate Solution

Dissolve 3 g of cobalt(II) nitrate and 20 g of ammonium thiocyanate in water in a volumetric flask to 100 ml.

Congo-Red

(cf. Vol. II, monograph No. 323)

Copper(II) Acetate

AGR
$Cu(CH_3COO)_2 \cdot H_2O$ (Mol. Wt. 199.64)

R-Copper(II) Acetate Solution

(about 0.25 **M**)
Dissolve in a volumetric flask in 5 ml of R-acetic acid and some water 5 g of copper(II) acetate, and dilute the solution with water to 100 ml.

Copper(II) Acetate Solution, 0.1 per cent

Dilute 10 ml of a 1 per cent solution of copper(II) acetate in a volumetric flask, with water to 100 ml.

Copper(II) Acetate Solution, 1 per cent

(about 0.05 **M**)
Dissolve 1 g of copper(II) acetate in a volumetric flask. in water to 100 ml.

Copper(II) Chloride

AGR
$CuCl_2 \cdot 2H_2O$ (Mol. Wt. 170.49)

Copper(II) Chloride Solution, Ammoniacal

Dissolve 22.5 g of Copper(II) Chloride in 200 ml of water, add 100 ml of concentrated R-Ammonia Solution, and mix.

R-Copper(II) Oxide-Ammonia Solution

Schweitzer's solution

Dissolve 10 g of crystalline copper(II) sulphate in 100 ml of water. To the solution add 20 per cent sodium hydroxide solution until the liquid becomes alkaline when tested with litmus paper. Decant the liquid and collect the formed precipitate on a filter. Wash the precipitate with cold water until it becomes free of sulphate, transfer into a porcelain dish. Add 10 per cent ammonia solution dropwise under stirring until the precipitate just dissolves.

The solution is a dark blue liquid with an odour of ammonia.

Copper(II) Sulphate

Crystalline Copper(II) Sulphate

(cf. Vol. II, monograph No. 141)

R-Copper(II) Sulphate Solution

(about 0.5 **M**)

Dissolve 12.5 g of crystalline copper(II) sulphate in a volumetric flask in water to 100 ml.

Copper(II) Sulphate, Anhydrous

Dry powdered crystalline copper(II) sulphate in a porcelain dish, under stirring, at first at 100° for an hour, then at a temperature not exceeding 300°, until it becomes completely white. Allow the substance to cool in a desiccator. Store in well-closed containers.

Cotton

(cf. Vol. III, monograph No. 810)

Cotton, Defatted

Extract cotton of good quality for 3 to 4 hours in a Soxhleth apparatus or in another suitable glass device with light petroleum. Gently press the cotton strips (between the palms), place them on filter paper and allow them to dry in a current of air on a dustfree place, covered with filter paper. During drying free flames must not burn in the room.

Cotton, Mercerised

Press a cotton plug into a funnel, treat it with 3 to 4 successive portions of 20 per cent sodium hydroxide, then wash with water.

Cyclohexane

AGR
C_6H_{12} (Mol. Wt. 84.16)

Dekaline

AGR
Dekahydronaphthalene
$C_{10}H_{18}$ (Mol. Wt. 138.25)

Denigés' Solution

Acid solution of R-mercury(II) sulphate.

Mix 5.0 g of mercury(II) oxide with 40 ml of water. To this mixture slowly add, during frequent shaking, 20 ml of concentrated R-sulphuric acid. Dilute the hot mixture with 40 ml of water, and shake frequently until all mercury(II) oxide dissolves.

Devarda's Alloy

AGR

An alloy prepared from 50 parts by weight of copper, 45 parts by weight of aluminium and 5 parts by weight of zinc.

Diethylamine

AGR
$C_4H_{11}N$ (Mol. Wt. 73.14)

Diethylene Dioxide

AGR
$C_4H_8O_2$

Digitonin

AGR
$C_{56}H_{92}O_{29}$ (Mol. Wt. 1229.30)

Dimethylaminobenzaldehyde

AGR
p-Dimethylaminobenzaldehyde
$C_9H_{11}ON$ (Mol. Wt. 149.19)

R-Dimethylaminobenzaldehyde Solution

Dissolve 0.25 g of dimethylaminobenzaldehyde in a volumetric flask, in 5 g of concentrated phosphoric acid. To the solution add 45 g of glacial acetic acid, and dilute the mixture with water to 100 ml.

Dimethyl Formamide

AGR
Dimethylamide of formic acid
C_3H_7NO (Mol. Wt. 73.10)

Dinitrobenzene

AGR
1,3-Dinitrobenzene
$C_6H_4O_4N_2$ (Mol. Wt. 168.11)

R-Dinitrobenzene Solution

Dissolve 1 g of dinitrobenzene in 100 ml of 96 per cent alcohol.

Dinitrophenylhydrazine

AGR
2,4-Dinitrophenyl hydrazine
$C_6H_6O_4N_4$ (Mol. Wt. 198.14)

R-Dinitrophenylhydrazine Hydrochloride Solution

To 0.500 g of dinitrophenyl hydrazine add 6 ml of concentrated hydrochloric acid, 37 per cent, and agitate the mixture until it turns canary yellow. Dilute the mixture with dehydrated alcohol to 100 ml and heat on a water-bath until the sample completely dissolves. Allow the cooled solution to stand overnight in a refrigerator. Filter the solution the next day when necessary.

When kept in a refrigerator the solution is suitable for use within 3 months.

Diphenylamine

AGR
$C_{12}H_{11}N$ (Mol. Wt. 169.23)

Diphenyl Carbazide

AGR
$C_{13}H_{14}ON_4$ (Mol. Wt. 242.28)

R-Diphenyl Carbazide Solution, Alcoholic

Dissolve 0.10 g of diphenyl carbazide in 10 ml of 96 per cent alcohol, under frequent shaking. Filter the solution if necessary. Prepare the solution freshly.

Dipotassium Hydrogen Phosphate

AGR
K_2HPO_4 (Mol. Wt. 174.18)

Dipyridyl

AGR
α,α'-Dipyridyl
$C_{10}H_8N_2$ (Mol. Wt. 156.18)

Dipyridyl Solution

Dissolve 0.1 g of Dipyridyl in 10 ml of 96 per cent alcohol

Disodium Hydrogen Phosphate Dihydrate

AGR
$Na_2HPO_4 \cdot 2H_2O$ (Mol. Wt. 178.01)

Dithizone

AGR
Diphenylthiocarbazone
$C_{13}H_{12}N_4S$ (Mol. Wt. 256.32)

Eriochrome Black "T"

AGR
Colour Index: 14645
Sodium-1-(1'-Hydroxy-2'-naphthylazo)-5-nitro-2-naphthol-4-sulphonate
$C_{20}H_{12}N_3O_7SNa$ (Mol. Wt. 461.38)

Ether

(cf. Vol. II, monograph No. 30)

R-Ether

To ether of pharmacopoeial grade add coarsely powdered potassium hydroxide until some of the later remains undissolved. Allow the mixture to stand for 1 to 2 days, under frequent shaking. Decant the ether from the potassium hydroxide and distil from a vessel filled with hot water, used as a water bath.

This operation is firehazardous, therefore, no open flames should be used in the distillation room!

Cool the ether vapours by a long condenser. To the end of the condenser tube attach a large suction flask with a one-hole stopper, placed in ice-water. Connect the suction pipe of the flask with a rubber hose and place the other end of the rubber hose into the waste-pipe of the laboratory desk.

If dehydrated ether is required, place freshly pressed sodium wire into the ether purified in the above-described way, until some of the sodium remains

undissolved, and seal the flask with a stopper equipped with a soda lime tube. From the ether stored in that way distil the required quantity as described above.

Ether prepared freshly in this way is free of peroxide.

Caution! Ether is inflammable!

Ethoxy Chrysoidine

p-Ethoxy chrysoidine
AGR
4-Ethoxy-2',4'-diamino-azobenzene hydrochloride
$C_{14}H_{16}ON_4 \cdot HCl$ (Mol. Wt. 292.77)

Ethyl Acetate

AGR
The ethyl ester of acetic acid
$C_4H_8O_2$ (Mol. Wt. 88.10)

Ethyl Piperidine

AGR
N-ethyl piperidine
$C_7H_{15}N$ (Mol. Wt. 113.20)

Ethylene Glycol

AGR
Ethanediol-1,2
$C_2H_6O_2$ (Mol. Wt. 62.07)

Fehling I Solution

Dissolve in a volumetric flask 69.28 g of crystalline copper(II) sulphate in water to 1000 ml.

Fehling II Solution

Dissolve 346 g of potassium sodium tartrate and 100 g of sodium hydroxide in a volumetric flask in water lo 1000 ml.

Fehling I + II Solution

Prepare freshly a 1 : 1 (by volume) mixture of Fehling I and Fehling II solutions.

Fluorescein Sodium

AGR
Colour Index: 45350
$C_{20}H_{10}O_5Na_2$ (Mol. Wt. 376.27)

Formaldehyde Solution

(cf. Vol. II, monograph No. 160)

Formaldehyde Solution, about 4 per cent

Dilute 11 g of formaldehyde solution with water to 100 ml.

Formaldehyde Solution with Concentrated Sulphuric Acid

Marquis' solution

A mixture of 1 ml of concentrated sulphuric acid and 1 drop of formaldehyde solution.

Prepare the solution freshly.

Formamide

AGR
Formic acid amide
CH_3NO (Mol. Wt. 45.04)

Formic Acid

AGR
HCOOH (Mol. Wt. 46.03)

Gentian Violet

(cf. Vol. II, monograph No. 230)

Glycerin

(cf. Vol. II, monograph No. 160)

Glycerin Solution, in Alcohol

A mixture of one volume of R-alcohol and one volume of glycerin.

Hexamethylenetetramine

(cf. Vol. II, monograph No. 171)

Hexamethylenetetramine Solution, 20 per cent

Dissolve 20 g of hexamethylenetetramine in a volumetric flask, in water, to 100 ml.

Hexane

AGR
n-Hexane
C_6H_{14} (Mol. Wt. 86.18)

Histamine Hydrochloride

(cf. Vol. II, monograph No. 174)

Hydrazine Sulphate

AGR
$N_2H_4 \cdot H_2SO_4$ (Mol. Wt. 130.13)

Hydrochloric Acid, Concentrated, 37 per cent

AGR

It must contain not less than 36 and not more than 39 per cent of HCl (Mol. Wt. 36.47).

R-Hydrochloric Acid

(about 2 **M**)

Dilute 36.5 g of 20 per cent hydrochloric acid in a volumetric flask with water to 100 ml.

Hydrochloric Acid, 1 per cent

(about 0.27 **M**)

Dilute 5 g of 20 per cent hydrochloric acid in a volumetric flask with water to 100 ml.

Hydrochloric Acid, 10 per cent

(about 2.9 **M**)

To 50 g of 20 per cent hydrochloric acid add 50 g of water, and mix.

Diluted hydrochloric acid of pharmacopoeial grade (Vol. III, monograph No. 449) may be also used.

Hydrochloric Acid, 20 per cent

(about 6 **M**)

To 20.0 g of concentrated hydrochloric acid 37 per cent add water until the mixture weighs as many grams as the percentage of HCl of the hydrochloric acid to be diluted.

Hydrochloric acid 20 per cent may be prepared from concentrated hydrochloric acid, 37 per cent, pharmacopoeial grade as follows: To concentrated hydrochloric acid, 37 per cent, add an equal volume of water. Pour the obtained diluted hydrochloric acid (of about 20 per cent) into the distilling flask of an adequate ground-glass apparatus. To the hydrochloric acid add 10 ml of freshly prepared chlorine solution and, in order to prevent retarded boiling, 1 to 2 glass beads and some pumice. Lubricate the ground joints of the flask and the distillation attachment with concentrated phosphoric acid, assemble the apparatus and start distillation. Discard the fractions of the distillate until they contain any chlorine. For purposes of checking, use test tubes as receivers, and perform the test with about

5 ml portions of the distillate diluted with equal volume of water, according to [*m*] Vol. II, monograph No. 15. (chlorine test). If the distillate already does not contain any chlorine, use a larger glass flask containing some water, as receiver, and distil 90 to 95 per cent of hydrochloric acid into the flask. Cool the receiver flask by placing it into a container filled with melting ice. Take care that, during the distillation process, the condenser tube must be immersed, just below the surface of the liquid.

Hydrochloric Acid, 25 per cent

Concentrated Hydrochloric Acid
(about 7.7 **M**)
(cf. Vol. II, monograph No. 15)

Hydrogen Peroxide Solution, Concentrated

AGR
Contains about 30 per cent of H_2O_2
(Mol. Wt. 34.01)

R-Hydrogen Peroxide Solution

A 3 per cent solution of hydrogen peroxide. Dilute in a volumetric flask, 10 g of concentrated hydrogen peroxide solution with water to 100 ml.

Prepare the solution freshly.

Hydrogen Sulphide Gas

H_2S (Mol. Wt. 34.08)

For laboratory purposes, develop hydrogen sulphide gas in an adequate apparatus from iron(II) sulphide (when very pure hydrogen sulphide gas is needed, from sodium sulphide) with 10 per cent hydrochloric acid. Purify the gas by allowing to bubble through a trap filled with water. Work with hydrogen sulphide gas only in free air or in a hood with proper ventilation. The prolonged inhalation of air which contains hydrogen sulphide more than 0.25 mg per litre may be poisonous. In higher concentrations hydrogen sulphide is highly perilous.

R-Hydrogen Sulphide Solution

(about 0.12 **M**)

An aqueous solution saturated with hydrogen sulphide gas at room temperature. Subject to rapid decomposition. Hydrogen sulphide water suitable for use has a strong odour of hydrogen sulphide. When mixed with an equal volume of R-iron(III) chloride solution an abundant precipitate of sulphur must be produced.

Store in a well-closed, completely filled flask, in a cool place, protected from light.

Hydroquinone

(cf. Vol. II, monograph No. 182)

Hydroxylamine Hydrochloride

AGR
Hydroxylammonium Chloride
$NH_2OH \cdot HCl$ (Mol. Wt. 69.50)

Hydroxlamine Hydrochloride Solution, Alcoholic, 3.5 per cent

(about 0.5 **M**)

Dissolve 3.50 g of hydroxylamine hydrochloride in a volumetric flask in 60 ml of 96 per cent alcohol. Dilute the solution with water, to 100 ml.

Hydroxylamine Hydrochloride Solution, Aqueous 3.5 per cent

(about 0.5 **M**)

Dissolve 3.50 g of hydroxylamine hydrochloride in a volumetric flask in water to 100 ml.

R-Invert Sugar Solution

(about 55 per cent)

Dissolve 300 g of sucrose in 100 ml of water. Boil the solution for a few minutes, remove the formed foam and immediately add 3.75 ml of R-sulphuric acid to the hot solution. After stirring for 30 seconds add 3.75 ml of R-sodium hydroxide solution, dilute with 150 ml of cold water, and cool.

Prior to use neutralise the solution with 0.1 **N** hydrochloric acid and 0.1 **N** sodium hydroxide, respectively, in the presence of **I**-phenolphthalein solution as indicator.

Iodine

(cf. Vol. II, monograph No. 191)

R-Iodine Solution

(about 0.4 **M**)

Dissolve 10 g of potassium iodide in a beaker in 25 ml of water. Add 5 g of powdered iodine. After the dissolution of iodine transfer the liquid by rinsing with water into a 100 ml volumetric flask, and complete the volume to 100 ml with water.

Iodine-Zinc Chloride Solution

To a solution of 6.6 g of zinc chloride in 3.4 g of water add 0.6 g of potassium iodide. Saturate the solution with powdered iodine.

Iron(II) Ammonium Sulphate

Mohr's salt
AGR
$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ (Mol. Wt. 392.16)

Iron(III) Ammonium Sulphate

AGR
$Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ (Mol. Wt. 482.21)

Iron(III) Chloride

(cf. Vol. II, monograph No. 158)

R-Iron(III) Chloride Solution

(about 0.1 **M**)
In a volumetric flask dissolve 2.70 g of crystalline iron(III) chloride in a mixture of 1 ml of R-hydrochloric acid and a small amount of water. Dilute the solution with water to 100 ml.

Iron(III) Chloride Solution, 1 per cent

Dissolve 1 g of iron(III) chloride in 0.1 **N** hydrochloric acid to 100 ml.

Iron(III) Chloride Solution, 50 per cent

(cf. Vol. III, monograph No. 480)

Iron(III) Nitrate

AGR
$Fe(NO_3)_3 \cdot 9H_2O$ (Mol. Wt. 404.02)

Iron(II) Sulphate

AGR
$FeSO_4 \cdot 7H_2O$ (Mol. Wt. 278.03)

R-Iron(II) Sulphate Solution

(about 0.36 **M**)
Dissolve 1 g of iron(II) sulphate in 10 ml of freshly boiled and cooled water. Prepare the solution freshly.

R-Iron(II) Sulphate Solution, Concentrated

(about 1.0 **M**)
Dissolve 27.8 g of iron(II) sulphate in a volumetric flask, in 50 ml of R-sulphuric acid and add water to 100 ml. Preserve the solution at daylight.

Iron(II) Sulphate Solution, 1 per cent

Dissolve 0.1 g of iron(II) sulphate in 10 ml of freshly boiled and cooled water. Prepare fresh solution.

Isobutanol

AGR
2-Methylpropanol-1
$C_4H_{10}O$ (Mol. Wt. 74.12)

Isooctane

AGR
2-Methylheptane
C_8H_{18} (Mol. Wt. 114.23)

Isopropanol

AGR
Propanol-2
C_3H_8O (Mol. Wt. 60.09)

Lead Acetate

AGR
$(CH_3COO)_2Pb \cdot 3H_2O$ (Mol. Wt. 379.35)

R-Lead Acetate Solution

(about 0.5 **M**)

Dissolve 196 g of lead acetate in a volumetric flask in freshly boiled and cooled water to 100 ml.

Lead Acetate Cotton

Moisten 3 g of degreased cotton in a mixture of 40 ml of R-lead acetate solution and 40 ml of freshly boiled and cooled water for 30 minutes. Press the liquid off the cotton plugs and dry them at 105 to 110°.

Lead Acetate Paper

Dilute 5 ml of R-lead acetate solution with 20 ml of freshly boiled and cooled water. Immerse strips of filter paper into the solution, place them on a glass plate and dry at 100°.

Lead Subacetate Solution

Triturate 10 g of lead oxide with 30 g of lead acetate. Heat this powder mixture with 10 g of freshly boiled hot water on a water-bath, under frequent shaking, until the yellow colour of the mixture turns white. Then pour 90 g of freshly boiled hot water into the flask. Shake the mixture and on cooling, allow it to sediment in a stoppered flask. Decant the supernatant from the sediment and use the clear liquid as reagent.

Lead Nitrate

AGR
$Pb(NO_3)_2$ (Mol. Wt. 331.23)

Lead Oxide

(cf. Vol. II, monograph No. 307)

Lether Powder

A white or yellowish white, finely flocculated, almost odourless powder, obtained from finest hide, grained with lime and thoroughly washed.

Lime Water

(about 0.02 **M**)
(cf. Vol. III, monograph No. 454)

Linalool

AGR
D-3,7-Dimethyl-octa-1,6-diene-3-ol
$C_{10}H_{18}O$ (Mol. Wt. 154.24)

Magenta

(cf. Vol. II, monograph No. 161)

Magenta Solution in Sulphurous Acid

Dissolve 0.100 g of magenta in 50 ml of water, under gentle heating. To the cooled solution add a solution of 1.00 g of sodium pyrosulphite in a mixture of 20 ml of water and 1 ml of concentrated hydrochloric acid, 37 per cent. Dilute the solution with water to 100 ml, seal the flask with the stopper, and keep it in a dark place for 2 hours. The liquid must be colourless.

Magnesium Chloride

AGR
$MgCl_2 \cdot 6H_2O$ (Mol. Wt. 203.33)

Magnesium Perchlorate

Anhydrous magnesium perchlorate
AGR
$Mg(ClO_4)_2$ (Mol. Wt. 223.23)

Magnesium Sulphate

Crystalline magnesium sulphate
(cf. Vol. II, monograph No. 215)

R-Magnesium Sulphate Solution

(about 0.5 M)

Dissolve 12.3 g of magnesium sulphate in a volumetric flask in water to 100 ml.

Malachite Green

AGR
Colour Index: 42000
p,p'-Dimethylamino-triphenylcarbonium chloride
$C_{23}H_{25}ClN_2$ (Mol. Wt. 364.91)

Mannitol

AGR
D-mannitol
$C_6H_{14}O_6$ (Mol. Wt. 182.17)

R-Mayer's Solution

Potassium-mercury(II) iodide solution

Dissolve 1.36 g of mercury(II) chloride and 5 g of potassium iodide in a volumetric flask in about 30 ml of water, and dilute the solution with water to 100 ml.

Menthol

(cf. Vol. II, monograph No. 219)

Mercury

(cf. Vol. II, monograph No. 176)

Mercury(II) acetate

AGR
$(CH_3COO)_2Hg$ (Mol. Wt. 318.70)

Mercury(II) Acetate Solution in Dehydrated Acetic Acid

(cf. Vol. I, p. 126)

Mercury(II) Bromide

AGR
$HgBr_2$ (Mol. Wt. 360.41)

Mercury(II) Bromide Paper

Immerse for 60 minutes, 15×25 millimetres strips of analytical filter paper in a methanolic solution of mercury(II) bromide. The dried reagent paper can be used for two days when kept in a dark place.

Mercury(II) Bromide Solution in Methanol

Dissolve 5.0 g of mercury(II) bromide in 100 ml of methanol, under heating to 30 to 40°.

Mercury(II) Chloride

AGR
$HgCl_2$ (Mol. Wt. 271.50)

R-Mercury(II) Chloride Solution

(about 0.1 **M**)
Dissolve 2.72 g of mercury(II) chloride in a volumetric flask in water to 100 ml.

Mercury(II) Chloride Solution, 6.5 per cent

(about 0.24 **M**)
Dissolve in a volumetric flask 6.5 g of mercury(II) chloride in water to 100 ml.

Mercury(II) Iodide

(cf. Vol. II, monograph No. 178)

Mercury(II) Oxide

(cf. Vol. II, monograph No. 180)

Methanol

AGR
CH_3OH (Mol. Wt. 32.04)

Methanol is highly poisonous!

Methanol, Dehydrated

It must contain not more than 0.03 per cent of water.

When the determined water content of methanol, tested according to Vol. I, p. 104, exceeds 0.03 per cent, refine it by distillation using a column, filled with glass rings. Discard the last third of the distillate. Protect the receiver flask using a drying trap against atmospheric moisture.

Methanyl Yellow

AGR
Colour Index: 13065
Sodium 4′-anilino-azobenzene-3-sulphonate
$C_{18}H_{14}O_3N_3SNa$ (Mol. Wt. 375.38)

Methylene Blue

(cf. Vol. II, monograph No. 225)

Methylene Blue Solution, 0.1 per cent

Dissolve 0.05 g of methylene blue in a volumetric flask in R-alcohol to 100 ml.

Methylene Blue Solution, 0.05 per cent, in Alcohol

Dissolve 0.05 g of methylene blue in a volumetric flask in R-alcohol to 100 ml.

Methyl Ethyl Ketone

AGR
Butanone
C_4H_8O (Mol. Wt. 72.11)

Methyl Orange

AGR
Colour Index: 13025
Sodium 4′-dimethylamino-azobenzene-4-sulphonate
$C_{14}H_{14}O_3N_3SNa$ (Mol. Wt. 327.34)

Methyl Red

AGR
Colour Index: 13020
4'-Dimethylamino-azobenzene-2-carboxylic acid
$C_{14}H_{15}O_2N_3$ (Mol. Wt. 269.29)

Methyl Testosterone

17α-Methyl-4-androsten-17β-ol-3-one
$C_{20}H_{30}O$ (Mol. Wt. 302.44)

Methyl Thymol Blue

AGR
Tetrasodium 2′,2″-dimethyl-3,′3″-bis (carboxymethyl)-aminomethyl/-5′,5″-isopropyl-phenolsulphophthaleinate
$C_{37}H_{40}O_{13}N_2SNa_4$ (Mol. Wt. 844.76)

R-Millon's Solution

A solution of mercury(II) nitrate in nitric and nitrous acid

Dissolve 65 g of mercury in 65 g of cold concentrated nitric acid, then gently heat the mixture. In a volumetric flask dilute the cooled solution with water to 100 ml.

Murexide

AGR
Colour Index: 56085
Acid ammonium purpurate
$C_8H_8O_6N_6 \cdot H_2O$ (Mol. Wt. 302.21)

Naphthalene

AGR
$C_{10}H_8$ (Mol. Wt. 128.16)

β-Naphthol

(cf. Vol. II, monograph No. 234)

α-Naphtholbenzeine

AGR
$C_{27}H_{20}O_3$ (Mol. Wt. 392.46)

α-Naphthylamine

AGR
$C_{10}H_9N$ (Mol. Wt. 143.18)

R-α-Naphthylamine Solution

Heat 0.2 g of α-naphthylamine with 20 ml of water to boiling. Decant the supernatant of the mixture, and dilute with a mixture of 75 ml of R-acetic acid and with 75 ml of water.

R-Nessler-Winkler Solution

An alkaline solution of mercury(II) iodide containing potassium bromide

Dissolve 1 g of mercury(II) iodide and 5 g of potassium bromide in 5 ml of water. To the solution add a solution of 2.5 g of sodium hydroxide in 20 ml of water, then 75 ml of water. On allowing the mixture to stand overnight, decant the clear supernatant.

The liquid is colourless and transparent.

Store in a glass-stoppered flask, protected from light. Lubricate the stopper with white soft paraffin.

Neutral Red

AGR
Colour Index: 50040
3-Amino-6-dimethylamino-2-methylphenazoniumchloride
$C_{15}H_{16}N_4 \cdot HCl$ (Mol. Wt. 288.80)

Ninhydrin

AGR
Triketohydrindene hydrate
$C_9H_4O_3 \cdot H_2O$ (Mol. Wt. 178.14)

Nitric Acid, Concentrated

AGR
It must contain not less than 64 and not more than 68 per cent of HNO_3 (Mol. Wt. 63.02).

R-Nitric Acid

(about 2 **M**)
To about 50 ml of water in a volumetric flask add 19 g of concentrated nitric acid and dilute the solution with water to 100 ml.

Nitric Acid, 30 per cent

(about 4.8 **M**)
To about 30 ml of water in a volumetric flask add 47 g of concentrated nitric acid, and dilute the solution with water, 1000 ml.

Nitric Acid, 50 per cent

(about 10.4 **M**)
To 24 g of water add 76 g of concentrated nitric acid.

Nitric Acid, Fuming

AGR
It must contain not less than 90 per cent of nitric acid and nitrous oxides together, expressed as HNO_3 (Mol. Wt. 63.02).

Nitrobenzene

AGR
$C_6H_5NO_2$ (Mol. Wt. 123.11)

Nitrogen Gas

(cf. Vol. II, monograph No. 267)

Nitroso-"R"-Salt

AGR
Disodium 1-nitroso-2-oxynaphthalene-3,6-disulphonate
$C_{10}H_5O_8NS_2Na_2$ (Mol. Wt. 377.27)

Oxalic Acid

AGR
$C_2H_2O_4 \cdot 2H_2O$ (Mol. Wt. 126.07)

Palladium(II) Chloride

AGR
$PdCl_2$ (Mol. Wt. 177.31)

R-Palladium(II) Chloride Solution

Dissolve 0.20 g of palladium(II) chloride in 10 ml of 10 per cent hydrochloric acid by gentle heating. In a volumetric flask dilute the cooled solution with water to 250 ml.

Pancreatin

(cf. Vol. II, monograph No. 286)

Paraffin, Hard

(cf. Vol. II, monograph No. 289)

Paraffin, Hard, Refined

Melt a sample of disintegrated hard paraffin with an equal amount of water on a water-bath. After repeated stirring allow the mass to solidify, and discard the wash-water. Repeat this refining procedure until the wash-water shows a neutral reaction.

Paraffin, Liquid

(cf. Vol. II, monograph No. 288)

Pentane

AGR
n-Pentane
C_5H_{12} (Mol. Wt. 72.15)

Pepsin

(cf. Vol. II, monograph No. 292)

Perchloric Acid Solution

AGR
It must contain about 60 per cent of $HClO_4$ (Mol. Wt. 100.47).

Perchloric Acid Solution, 5 per cent

(about 0.5 **M**)

To 5 g of perchloric acid solution add water until the mixture weighs as many grams as the percentage of $HClO_4$ in the solution to be diluted.

Periodic Acid

AGR
$HIO_4 \cdot 2H_2O$ (Mol. Wt. 227.95)

R-Periodic Acid Solution

(about 0.3 **M**)

Dissolve in a volumetric flask 6.8 g of periodic acid in water to 100 ml.

Peroxide-Sulphuric Acid

To 2 volumes of concentrated sulphuric acid add one volume of concentrated hydrogen peroxide solution.

Prepare the solution freshly.

Petrol Ether

AGR
Boling point: from 40° to 60°

Caution, inflammable!

Phenanthroline Hydrochloride

AGR
o-Phenoanthroline hydrochloride
$C_{12}H_8N_6 \cdot HCl \cdot H_2O$ (Mol. Wt. 234.68)

Phenol

(cf. Vol. II, monograph No. 299)

Phenol, Liquified

(cf. Vol. III, monograph No. 581)

Phenol Solution, 5 per cent

Dissolve 5.5 g of liquified phenol in a volumetric flask, in water, to 100 ml of solution.

Phenol Red

AGR
Phenol sulphotphthalein
$C_{19}H_{14}O_5S$ (Mol. Wt. 354.37)

Phenolphthalein

(cf. Vol. II, monograph No. 298)

Phenylhydrazine

AGR
$C_6H_8N_2$ (Mol. Wt. 108.14)

R-Phenylhydrazine

Phenylhydrazine distilled in vacuo.

Phenylhydrazine Solution, Alcoholic, 2 per cent

Dissolve in a volumetric flask, 1 ml of phenylhydrazine in 96 per cent alcohol to 50 ml.

Prepare the solution freshly.

Phloroglucinol

AGR
1,3,5-Trihydroxybenzene
$C_6H_6O_3 \cdot 2H_2O$ (Mol. Wt. 162.14)

R-Phloroglucinol Solution in Hydrochloric Acid

Dissolve 1 g of phloroglucinol in a mixture of 60 g of 96 per cent alcohol and 40 g of a 25 per cent hydrochloric acid.

R-Phloroglucinol Solution in Alcohol

Dissolve 5 g of phloroglucinol in a volumetric flask, in R-alcohol, to 100 ml.

Phosphomolybdic Acid

AGR
$20MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$
(Mol. Wt. 3939.78)

Phosphormolybdic Acid Solution, 1 per cent

Dissolve in a volumetric flask 1 g of phosphomolybdic acid in water, to 100 ml.

R-Phosphomolybdic Acid–Tungstic Acid Solution

Dissolve 5 g of sodium tungstate and 1.2 g of phosphomolybdic acid in a mixture of 2.5 ml of concentrated phosphoric acid and 35 ml of water under heating for 2 hours, using a reflux condenser. Dilute the solution on cooling, with water to 50 ml.

Phosphoric Acid, Concentrated

(cf. Vol. II, monograph No. 20)

R-Phosphoric Acid

(about 1.0 **M**)

Dilute 11.5 g of concentrated phosphoric acid in a volumetric flask, with water to 100 ml.

Phosphoric Acid, 20 per cent

Dilute 23.5 g of concentrated phosphoric acid in a volumetric flask, with water to 100 ml.

Phosphoric Acid, 25 per cent

(about 2.6 **M**)

Dilute in a volumetric flask 29 g of concentrated phosphoric acid with water to 100 ml.

Phosphoric Acid, Concentrated, Iron-free

In order to check the preparation add 9 ml of water to 1 g of concentrated phosphoric acid in a 50 ml colourless, tall beaker. Neutralise the liquid with a 10 per cent ammonia solution, and dilute the mixture with water to 20 ml. On adding 2 ml of R-sulphuric acid, 6 ml of a 10 per cent solution of hydroxylammonium chloride, 7.5 ml of a 20 per cent solution of ammonium acetate and 0.5 ml of dipyridyl solution, make up the volume of the mixture with water to 50 ml.

Evaporate simultaneously on a water-bath to dryness a volume of a 10 per cent ammonia solution identical with that consumed for the neutralisation of the sample of phosphoric acid being tested. Dissolve the dry residue in 10 ml of water and transfer by rinsing into another 50 ml, colourless, tall beaker. Add to the solution, 1.00 ml of iron limit solution, 2 ml of R-sulphuric acid, 6 ml of a 10 per cent hydroxylammonium chloride solution, 7.5 ml of a 20 per cent ammonium acetate solution and 0.5 ml of dipyridyl solution. Dilute the mixture with 14 ml of water and allow the liquid to stand for an hour. Any reddish colour of the test sample must not exceed that of the reference solution.

Phosphoric Acid, 20 per cent, Iron-free

(about 2 **M**)

Dissolve 23.5 g of iron-free concentrated phosphoric acid in a volumetric flask, in water to 100 ml.

Phosphorus Pentoxide

AGR

P_2O_5 (Mol. Wt. 141.96)

Picric Acid

AGR
2,4,6-Trinitrophenol
$C_6H_3O_7N_3$ (Mol. Wt. 229.11)

Caution! On impact or on rapid heating, picric acid explodes vehemently!

Picric Acid Solution, 1 per cent

(about 0.04 **M**)
Dissolve 1 g of picric acid in a volumetric flask in water to 100 ml.

Picric Acid Solution, 5 per cent, in Alcohol

(about 0.22 **M**)
Dissolve 5 g of picric acid in a volumetric flask, in R-alcohol to 100 ml.

Potassium Bromate

AGR
$KBrO_3$ (Mol. Wt. 167.01)

Potassium Bromide

AGR
KBr (Mol. Wt. 119.01)

Potassium Carbonate

AGR
K_2CO_3 (Mol. Wt. 138.21)

Potassium Carbonate Solution, 20 per cent

(about 1.5 **M**)
Dissolve 1 g of potassium carbonate in 5 ml of water.
Prepare the solution freshly.

Potassium Chlorate

AGR
$KClO_3$ (Mol. Wt. 122.55)

Potassium Chlorate Solution, 5 per cent

Dissolve 5 g of potassium chlorate in a volumetric flask in water to 100 ml.

Potassium Chloride

AGR
KCl (Mol. Wt. 74.56)

Potassium Chromate

AGR
K_2CrO_4 (Mol. Wt. 194.20)

R-Potassium Chromate Solution

(about 0.25 **M**)
Dissolve 4.86 g of potassium chromate in a volumetric flask in water to 100 m

Potassium Cyanide

AGR
KCN (Mol. Wt. 65.11)
Caution! Both potassium cyanide and the hydrogen cyanide gas which evolves from it on the effect of acids, are extremely poisonous. Thus, great care must be taken when a solution of potassium cyanide is being acidified or poured to an acid. Work only under hood with a good ventilation. Do not pipet solutions of potassium cyanide!
Store solutions of potassium cyanide in wellclosed glass bottles, labelled as "Poison!", protected from light, locked up in the poison cabinet.

Potassium Cyanide Solution, 1 per cent

Dissolve 0.1 g of potassium cyanide in 10 ml of freshly boiled and cooled water.
Prepare the solution freshly.

Potassium Cyanide Solution, 5 per cent

Dissolve 0.5 g of potassium cyanide in 10 ml of freshly boiled and cooled water.
Prepare the solution freshly.

Potassium Cyanide Solution, 10 per cent

Dissolve 1 g of potassium cyanide in 10 ml of freshly boiled and cooled water.
Prepare the solution freshly.

Potassium Dichromate

AGR
$K_2Cr_2O_7$ (Mol. Wt. 294.21)

R-Potassium Dichromate Solution

(about 0.1 **M**)
Dissolve 2.94 g of potassium dichromate in a volumetric flask in water, to 100 ml.

Potassium Dichromate Solution, 1 per cent

Dissolve 1 g of potassium dichromate in a volumetric flask in water to 100 ml.

Potassium Dichromate Solution, 10 per cent

(about 0.3 **M**)
Dissolve 10 g of potassium dichromate in a volumetric flask to 100 ml.

Potassium Dihydrogen Phosphate

AGR
KH_2PO_4 (Mol. Wt. 136.09)

Potassium Guaiacol Sulphonate

Pharmacopoeial quality.

Potassium Hydrogen Carbonate

AGR
$KHCO_3$ (Mol. Wt. 100.11)

Potassium Hydrogen Carbonate Solution, Saturated

Repeatedly shake for a day 15 g of potassium hydrogen carbonate with 50 ml of water. Use as reagent the clear supernatant of the solution.

Potassium Hydrogen Iodate

AGR
$KH(IO_3)_2$ (Mol. Wt. 389.94)

Potassium Hydrogen Phthalate

AGR
$C_8H_5O_4K$ (Mol. Wt. 204.23)

Potassium Hydrogen Sulphate

AGR
$KHSO_4$ (Mol. Wt. 136.17)

Potassium Hydrogen Tartarate

AGR
$C_4H_5O_6K$ (Mol. Wt. 188.18)

Potassium Hydroxide

AGR
KOH (Mol. Wt. 56.11)

Potassium Hydroxide Solution, 5 per cent

(about 0.9 **M**)

Dissolve 5 g of potassium hydroxide in a volumetric flask in freshly boiled and cooled water, to 100 ml.

Store the solution in a glass bottle sealed with a cork stopper saturated with paraffin.

Potassium Hydroxide Solution, 20 per cent

(about 3.6 **M**)

Dissolve 20 g of potassium hydroxide in a volumetric flask in freshly boiled and cooled water, to 100 ml.

Store the solution in a glass bottle sealed with a cork stopper saturated with paraffin.

Potassium Hydroxide Solution, about 1.0 N, in Dehydrated Alcohol

Dissolve 5.6 g of potassium hydroxide in a volumetric flask, in dehydrated alcohol to 100 ml.

Potassium Hydroxide Solution 3 per cent, in Alcohol

(about 0.43 **M**)

Dissolve 3 g of potassium hydroxide in a volumetric flask in 96 per cent R-alcohol, to 100 ml.

Store the solution in a glass bottle sealed with a cork stopper saturated with paraffin.

Potassium Hydroxide Solution, 15 per cent, in Alcohol

(about 2.6 **M**)

Dissolve 15 g of potassium hydroxide in a volumetric flask in 96 per cent R-alcohol, to 100 ml.

Store the solution in a glass bottle sealed with a cork stopper saturated with paraffin.

Potassium Hydroxide Solution, 10 per cent, in Ethylene Glycol

Dissolve 10 g of potassium hydroxide in a volumetric flask in ethylene glycol to 100 ml.

R-Potassium Hydroxide, Halogen-free

The preparation must not contain halogens. In order to check the purity, dissolve 1 g in 4 ml of water and add 10 ml of R-nitric acid. To the cooled, clear liquid add 1.0 ml of R-silver nitrate solution and shake the mixture. No change must be perceptible in 5 minutes.

When in the determination of organic halogens (Vol. I, p. 121), digestion is performed with potassium hydroxide, establish any halogen content of potassium hydroxide in a blank run.

Store the preparation in well-closed glass bottles, sealed with paraffin.

Potassium Iodide

AGR
KI (Mol. Wt. 166.01)

Potassium Iodide Solution, 10 per cent

(about 0.6 **M**)
Dissolve 1.0 g of potassium iodide in 10 ml of freshly boiled and cooled water. Prepare the solution freshly.

Potassium Iron(II) Cyanide

AGR
$K_4[Fe(CN)_6] \cdot 3H_2O$ (Mol. Wt. 422.39)

R-Potassium Iron(II) Cyanide Solution

(about 0.25 **M**)
Dissolve 1.05 g of potassium iron(II) cyanide in 10 ml of water. Prepare the solution freshly.

Potassium Iron(III) Cyanide

$K_3[Fe(CN)_6]$ (Mol. Wt. 329.25)

R-Potassium Iron(III) Cyanide Solution

(about 0.33 **M**)
Dissolve 1.09 g of potassium iron(III) cyanide in 10 ml of water. Prepare the solution freshly.

Potassium Nitrate

AGR
KNO_3 (Mol. Wt. 101.11)

R-Potassium Nitrate, Halogen-free

The preparation must not contain halogens. In order to check the preparation, perform the test according to Vol. I, p. 107, omitting the use of the chloride limst solution. When reagents containing halogens are used, their halogen content must be taken into account.

Potassium Permanganate

AGR
$KMnO_4$ (Mol. Wt. 158.03)

R-Potassium Permanganate, Sulphate-free

A preparation of pharmacopoeial grade, free of sulphate. In order to check the preparation, dissolve 5 g in 50 ml of water under gentle heating. To the hot solution cautiously add 10 ml of R-alcohol, and heat the liquid until the supernatant liquid above the formed brown precipitate becomes quite colourless. On cooling, filter the precipitous liquid and wash the precipitate with 3 successive 10 ml portions of water. Reduce the volume of the filtrate combined with the wash-water portions on a water-bath to about 10 ml and perform the test with the concentrate, according to Vol. I, p. 99, omitting the use of the sulphate limit test. The reaction mixture must not change.

Note. During the test, the reaction mixture must not be heated with a gas flame (because of possible sulphurous combustion products).

R-Potassium Permanganate Solution

(about 0.32 **M**)

Dissolve 5 g of potassium permanganate in a volumetric flask in water to 100 ml.

Potassium Sodium Tartrate

(cf. Vol. II, monograph No. 199)

Potassium Sulphate

AGR

K_2SO_4 (Mol. Wt. 174.27)

Potassium Tetraoxalate

AGR

$C_4H_3O_8K \cdot 2H_2O$ (Mol. Wt. 254.20)

Potassium Thiocyanate

(cf. Vol. II, monograph No. 203)

R-Potassium Thiocyanate Solution

(about 0.5 **M**)

Dissolve 4.86 g of potassium thiocyanate in a volumetric flask in water to 100 ml.

Potassium Thiocyanate Solution, 25 per cent

(about 2.6 **M**)

Dissolve 2.5 g potassium thiocyanate in 10 ml of water.

Prepare the solution freshly.

Propanol

AGR
n-Propylalcohol
C_3H_8O (Mol. Wt. 60.09)

R-Propanol

Allow propanol to stand for a few days over 10 per cent of coarsely powdered potassium hydroxide, under frequent shaking, then distil the liquid. Use only the fraction distilling between 96° and 99°.

Propylene Glycol

(cf. Vol. II, monograph No. 317)

Pumice

Lapis pumicis

Coarsely disintegrate commercial pumice. Remove the fine powder by screening through sieve No. V, then boil with diluted nitric acid the coarse powder which passes through sieve IV. Wash the coarse powder with water, dry at 130° and ignite.

Pyridine

AGR
C_5H_5N (Mol. Wt. 79.10)

R-Pyridine

Boil pyridine for 4 to 5 hours over 5 to 6 per cent of coarsely powdered potassium hydroxide, using a reflux condenser, then distil pyridine. Use only the fraction distilling between 114° and 116°.

Perform the distillation under a hood with proper ventilation, and apply effective cooling.

Pyridine, Dehydrated

It must contain not more than 0.1 per cent of water. If the water-content of R-pyridine, determined according to Vol. I, p. 112, exceeds 0.1 per cent, add 10 per cent (v/v) of benzene and distil the mixture using a column, filled with glass rings. Use the fraction distilling between 114° and 116°.

Pyridylazonaphthol

Abbreviated name: PAN
AGR
$C_{15}H_{11}ON_3$ (Mol. Wt. 249.28)

Pyrogallol

AGR
1,2,3-Trihydroxybenzene
$C_6H_3(OH)_3$ (Mol. Wt. 126.11)

Quinine Sulphate

(cf. Vol. II, monograph No. 124)

Quinine Sulphate Solution, Aqueous, Saturated

Dissolve 0.1 g of quinine sulphate in 50 ml of water under heating. Cool the solution to room temperature under frequent shaking, and filter through a small paper filter. Use the clear filtrate.

Prepare the solution freshly.

Resorcinol

(cf. Vol. II, monograph No. 321)

Rubeanic Acid

AGR
Dithio-oxalic diamide
$C_2H_4N_2S_2$ (Mol. Wt. 120.17)

Salicyl Aldehyde

AGR
2-Hydroxy-benzaldehyde
$C_7H_6O_2$ (Mol. Wt. 122.12)

Salicyl Aldehyde Solution, 1 per cent, Alcoholic

Dissolve 0.1 g of salicyl aldehyde in 10 ml of R-alcohol.

Prepare the solution freshly.

Sand, Marine

Arena marina

From sea sand remove the fine powder by screening through sieve No. VI. Repeatedly boil the sand with diluted nitric acid, wash with water, dry and ignite.

Saponin

AGR

Saponin obtained from Saponaria, and refined.

A white powder. Soluble in water. Its aqueous solution shows a strong foaming.

It must not contain any Quillaja saponins.

Its hemolytic index (HI) = 25 000 (Vol. I, p. 210).

Selenium

AGR
Se (At. Wt. 78.96)

Semioxamazide

AGR
Oxymic hydrazide
$C_2H_5O_2N_3$ (Mol. Wt. 103.08)

Silica Gel Stained with a Cobalt Salt

The colour of blue silica gel turns pinkish on the effect of adsorbed moisture. Pink silica gel can be regenerated by heating it to 130 to 150°. Heating to higher temperatures must be carefully avoided, because overheated silica gel loses its capability of adsorbing water.

Silicotungstic Acid

AGR
$SiO_2 \cdot 12WO_3 \cdot 26H_2O$ (Mol. Wt. 331.12)

Silver Nitrate

AGR
$AgNO_3$ (Mol. Wt. 169.89)

R-Silver Nitrate Solution

(about 0.1 **M**)
Dissolve in a volumetric flask 1.7 g of silver nitrate in water to 100 ml.

Sodium

AGR
Na (At. Wt. 22.99)

Just prior to use, take out sodium from under the petroleum medium, by means of a pincette, dry it between strips of filter paper and remove the superficial crust. Do not touch metallic sodium with the hand; cut it with a stainless steel knife!

Store sodium in a well-closed, glass-stoppered flask, filled with petroleum, in a cool place.

Caution, sodium is fire hazardous!

Sodium Acetate

Crystalline sodium acetate

AGR
$C_2H_3O_2Na \cdot 3H_2O$ (Mol. Wt. 136.09)

R-Sodium Acetate Solution

(about 2 **M**)

Dissolve 27.2 g of crystalline sodium acetate in a volumetric flask in water to 100 ml.

Sodium Acetate Solution, 3 per cent

(about 0.22 **M**)

Dissolve 3 g of crystalline sodium acetate in a volumetric flask in water to 100 m.

Sodium Acetate Solution, 10 per cent

(about 0.74 **M**)

Dissolve 10 g of crystalline sodium acetate in a volumetric flask in water to 100 ml.

Sodium Acetate Solution, 20 per cent

(about 1.47 **M**)

Dissolve in a volumetric flask 20 g of crystalline sodium acetate in water to 100 ml.

R-Sodium Acetate

Anhydrous Sodium Acetate

$C_2H_3O_2Na$ (Mol. Wt. 82.04)

Heat in a porcelain dish placed on an asbestos wire net, powdered crystalline sodium acetate under continuous stirring with a glass rod, until the water is removed and the residue just begins to melt. Powder the partially cooled substance, and immediately transfer it into airtightly closed flasks. Lubricate the glass stoppers of the flasks with white soft paraffin. Anhydrous sodium acetate may be stored also in flasks sealed with cork stoppers coated with paraffin.

R-Sodium Ammonium Acetate Solution

Dissolve 136.09 g of crystalline sodium acetate and 77.09 g of ammonium acetate in a volumetric flask, in water to 1000 ml.

R-Sodium Arsenite Solution

(about 0.05 **M**)

Dissolve 0.50 g of arsenic trioxide in 10 ml of R-sodium hydroxide solution under gentle heating. Rinse the cooled solution with water into a 100 ml volumetric flask, neutralise it with R-hydrochloric acid and dilute it with water to 100 ml.

Sodium Bromide

AGR

NaBr (Mol. Wt. 102.91)

Sodium Carbonate

Crystalline sodium carbonate
(cf. Vol. II, monograph No. 241)

Sodium Carbonate, Anhydrous

AGR
Na_2CO_3 (Mol. Wt. 106.00)

R-Sodium Carbonate Solution

(about 1.0 **M**)
Dissolve 28.6 g of crystalline sodium carbonate in a volumetric flask in water to 100 ml.

R-Sodium Carbonate, Anhydrous, Halogen-free

In order to check the preparation for freedom of halogens, dissolve 1 g in 11 ml of water. To the solution add cautiously 3 ml of 50 per cent nitric acid in small portions. To the cooled clear solution add 1.0 ml of R-silver nitrate solution and shake the mixture. No change must be perceptible in 5 minutes.

Sodium Chloride

AGR
NaCl (Mol. Wt. 58.45)

Sodium Chloride Solution, 5 per cent

(about 0.8 **M**)
Dissolve 5 g of sodium chloride in a volumetric flask in water to 100 ml.

Sodium Chorlide Solution, Saturated

To 100 ml of water add 40 g of powdered sodium chloride, and boil the liquid for 5 minutes, under stirring with a glass rod. Allow the liquid to cool under frequent shaking. Use the clear supernatant above the crystals as the reagent solution.

Sodium Choleinate

Fel tauri depuratum siccum

A substance obtained from fresh cattle bile by extraction with alcohol, adequately decolourised and refined. It consists in major part of a mixture of sodium taurocholate and glucocholate. A yellowish-white, hygroscopic mass. Taste sweetish, followed by a sensation of bitter. Clearly soluble in water, in R-alcohol. Its residue on ignition must be not less than 12 and not more than 14 per cent. The residue shows an alkaline reaction.

Sodium Chromate

AGR
$Na_2CrO_4 \cdot 4H_2O$ (Mol. Wt. 234.07)

Sodium Citrate

(cf. Vol. II, monograph No. 245)

Sodium Cobalt(III) Nitrite

AGR
$Na_3[Co(NO_2)_6]$ (Mol. Wt. 403.98)

Sodium Dithionite

AGR
$Na_2S_2O_4$ (Mol. Wt. 174.13)

Sodium Edetate

AGR
Disodium Ethylenediamine Tetraacetate
$C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$ (Mol. Wt. 372.25)

Sodium Hydrogen Carbonate

(cf. Vol. II, monograph No. 237)

Sodium Hydrogen Carbonate Solution, 4 per cent

(about 0.48 **M**)

Dissolve 4 g of sodium hydrogen carbonate in a volumetric flask in water of room temperature to 100 ml.

Sodium Hydrogen Carbonate Solution, 5 per cent

(about 0.6 **M**)

Dissolve 5 g of sodium hydrogen carbonate in a volumetric flask in water of room temperature to 100 ml.

Sodium Hydrogen Carbonate Solution, Saturated

To 10 g of sodium hydrogen carbonate in a glass-stoppered flask add 100 ml of water. In a 2-hour period frequently shake the contents of the flask. Use the supernatant liquid as reagent.

R-Sodium Hydrogen Carbonate, Iron-free

AGR

Sodium Hydrogen Sulphite Solution, 5 per cent

(about 0.5 **M**)

Dissolve 5 g of sodium pyrosulphite in a volumetric flask in water to 100 ml. Prepare the solution freshly.

Sodium Hydroxide

AGR
NaOH (Mol. Wt. 40.01)

R-Sodium Hydroxide, Halogen-free

In order to check the preparation for freedom of halogens, dissolve 1 g in 10 ml of water, and add 4 ml of 50 per cent nitric acid. Shake the cooled clear liquid with 1.0 ml of R-silver nitrate solution. No change must be perceptible in 5 minutes.

R-Sodium Hydroxide Solution

(about 2 **M**)

Dissolve 8 g of sodium hydroxide in a volumetric flask, in freshly boiled and cooled water to 100 ml.

Store in a glass-stoppered flask. Lubricate with white soft paraffin the stopper.

Sodium Hydroxide Solution, 10 per cent

(about 2.5 **M**)

Dissolve in a volumetric flask 10 g of sodium hydroxide in freshly boiled and cooled water to 100 ml.

Store in a glass-stoppered flask. Smear a thin layer of white soft paraffin on the stopper.

Sodium Hydroxide Solution, 20 per cent

(about 5 **M**)

Dissolve 20 g of sodium hydroxide in a volumetric flask in freshly boiled and cooled water to produce 100 ml of solution.

Store in a glass-stoppered flask. Lubricate the stopper with white soft paraffin.

Sodium Hydroxide Solution, 30 per cent

(about 7.5 **M**)

Dissolve 30 g of sodium hydroxide in a volumetric flask, in freshly boiled and cooled water, to 100 ml.

Store in a glass-stoppered flask. Lubricate the stopper with white soft paraffin.

R-Sodium Hydroxide Solution, 5 per cent, in Alcohol

Dissolve 5 g of sodium hydroxide in a volumetric flask, in R-alcohol to 100 ml.

Sodium Iodate

AGR
$NaIO_3$ (Mol. Wt. 197.90)

Sodium Iodide

AGR
NaI (Mol. Wt. 149.92)

Sodium Nitrite

(cf. Vol. II, monograph No. 253)

Sodium Nitrite Solution, 1 per cent

(about 0.14 **M**)
Dissolve 0.1 g of sodium nitrite in 10 ml of water.
Prepare the solution freshly.

Sodium Nitrite Solution, 5 per cent

(about 0.7 **M**)
Dissolve 5 g of sodium nitrite in a volumetric flask, in water to 100 ml.

Sodium Nitroprusside

AGR
$Na_2[(Fe)CN_5NO] \cdot 2H_2O$ (Mol. Wt. 297.97)

Sodium Nitroprusside Solution, 2 per cent

(about 0.07 **M**)
Dissolve 0.20 g of sodium nitroprusside in 10 ml of water.
Prepare the solution freshly.

Sodium Nitroprusside Solution, 10 per cent

(about 0.34 **M**)
Dissolve 1 g of sodium nitroprusside in 10 ml of water.
Prepare the solution freshly.

Sodium Oxalate

AGR
$Na_2(COO)_2$ (Mol. Wt. 134.01)

Sodium Periodate

AGR
$NaIO_4$ (Mol. Wt. 213.90)

R-Sodium Periodate Solution

(about 0.05 **M**)
Dissolve in a volumetric flask 1.07 g of sodium periodate in water to 100 ml.
Prepare the solution freshly.

Sodium Phosphate

(cf. Vol. II, monograph No. 256)

Sodium Picrate Solution in Methanol

Baljet's reagent

Dissolve 1 g of picric acid in 50 ml of methanol. To the solution add 45 ml of water and 5 ml of a 10 per cent sodium hydroxide solution.

Prepare the solution freshly.

Sodium Plumbite Solution

Dissolve 1.64 g of lead acetate in 100 ml of R-sodium hydroxide solution.

Sodium Pyrosulphite

(cf. Vol. II, monograph No. 257)

Sodium Sulphate

Crystalline sodium sulphate

(cf. Vol. II, monograph No. 259)

Sodium Sulphate, Anhydrous

Allow powdered crystalline sodium sulphate to stand for several days at room temperature in a place protected from dust. Heat the effloresced preparation at 40 to 50° until it loses the half of its original weight. Dry the residue at 130°, then gently ignite it under frequent stirring.

Sodium Sulphide

AGR

$Na_2S \cdot 9H_2O$ (Mol. Wt. 240.18)

R-Sodium Sulphide Solution

(about 0.5 **M**)

Dissolve 5 g of sodium sulphide in a well-stoppered flask, in 10 ml of water and add 30 ml of glycerin. After a few days filter the solution through a small cotton plug previously washed with alcohol. Return the filtrate if necessary on the filter until a clear filtrate is obtained.

Do not use solutions older than 6 months.

Sodium Sulphite

AGR

$Na_2SO_4 \cdot 7H_2O$ (Mol. Wt. 252.18)

Sodium Tartrate

AGR
$C_4H_4O_6Na_2 \cdot 2H_2O$ (Mol. Wt. 230.08)

Sodium Tetraphenyl Borate

AGR
$(C_6H_5)_4BNa$ (Mol. Wt. 342.23)

R-Sodium Tetraphenyl Borate Solution

(about 0.02 **M**)

Dissolve 0.6 g of sodium tetraphenyl borate in a volumetric flask in water to 100 ml. If the solution is turbid, agitate it with 0.5 g of alumina for 5 minutes, and filter it through a dense filter paper, if necessary, returning the liquid again on the filter, until a clear liquid is obtained. When the solution turns turbid on standing, refilter until a clear solution is obtained.

When stored in a flask of resistent glass at room temperature, protected from light, the solution is suitable for use for about 2 months.

Sodium Tetraphenyl Borate, Solution for Precipitation

(about 0.1 **M**)

Dissolve 3.4 g of sodium tetraphenyl borate in 100 ml of water. If the solution is turbid, vigorously shake it with 0.5 g of alumina for 5 minutes, and filter through a dense filter paper, when necessary, returning the liquid again on the filter, until a clear liquid is obtained. When the solution turns turbid on standing, refilter it until a clear solution is obtained.

When stored in a flask of resistent glass, at room temperature, protected from light, the solution is suitable for use for about 6 weeks.

Sodium Thiosulphate

AGR
$Na_2S_2O_3 \cdot 5H_2O$ (Mol. Wt. 248.21)

Sodium Tungstate

AGR
$Na_2WO_4 \cdot 2H_2O$ (Mol. Wt. 329.95)

Sorboxethene Stearate

(cf. Vol. II, monograph No. 333)

Starch

(cf. Vol. III, monograph No. 381)

Starch Solution, 1 per cent

Heat in a 50 ml conical flask 8 ml of water to boiling. Agitate 0.1 g of potato starch with 2 ml of water and add to the hot water. Boil the liquid, under periodical replacement of the evaporated water, until an opalescent solution is obtained. Filter the cooled solution if necessary through a small paper filter.

Prepare the solution freshly.

Sucrose

(cf. Vol. II, monograph No. 326)

Sudan III

AGR
Colour Index: 26100
Consists mainly of 1-(p-phenylazo-phenylazo)-2-naphthol
$C_{22}H_{16}ON_4$ (Mol. Wt. 352.38)

Sudan III Solution

Dissolve 0.05 g of Sudan III in 5 ml of 96 per cent alcohol and add 5 ml of glycerin.

Sulphanylamide

Pharmacopoeial quality.

Sulphanylic Acid

AGR
p-Aminobenzenesulphonic acid
$C_6H_7O_3NS$ (Mol. Wt. 173.19)

Sulphanilic Acid Solution

Dissolve 0.5 g of sulphanilic acid in a mixture of 75 ml of R-acetic acid and 75 ml of water.

Sulphosalicylic Acid

AGR
5-Sulphosalicylic acid
$C_7H_6O_6S \cdot 2H_2O$ (Mol. Wt. 254.22)

Sulphosalicylic Acid Solution, 20 per cent

Dissolve 20 g of sulphosalicylic acid in 80 g of water.

Sulphur Dioxide Gas

SO_2 (Mol. Wt. 64.07)

It must contain not more than 0.01 per cent of water. Determine the water content of sulphur dioxide gas by titration according to Vol. I, p. 112. If the water content of the gas exceeds 0.01 per cent, allow the gas, prior to use, to pass in a slow rate through a U-shaped trap tube filled up by 2 to 3 alternating layers of glass wool and phosphorus pentoxide.

Sulphuric Acid, Concentrated

AGR

It must contain not less than 95 and not more than 98 per cent of H_2SO_4 (Mol. Wt. 98.08).

R-Sulphuric Acid, Concentrated

To 300 ml of concentrated sulphuric acid add in a 500 ml long-necked digestion flask 10 ml of concentrated hydrogen peroxide solution. In order to prevent retarded boiling, add 2 to 3 glass beads and vigorously heat the sulphuric acid with a free flame until the last traces of hydrogen peroxide are removed. When the white fumes of sulphuric acid had completely filled the flask, continue the boiling for further 5 minutes.

Distil in an adequate distillation apparatus the cooled sulphuric acid thus prepared, clean the distillation flask and the receiver flask of the apparatus prior to use with peroxide-containing sulphuric acid.

An about 150 ml Jena-glass fractionation flask is suitable for the distillation. The slightly downwards bending side pipe of this flask deeply intrudes into the about 200-ml *silicaglass* flask serving as a receiver. However, this pipe must not touch the walls of the flask. In the neck of the distillation flask, insert an unground, loosely–fit glass stopper, and fix the neck, thickly surrounded by asbestos thread, to a stand. Place the receiver into the glass funnel serving as outlet for cooling water, and arrange it in a way to secure the continuous outflow of the cooling water.

Add 1 to 2 glass beads into the distillation flask, pour about 120 ml of the prepared sulphuric acid, and vigorously boil the sulphuric acid over free flame, under cooling the receiver with an abundant water current. Continue distillation until only about 10 ml of acid remains in the flask. Then pour another fresh portion of the prepared sulphuric acid into the distillation flask and continue distillation; work always under a hood with proper ventilation.

Store Sulphuric acid AGR in a glass-stoppered flask equipped with a glass cap, previously rinsed with peroxide-containing sulphuric acid and with water.

Sulphuric Acid, about 1.0 N

(about 0.5 N)

To about 50 ml of water in a 100 ml volumetric flask add 5.0 g of concentrated R-sulphuric acid in a thin jet. Complete the cooled solution with water to 100 ml

Sulphuric Acid, about 9 N

(about 4.5 **M**)

Dilute 88 g of 50 per cent sulphuric acid in a volumetric flask with water to 100 ml.

R-Sulphuric Acid

(about 1.0 **M**)

To about 50 ml of water in a 100 ml volumetric flask add cautiously, in a thin jet, 10.0 g of concentrated R-sulphuric acid in small portions, under frequent shaking. Dilute the cooled liquid with water to 100 ml.

Sulphuric Acid, 1 per cent

(about 0.1 **M**)

To 90 g of water, add 10 g of R-sulphuric acid.

Sulphuric Acid, 50 per cent

(about 7.1 **M**)

To 50 g of concentrated R-sulphuric acid add water until the mixture weighs as many grams as the percentage of H_2SO_4 of the undiluted sulphuric acid. For that purpose, transfer at first the calculated amount of water into an about 200 ml dry conical flask, then cautiously, during stirring and cooling, in small portions, in a thin jet, add the calculated amount of concentrated R-sulphuric acid.

Sulphuric Acid, 70 per cent

(about 11.5 **M**)

Dilute 70 g of concentrated R-sulphuric acid as directed in the paragraph of sulphuric acid, 50 per cent.

Sulphuric Acid, 80 per cent

(about 14 **M**)

Dilute 80 g of concentrated R-sulphuric acid as directed in the paragraph of sulphuric acid, 50 per cent.

Sulphuric Acid, 95 per cent

(about 18 **M**)

Dilute 95 g of concentrated R-sulphuric acid as directed in the paragraph of sulphuric acid, 50 per cent.

Tannic Acid

(cf. Vol. II, monograph No. 23)

Tannic Acid Solution, 10 per cent

Dissolve 1 g of tannic acid in 10 ml of water.
Prepare this solution always freshly.

Tartaric Acid

(cf. Vol. II, monograph No. 24)

Tartaric Acid Solution, 1 per cent

Dissolve 1 g of tartaric acid in water in a volumetric flask to 100 ml.

Tetrahydrofuran

AGR
C_4H_8O (Mol. Wt. 72.11)

Tetramethylammonium Hydroxide Solution

AGR
An aqueous solution containing about 10 per cent of $C_4H_{13}ON$ (Mol. Wt. 91.15)

Tetramethylammonium Hydroxide Solution, 1 per cent

In a volumetric flask dilute 10 g of tetramethylammonium hydroxide solution with 96 per cent alcohol to 100 ml.

Tetrazolium Blue

AGR
3,3'-Dianisol-bis-[4,4'-(3,5-diphenyl)-tetrazolium chloride]
$C_{40}H_{32}O_2N_8Cl_2$ (Mol. Wt. 726.66)

Tetrazolium Blue Solution, 0.5 per cent, Alcoholic

Dissolve 0.05 g of tetrazolium blue in 10 ml of 96 per cent alcohol.
Prepare the solution freshly.

Thiourea

AGR
H_2NCSNH_2 (Mol. Wt. 76.12)

Thiourea Solution, 10 per cent

Dissolve 10 g of thiourea in a volumetric flask, in 50 per cent alcohol to 100 ml.

Thymol

(cf. Vol. II, monograph No. 358)

Thymol Blue

AGR
Thymol sulphophthalein
$C_{27}H_{30}O_5S$ (Mol. Wt. 466.58)

Thymolphthalein

AGR
$C_{28}H_{30}O_4$ (Mol. Wt. 430.52)

Tin(II) Chloride

AGR
$SnCl_2 \cdot 2H_2I$ (Mol. Wt. 225.65)

Tin(II) Chloride, Hydrochloric Acid Solution

Dissolve in a volumetric flask 0.40 g of tin(II) chloride in 68 ml of concentrated hydrochloric acid, 37 per cent. Dilute the solution with water to 100 ml.

Toluene

AGR
$C_6H_5 \cdot CH_3$ (Mol. Wt. 92.13)

Toluidine Blue

AGR
Colour Index: 52040
2-Methyl-3-amino-6-dimethylamino-phenazthionium chloride or its double salt with zinc chloride
$C_{15}H_{16}N_3SCl$ (Mol. Wt. 305.83)
$C_{15}H_{16}N_3SCl \cdot ZnCl_2$ (Mol. Wt. 442.12)

Toluidine Blue Solution

Dissolve 0.02 g of toluidine blue in 100 ml of water.

Tragacantha

(cf. Vol. III, monograph No. 442)

Triacetyl Oxyhydroquinone

Suspend 250 g of hydroquinone in a mixture of 250 g of concentrated sulphuric acid and 1000 ml of water. Under vigorous stirring and cooling, add 225 g of finely powdered potassium dichromate in small portions. The temperature must not rise above +10°. Gradually dilute the reaction mixture with ice water to 3 litres. After the addition of the last portion of potassium dichromate, continue

stirring for about an hour, then filter the mixture. Extract the quinone from the mother liquor with ether (melting-point of the quinone: 115.7°).

Dissolve 150 g of quinone in a mixture of 400—450 g of acetic anhydride and 10 ml of concentrated sulphuric acid, under stirring. Add the quinone gradually in small portions, under cooling, and maintain the temperature between 40 an 50°. When all the quinone has been added, and the temperature does not rise any more, pour the contents of the flask in a large volume of water. Triacetyl oxyhydroquinone separates as a solidifying oil. On recrystallisation from methanol, its melting-point is 96.5—97°.

Trichloroacetic Acid

(cf. Vol. II, monograph No. 25)

Trichloroacetic Acid Solution, 50 per cent

(cf. Vol. III, monograph No. 451)

Triphenyltetrazolium Chloride

AGR
2,3,5-Triphenyl-1,2,3,4-tetrazolium chloride
$C_{19}H_{15}N_4Cl$ (Mol. Wt. 334.80)

Triphenyltetrazolium Chloride Solution, 0.5 per cent

Dissolve 0.05 g of triphenyltetrazolium chloride in 10 ml of dehydrated alcohol.

Prepare the solution freshly.

Tropeolin "OO"

AGR
Colour Index: 13080
Sodium 4'-phenylamino-azobenzene-4-sulphonate
$C_{18}H_{14}O_3N_3SNa$ (Mol. Wt. 375.38)

Urea

Uric Acid

AGR
$C_5H_4O_3N_4$ (Mol. Wt. 168.11)

Vanadium(V) Oxide

AGR
V_2O_5 (Mol. Wt. 181.90)

Vanadium Solution, in Sulphuric Acid

Dissolve 0.20 g of vanadium(V) oxide in a volumetric flask, in 4 ml of concentrated sulphuric acid. To the solution add water to 100 ml.

Vanillin

(cf. Vol. II, monograph No. 367)

Vanillin Solution, in Hydrochloric Acid

Dissolve in 0.1 g of vanillin in 10 ml of R-alcohol. Add to the solution 5 ml of 25 per cent hydrochloric acid and 5 ml of water.

Vanillin Solution, 1 per cent, in Concentrated Sulphuric Acid

Dissolve 0.1 g of vanillin in 10 ml of concentrated sulphuric acid. Prepare the solution freshly.

Variamine Blue

AGR
4-Amino-4'-methoxy-diphenylamine hydrochloride
$C_{13}H_{14}ON_2 \cdot HCl$ (Mol. Wt. 250.73)

Xylene

AGR
Mixture of the three isomers of xylene
$C_6H_4(CH_3)_2$ (Mol. Wt. 106.17)

Zinc, Granulated

AGR

Zinc, Powdered

AGR
Zn (At. Wt. 65.37)

Zinc Chloride

(cf. Vol. II, monograph No. 373)

Zinc Oxide

(cf. Vol. II, monograph No. 374)

Zinc Sulphate

(cf. Vol. II, monograph No. 375)

II CHROMATOGRAPHIC MATERIALS AND REAGENTS

Chromatographic Papers

Chromatographic paper *Macherey–Nagel* No. 263
Chromatographic paper *Schleicher–Schüll* No. 2043/b
Chromatographic paper *Whatman* No. 1

Adsorbents

"Kieselgur-G"

Diatomaceous earth, containing also dried calcium sulphate (gypsum), produced for the purposes of thin-layer chromatography.

"Silica Gel G"

Silica gel, containing also dried calcium sulphate (gypsum), produced for the purposes of thin-layer chromatography.

Solvents

For chromatographic investigations only solvents of analytical purity can be used. Test dimethyl formamide and formamide prior to use as follows:

Dimethyl Formamide

AGR

A strip of red litmus paper immersed in a freshly prepared mixture of 5 ml of dimethylformamide and 20 ml of water must not turn blue, and a strip of blue litmus paper must not turn red. After the elapse of 5 minutes add 2 drops of **I**-phenolphthalein solution and 0.05 ml of 0.1 **N** sodium hydroxide solution to the liquid. The mixture must turn pale pink.

Formamide

AGR

To 2 volumes of formamide add 8 volumes of acetone. No white precipitate must be produced.

Developer Solutions

Chlorogen Developer Solution

I. Dissolve 25 g of trichloroacetic acid in 100 ml of 96 per cent alcohol.
II. Dissolve 0.30 g of chlorogen in 10 ml of water.
Combine 15 volumes of solution I with 1 volume of solution II directly prior to use.

Dragendorff's Developer Solution

Preparation of the stock solution:
Dissolve 1 g of basic bismuth nitrate in 10 ml of concentrated acetic acid; dissolve 8 g of potassium iodide in 20 ml of water.

Mix the two solutions, shake the mixture gently, and filter the liquid after becoming clear. This stock solution is suitable for use, after 2 days of preparation. When protected from direct light, it remains suitable for use for 2 to 3 months.

Preparation of the developer solution:

Immediately prior to use add 5 ml of concentrated acetic acid and 25 ml of water to a 10 ml portion of the stock solution.

Dragendorff's Developer Solution Containing Ethyl Acetate

Preparation of the stock solution:

To 2.6 g of basic bismuth carbonate add 7 g of sodium iodide and 25 ml of concentrated acetic acid, and boil the mixture for a few minutes. On allowing the mixture to stand overnight, filter the eventually formed sodium acetate crystals. To a 20 ml portion of the filtrate add 80 ml of ethyl acetate. This stock solution is ready for use after 2 days, and, when protected from direct light, it remains suitable for use for 2 to 3 months.

Preparation of the developer solution:

Immediately prior to use, add 25 ml of concentrated acetic acid and 60 ml of ethyl acetate to a 10 ml portion of the stock solution.

Ninhydrin Developer Solution

Dissolve 0.25 g of ninhydrin in a volumetric flask, in 1 ml of concentrated acetic acid, and add acetone to 100 ml.

Sulphuric Acid, about 0.05 N

To 5 ml of R-sulphuric acid, add water to 100 ml.

Tetrazolium Blue Developer Solution

Dissolve 10 mg of tetrazolium blue in 25 ml of a 3 per cent alcoholic potassium hydroxide solution.

Prepare the solution freshly.

Vanillin Developer Solution in Sulphuric Acid

Dissolve 1 g of vanillin in concentrated R-sulphuric acid in a volumetric flask, to 100 ml.

III CHEMICAL REFERENCE STANDARDS

In the present Pharmacopoeia the use of various chemical reference standards is officially specified in certain physico-chemical and chemical tests. In these tests, the hereunder listed Pharmacopoeial Reference Standards are used which are of extremely pure grade as prescribed and distributed by the National Pharmaceutical Institute of Hungary.

Acetyldigitoxin
Adrenaline
Chloramphenicol
Chloramphenicol palmitate
Chloropromazine hydrochloride
Cholecalciferol
Cyanocobalamin
Deoxycortone acetate

Dienoestrol
Digitoxin
Digoxin
Ergocalciferol
Ergometrine maleate
Ergotamine tartrate
Ethinyloestradiol
Hydrocortisone
Hydrocortisone acetate
Lanatoside C
Lidocaine hydrochloride
Methandrostenolone
Nalorphine hydrobromide
Neostigmine methyl sulphate
Noradrenaline hydrogen tartrate
Oestradiol benzoate
Oestradiol propionate
Oestrone
Prednisolone
Progesterone
Promethazine hydrochloride
Testosterone propionate
Trimetozine

The below listed microbiological antibiotic reference standards, enumerated in Table 16, Vol. I, page 133, are used also as chemical reference standards:

Oxytetracycline (oxytetracycline dihydate)
Penicillin (benzylpenicillin sodium)

Tetracycline (tetracycline hydrochloride)

For the determination of the melting-point (4.2, Vol. I, p. 68), use the following reference standards:

Acetanilide
Azobenzene
Caffeine
Norcaine
Phenacetine
Salicylic Acid
Sulphaguanidine
Sulphanylamide
Vanillin

IV LIMIT TEST SOLUTIONS

(A) LIMIT TEST SOLUTIONS FOR CONTAMINATIONS

Lead Limit Solution

Each ml of solution contains 10 μg of Pb.

Preparation

Dissolve 0.160 g of lead nitrate in a volumetric flask in 0.5 ml of R-nitric acid, and water added successively, to produce 100 ml of solution. Dilute 1 ml of this stock solution in a volumetric flask to 100 ml. The obtained liquid serves as the lead limit solution being suitable for use for one day.

This limit solution is to be applied also in cases when, in addition to lead, also the presence of other heavy metals are tested.

Arsenic Limit Solution

Each ml contains 0.5 μg of As.

Preparation

Dissolve 0.066 g of arsenic trioxide in 3 ml of R-sodium hydroxide solution by gentle heating. Rinse the cooled solution with water into a 1000 ml volumetric flask, add 5 ml of R-hydrochloric acid and water, to produce 1000 ml of stock solution.

In a volumetric flask dilute a 1.00 ml portion of the stock solution with water, to produce 100 ml of solution which serves as the arsenic limit solution.

Iron Limit Solution

Each ml of solution contains 10 μg of Fe.

Preparation

Dissolve 0.351 g of iron(II) ammonium sulphate hexahydrate in a volumetric flask, in 2 ml of R-sulphuric acid, and water, to produce 100 ml of stock solution.

Dilute a 2.00 ml portion of the stock solution in a volumetric flask, with water to produce 100 ml of solution which serves as the iron limit solution.

Use this limit solution also in tests for heavy metal contamination. On the effect of hydrogen sulphide the liquid turns greyish green.

Calcium Limit Solution

Each ml of the solution contains 50 μg of Ca.

Preparation

Dissolve 0.125 g of calcium carbonate in a 100 ml beaker, in 10 ml of water and 2 ml of R-hydrochloric acid. Meat the solution to boiling, in order to remove carbon dioxide. Transfer the cooled liquid into a 100 ml volumetric flask, add 0.30 g of sodium acetate and water, to produce 100 ml of stock solution.

Dilute a 10.00 ml portion of the stock solution in a volumetric flask, with water, to produce 100 ml of solution which serves as the calcium limit solution.

Ammonium Limit Solution

Each ml of solution contains 10 μg of NH_3.

Preparation

Dissolve 0.314 g of ammonium chloride in a volumetric flask in water, to produce 100 ml of stock solution.

Dilute a 1.00 ml portion of the stock solution in a volumetric flask, with water to produce 100 ml of solution which serves as the ammonium limit solution.

Use this limit solution also in the case when nitrate is detected after being converted into ammonia. In that case, each ml of ammonium limit solution is equivalent to 36.4 μg of NO_3.

otassium Limit Solution

Each ml of solution contains 100 μg of K.

Preparation

Dissolve 0.223 g of potassium sulphate in a volumetric flask in water, to produce 100 ml of stock solution.

Dilute a 10.00 ml portion of the stock solution in a volumetric flask, with water to produce 100 ml of solution which serves as the potassium limit solution.

Sulphate Limit Solution

Each ml of solution contains 50 μg of SO_4.

Preparation

Dilute 5.21 ml of 0.02 **N** sulphuric acid in a volumetric flask in water, to produce 100 ml of solution.

Chloride Limit Solution

Each ml of solution contains 25 μg of Cl.

Preparation

Dilute 7.05 ml of 0.01 **N** hydrochloric acid in a volumetric flask, with water to produce 100 ml of solution.

Nitrate Limit Solution

Each ml of solution contains 100 μg of NO_3.

Preparation

Dissolve 0.815 g of potassium nitrate in a volumetric flask, in 50 per cent sulphuric acid, to produce 100 ml of stock solution.

Dilute a 2.00 ml portion of the stock solution in a volumetric flask, with 50 per cent sulphuric acid, to produce 100 ml of solution which serves as the nitrate limit solution.

If in order to detect nitrate, it is reduced to ammonia and detected in that form, the ammonia limit solution should be used.

(B) COLORIMETRIC MATCHING FLUIDS

The 8-member series of matching fluids which serve as reference standards in the various qualitative tests checking the degree of colouration, are prepared in 4 different tints by diluting the colour stock solutions of the corresponding tint in the ratio specified in Table 33, using 1 per cent sulphuric acid as diluting agent.

The various colorimetric matching fluids are denoted by a letter (initials of the colours Y = yellow, P = pink, G = green, and B = brown) and a number within the series itself. The colorimetric matching fluids are stable when stored in sealed colourless test tubes of resistent glass, protected from direct sunlight.

The colour stock solutions of various tint are prepared from four different salt solutions and from 1 per cent sulphuric acid according to the ratios specified in Table 34 as follows:

TABLE 33

Colorimetric Matching Fluids

Serial number of colorimetric matching fluid	Colour stock solution ml	1 per cent sulphuric acid ml
0	0.78	99.22
1	1.56	98.44
2	3.12	96.88
3	6.25	93.75
4	12.50	87.50
5	25.00	75.00
6	50.00	50.00
7	100.00	—

TABLE 34

Basic Colour Solutions

Tint	Cobalt solution ml	Copper solution ml	Dichromate solution ml	Iron solution ml	1 per cent sulphuric acid ml
Yellow (Y)	9.5	1.9	10.7	4.0	73.9
Pink (P)	40.5	6.1	6.3	12.0	35.1
Green (G)	3.5	20.1	10.4	4.0	62.0
Brown (B)	35.0	17.0	8.0	40.0	—

Cobalt Solution

Each ml of the solution contains 60.0 mg of $CoCl_2 \cdot 6H_2O$.

Preparation

Dissolve 8 g of R-cobalt(II) chloride in 120 ml of 1 per cent sulphuric acid. Filter the solution if necessary. In order to determine the accurate content of cobalt(II) chloride of the solution, dilute a 5.00 ml portion of the liquid with water in a volumetric flask, to 100 ml. To a 10.00 ml portion of this diluted solution add in a glass-stoppered conical flask 10 ml of water, 1 ml of R-hydrogen peroxide solution and 10 ml of R-sodium hydroxide solution. On adding some pumice, boil the contents of the flask until hydrogen peroxide completely decomposes (for about 10 minutes). To the cooled solution add 20 ml of water, 1 g of potassium iodide and 25 ml of R-hydrochloric acid. Seal the flask with its stopper and allow to stand until the precipitate dissolves. Titrate the liberated iodine with 0.01 **N** sodium thiosulphate using **I**-starch solution as indicator.

Each ml of 0.01 **N** sodium thiosulphate is equivalent to 2.380 mg (lg .37649) of $CoCl_2 \cdot 6H_2O$.

Calculate the grams of $CoCl_2 \cdot 6H_2O$ present in 100 ml of the original solution, and prepare a solution containing 6.000 g of $CoCl_2 \cdot 6H_2O$ per 100 ml.

The mode of calculation and dilution is as follows.

If, according to the determination, 100 ml of the cobalt(II) chloride solution contains 6.240 g of $CoCl_2 \cdot 6H_2O$, then the required 6.000 g of $CoCl_2 \cdot 6H_2O$ are present in 96.15 ml of the solution. Transfer, consequently, into a dry 100 ml volumetric flask accurately 3.85 ml of 1 per cent sulphuric acid from a burette, and make up the volume to 100 ml with the cobalt(II) chloride solution of known concentration.

Copper Solution

Each ml of the solution contains 60.0 mg of $CuSO_4 \cdot 5H_2O$.

Preparation

Dissolve 8 g of copper(II) sulphate in 120 ml of 1 per cent sulphuric acid. Filter the solution if necessary. In order to determine the accurate content of copper sulphate of the solution, dilute a 5.00 ml portion of the liquid with water in a volumetric flask to 100 ml. To a 10.00 ml portion of this diluted solution add in a glass-stoppered conical flask 20 ml of water, 1 g of potassium iodide and 5 ml of concentrated R-acetic acid. After the elapse of 10 minutes titrate the liberated iodine with 0.01 **N** sodium thiosulphate, using **I**-starch solution as indicator.

Each ml of 0.01 **N** sodium thiosulphate is equivalent to 2.497 mg (lg .39739) of $CuSO_4 \cdot 5H_2O$.

Calculate the grams of $CuSO_4 \cdot 5H_2O$ present in 100 ml of the original solution and prepare a solution containing 6.000 g of $CuSO_4 \cdot 5H_2O$ per 100 ml.*

Dichromate Solution

Each ml of the solution contains 4.9 mg of $K_2Cr_2O_7$.

Preparation

The dichromate solution is in fact 0.1 **N** potassium dichromate solution prepared with 1 per cent sulphuric acid. Prepare it according to Vol. I, p. 337, with the difference of using a 1 per cent sulphuric acid both as solvent and as diluent. Check the titer of the solution according to Vol. I, p. 337.

Iron Solution

Each ml of the solution contains 45.0 mg of $FeCl_3 \cdot 6H_2O$.

Preparation

Dissolve 6.6 g of iron(III) chloride in 120 ml of 1 per cent sulphuric acid. Filter the solution if necessary. In order to determine the accurate content of iron(III) chloride of the solution, dilute a 5.00 ml portion of the liquid with water in a volumetric flask to 25 ml. To a 10.00 ml portion of this diluted solution add in a 200 ml conical flask 60 ml of water. Adjust the pH value of the liquid between 2 and 3 with 1.0 **N** hydrochloric acid and R-ammonia solution, using a small strip (3×5 millimetres) of Congo paper as indicator. Heat the solution

* As regards the way of calculation and the mode of dilution, cf. the specifications under the title: Cobalt solution, p. 329, Vol. I.

to 40 to 50°, and titrate it with 0.05 **M** Sodium Edetate solution, using 2 ml of a 20 per cent solution of sulphosalicylic acid as indicator, until the lilac tint of the liquid turns just straw-yellow.

Each ml of 0.05 **M** Sodium Edetate solution is equivalent to 13.516 mg (lg .13085) of $FeCl_3 \cdot 6H_2O$.

Calculate the grams of $FeCl_3 \cdot 6H_2O$ present in 100 ml of the original solution, and prepare a solution which contains 4.500 g of $FeCl_3 \cdot 6H_2O$ per 100 ml.*

V VOLUMETRIC SOLUTIONS

The volumetric solutions of this Pharmacopoeia contain at 20°, per millilitre, the milliequivalents of the substances (Normal solutions), and, respectively, 0.5, 0.1, 0.02, 0.01 or 0.002 times the milligram-equivalent amounts of the same (0.5 **N**, 0.1 **N**, 0.02 N, 0.01 **N** and 0.002 **N** solutions).

In practice, however, the actual normality of solutions does not always accurately agree with the specified normality. The numeral indicating the number of millilitres of the volumetric solution of the specified normality equivalent to 1 ml of the tested volumetric solution is denoted as the *titer*.

Calculate the titer of volumetric solutions from the results of at least two consecutive determinations. The difference between the results of the single determinations must not exceed 0.1 per cent.

In general, give the titer to 3 decimals. The third decimal must be accurate to at least ± 1 unit. In the case of measurements of high accuracy, give the titer to 4 decimals. In that case, the 3rd decimal must be accurate.

Indicate the titer, the logarithm of the titer, the indicator used and the date of establishing the titer on the label of the flask which contains the volumetric solution.

*

Periodically check the titer of the volumetric solutions of iodine, potassium hydroxide, potassium permanganate, potassium thicyanate, sodium thiosulphate and sodium hydroxide. If in a titration, the titer of the volumetric solution is to be determined separately in a blank run, neglect the titer indicated on the label of the flask.

Keep the volumetric solutions in flasks of resistant glass, sealed with glass stoppers or with closable suction devices. Flasks containing alkali hydroxide solutions may practically be sealed by glass stoppers lubricated with a thin layer of soft paraffin or by cork stoppers coated with melted paraffin or the solutions kept in flasks equipped with a soda-lime trap and with a closable suction device.

For the tests specified in the present Pharmacopoeia, use volumetric solutions of 20°. The volume of solutions of a temperature other than 20° may be corrected to the volume corresponding to 20°, in the way specified in Vol. I, page 66.

* As regards the way of calculation and the mode of dilution, cf. the specifications under the title: Cobalt solution, Vol. I, p. 329.

1 Calcium Chloride Solution 0.05 **M** *(= 0.1* **N** *or 1/20* **M**)

Each ml of the solution contains 10.9545 mg of $CaCl_2 \cdot 6H_2O$.

Preparation: Dissolve 11.0 g of crystalline calcium chloride ($CaCl_2 \cdot 6H_2O$) weighed with 10 mg accuracy, in a beaker in water, transfer the solution into a 1000 ml volumetric flask, and dilute to 1000 ml with water.

Standardization of the solution

Transfer a 10.00 ml portion of the solution from a burette, into a 200 ml beaker, add a small amount of pumice, 50 ml of water, 5 ml of R-nitric acid, 20.00 ml of 0.1 **N** silver nitrate, and 2 g of R-potassium nitrate free of halogens. Boil the mixture vigorously for 3 minutes and cool. Titrate the cooled liquid with 0.1 **N** potassium thiocyanate, using 1 ml of **I**-iron(III) nitrate solution as indicator.

Calculate the titer (T) of 0.05 **M** calcium chloride solution by the formula-

$$T = \frac{a \times T_a - a \times T_b}{10}$$

where a = the volume (in ml) and T_a the titer of 0.1 **N** silver nitrate applied in excess,

b = the volume (in ml) and T_b the titer of 0.1 **N** potassium thicyanate consumed in the titration.

2 Cerium(IV) Sulphate Solution, 0.1 **N** *(1/10* **M**)

Each ml of the solution contains 40.433 mg of $Co(SO_4)_2 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acid solution.

Instead of this solution, the below given solution may be also used.

Cerium(IV) Ammonium Sulphate Solution 0.1 **N** *(1/10* **M**)

Each ml of the solution contains 66.862 mg of $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acidn solutio.

Dissolve 42.0 g of cerium(IV) sulphate or 70.0 g of cerium(IV) ammonium sulphate, weighed on hand scales, in a mixture of 500 ml of water and 28 ml of concentrated R-sulphuric acid, when necessary, under heating. Dilute the cooled solution with water in a volumetric flask to 1000 ml. After sedimentation for two days, filter the solution through a sintered glass filter (G4) if necessary.

Standardization of the solution

To 20 ml of R-sulphuric acid in a 200 ml glass-stoppered conical flask add from a burette 10.00 ml of 0.1 **N** cerium(IV) sulphate solution. On adding 2 g of potassium hydrogen carbonate in large crystals, loosely close the flask with its moistened stopper. After ceasing of gas development add 1 g of potassium iodide, close the flask with its stopper and allow it to stand for 5 minutes, then dilute the solution with 30 ml of water and titrate with 0.1 **N** sodium thiosulphate, using **I**-starch solution as indicator.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = the volume (in ml) and T_a the titer of 0.1 **N** sodium thiosulphate,
b = the volume (in ml) of cerium(IV) sulphate or cerium(IV) ammonium sulphate.

Store the solution, protected from light.

3 Cerium(IV) Sulphate Solution, 0.02 **N** *(1/50* **M**)

Each ml of solution contains 8.0866 mg of $Ce(SO_4)_2 \cdot 4H_2O$ in about 1.0 **N** sulphuric acid solution.

Instead of this solution, the below given solution may be also used:

Cerium(IV) Ammonium Sulphate Solution, 0.02 **N** *(1/50* **M**)

Each ml of the solution contains 13.3724 mg of $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acid solution.

Dilute 50.00 ml of 0.1 **N** cerium(IV) sulphate or 0.1 **N** cerium(IV) ammonium sulphate solution in a volumetric flask to 250 ml, with approximately 1.0 **N** sulphuric acid of the same temperature (prepared from 1 volume of R-sulphuric acid and 1 volume of water). The titer of this solution is practically the same as that of the diluted 0.1 **N** volumetric solution. Check the titer of the solution if necessary, in the way specified under *2* (p. 332), using 0.01 **N** sodium thiosulphate.

Store the solution, protected from light.

4 Cerium(IV) Sulphate Solution, 0.01 **N** *(1/100* **M**)

Each ml of the solution contains 4.0433 mg of $Ce(SO_4)_2 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acid solution.

Instead of this solution, the below given solution can be also used:

Cerium(IV) Ammonium Sulphate Solution, 0.01 **N** *(1/100* **M**)

Each ml of the solution contains 6.6862 mg of $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acid solution.

Dilute 25.00 ml of 0.1 **N** cerium(IV) sulphate or 0.1 **N** cerium(IV) ammonium sulphate solution in a volumetric flask, to 250 ml, with approximately 1.0 **N** sulphuric acid of the same temperature (prepared from 1 volume of R-sulphuric acid and 1 volume of water). The titer of this solution is practically the same as that of the diluted 0.1 **N** volumetric solution. Check the titer of the solution if necessary, in the way specified under *2* (p. 332), using 0.01 **N** sodium thiosulphate solution.

Store the solution, protected from light.

5 Cerium(IV) Sulphate Solution, 0.005 **N** *(1/200* **M**)

Each ml of the solution contains 2.0217 mg of $Ce(SO_4)_2 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acid solution.

Instead of this solution, the below given solution may be also used.

Cerium(IV) Ammonium Sulphate Solution, 0.005 **N** *(1/200* **M**)

Each ml of the solution contains 3.3431 mg of $Ce(SO_4)_2 \cdot 2(NH_4)_2SO_4 \cdot 4H_2O$ in an about 1.0 **N** sulphuric acid solution.

Dilute 10.00 ml of 0.1 **N** cerium(IV) sulphate or 0.1 **N** cerium(IV) ammonium sulphate solution in a volumetric flask to 200 ml, with approximately 1.0 **N**

sulphuric acid of the same temperature (prepared from 1 volume of R-sulphuric acid and 1 volume of water). The titer of this solution is practically the same as that of the diluted 0.1 **N** volumetric solution. Check the titer of the solution if necessary in the way specified under *2* (p. 332), using 0.005 **N** sodium thiosulphate solution.

Store the solution protected from light.

6 Copper(II) Sulphate Solution, 0.05 **M**

Each ml of the solution contains 12.485 mg of $CuSO_4 \cdot 5H_2O$.

Dissolve 12.5 g of copper(II) sulphate weighed on a hand scales in a volumetric flask, in water, to 1000 ml.

Standardization of the solution

Transfer 10.00 ml of 0.05 **M** Sodium Edetate solution in a 100 ml conical flask, add 20 ml of water, 6.0 ml of R-sodium ammonium acetate solution, 1.0 ml of 20 per cent acetic acid and 5 drops of **I**-pyridyl-azonaphthol solution. Heat the solution to boiling and titrate the hot liquid with 0.05 **M** copper(II) sulphate until the yellow solution turns violet.

Calculate the titer of the solution by the formula:

$$T = \frac{a}{b} \times T_a$$

where a = the number of millilitres and T_a the titer of the 0.05 **M** Sodium Edetate solution,

b = the number of millilitres of the 0.05 **M** copper(II) solution sulphate.

7 Hydrochloric Acid, 1.0 **N** *(1* **M***)*

Each ml of the solution contains 36.465 mg of HCl.

Preparation of an approximately 1.0 **N** *hydrochloric acid stock solution*

Establish the density of hydrochloric acid purified according to Vol. I, p. 286, with the aid of a Westphal balance or of an areometer. Calculate the HCl content of the acid from the value of density, with the aid of Table XXI in Vol. IV, then dilute the acid to obtain an about 4 per cent hydrochloric acid. Preserve this hydrochloric acid stock solution in a flask closed with a cork stopper coated with paraffin. Seal the stoppered flask with melted paraffin.

Determination of the HCl content of hydrochloric acid stock solution

Dissolve in a 300 ml conical flask, in 100 ml of water, about 10 g of potassium hydrogen carbonate prepared in the way specified in *44*, Vol. I, p. 348, and weighed accurately. Titrate this solution with the hydrochloric acid stock solution to be tested, in the way specified for the determination of the sulphuric acid content of sulphuric acid stock solution (*44*, Vol. I, p. 348) (beginning with the text "In a dry weight-buret, add about 120 g of the stock solution of sulphuric acid").

Calculate the weight (K) of hydrochloric acid stock solution, necessary for the preparation of 1000 ml of 1.0 **N** hydrochloric acid by the formula

$$K = 100.114 \times \frac{b}{a}$$

where a = the weight (in grams) of potassium hydrogen carbonate weighed for the titration,

b = the weight (in grams) of the hydrochloric acid stock solution consumed in the titration

(lg 10.0114 = .00049)

Determine the HCl content of the stock solution in at least three consecutive parallel tests. From the results calculate the relevant K values, and establish their mean value. The single K values should not deviate from the mean value by more than 0.1 per cent.

Note, on the label of the flask, the weight (K) of the hydrochloric acid stock solution required for the preparation of 1000 ml of 1.0 **N** hydrochloric acid, the date and method of checking the contents. Seal the flask carefully with a paraffin coat. A repeated checking of the stock solution stored in this way is required only in exceptional cases.

Preparation of the exact 1.0 **N** *hydrochloric acid*

Weigh, with 10 mg accuracy, in an adequate flask, the calculated amount (K) for 1 litre of the hydrochloric acid stock solution of known K value, shaken previously carefully. Smear a thin layer of tap lubricant on the rim of the flask and transfer its content by rinsing with 20° water, into a 1000 ml volumetric flask. Homogenize the contents of the flask by swinging, and dilute with 20° water to exactly 1000 ml. The titer of the solution prepared in this way is practically 1.000.

Standardization of 1.0 **N** *hydrochloric acid*

Check the titer of the solution if necessary in the way described under *44*, in connection with the determination of the titer of 1.0 **N** sulphuric acid.

8 Hydrochloric Acid, 0.5 **N** *(1/2* **M***)*

Each ml of the solution contains 18.233 mg of HCl.

Fill a 500 ml dry volumetric flask with 1.0 **N** hydrochloric acid up to the mark. Transfer the solution by rinsing with freshly boiled water cooled to the temperature of hydrochloric acid, into a 1000 ml volumetric flask and dilute it with water to 1000 ml.

The titer of the hydrochloric acid prepared in this way is practically the same as that of 1.0 **N** hydrochloric acid.

Standardization of the solution

Check the titer of the solution, if necessary, with the use of about 0.5 g of potassium hydrogen carbonate, in the way described in paragraph *44*, in connection with 1.0 **N** sulphuric acid.

9 Hydrochloric Acid, 0.1 **N** *(1/10* **M***)*

Each ml of the solution contains 3.6465 mg of HCl.

(a) Weigh, with 10 mg accuracy in an adequate flask, an amount corresponding to one tenth part of the K value, of the hydrochloric acid stock solution of known K value, described in paragraph 7. Dilute it with freshly boiled water cooled to 20° to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 100.00 ml of 1.0 **N** hydrochloric acid (the titer of which has been checked prior to dilution) with water of the same temperature, to 1000 ml.

The titer of the solution prepared in this way is practically the same as that of 1.0 **N** hydrochloric acid.

Standardization of the solution

Check the titer of the solution, if necessary, in the way described in paragraph *44* in connection with the checking of the titer of sulphuric acid (Vol. I, p. 348), with the difference of dissolving the prescribed amount of potassium hydrogen carbonate in a volumetric flask in water of, 20° to 100 ml and then titrate 10.00 ml portions of that solution with the 0.1 **N** hydrochloric acid to be checked.

Calculate the titer of the solution by the formula

$$T = \frac{g \times 100}{10.0114 \times b}$$

where g = the weight (in grams) of potassium hydrogen carbonate dissolved in 100 ml of water,

b = the amount (in ml) of 0.1 **N** hydrochloric acid consumed in the test (lg 10.0114 = .00049)

10 Hydrochloric Acid, 0.02 **N** *(1/50* **M***)*

Each ml of the solution contains 0.72930 mg of HCl.

Dilute 50.00 ml of 0.1 **N** hydrochloric acid in a volumetric flask, with freshly boiled water cooled to the temperature of hydrochloric acid, to 250 ml.

The titer of the solution prepared in this way is practically the same as that of the 0.1 **N** hydrochloric acid.

Standardization of the solution

Check the titer of the solution, if necessary, in the way described in connection with the checking of the titer of 0.02 **N** Sulphuric Acid (Vol. I, p. 350).

11 Hydrochloric Acid, 0.01 **N** *(1/100* **M***)*

Each ml of the solution contains 0.36465 mg of HCl.

Dilute 10.00 ml of 0.1 **N** hydrochloric acid in a volumetric flask, with freshly boiled water cooled to the temperature of the acid to 100 ml.

The titer of the solution prepared in this way is practically the same as that of the 0.1 **N** hydrochloric acid.

Standardization of the solution

Check the titer of the solution, if necessary, in the way described for checking the titer of 0.02 **N** sulphuric acid (Vol. I, p. 350), with the difference, that 5.00 ml portions of potassium hydrogen carbonate solution are titrated.

Calculate the titer of the solution by the formula

$$T = \frac{50 \times g}{1.00114 \times b}$$

where g = the weight (in grams) of potassium hydrogen carbonate dissolved in 100 ml of water,

b = the amount of 0.01 **N** hydrochloric acid (in ml), consumed in the titration
(lg 1.00114 = .00049)

12 Iodine Solution, 0.1 **N**

Each ml of the solution contains 12.692 mg of I in a 2.5 per cent solution of potassium iodide.

Dissolve 25 g of iodide potassium in a 100 ml beaker, in 25 ml of water. Add to the solution 12.7 g of iodine, powdered in a porcelain mortar and weighed on hand scales. When the iodine has been completely dissolved, transfer the solution by rinsing with water into a 1000 ml volumetric flask, and dilute it with water to 100 ml.

Standardization of the solution

To about 40 ml of water in a glass-stoppered conical flask add 10.00 ml of 0.1 **N** iodine solution and 5 ml of R-sulphuric acid. Titrate the mixture with 0.1 **N** sodium thiosulphate solution, using **I**-starch solution as indicator.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = the number of millilitres and T_a the titer of the 0.1 **N** sodium thiosulphate solution,

b = the number of millilitres of 0.1 **N** iodine solution.

Preserve the solution in well-closed, glass-stoppered flasks, protected from light.

Frequently check the titer of the solution.

13 Iodine Solution, 0.01 **N**

Each ml of the solution contains 1.2692 mg of I.

To 10.00 ml of 0.1 **N** iodine solution in a 100 ml volumetric flask, add 0.5 g of potassium iodide. Dilute the solution with freshly boiled and cooled water to 100 ml. Determine the titer of the 0.01 **N** iodine solution from time to time, in the way described in paragraph *12*, using 0.01 **N** sodium thiosulphate.

Preserve the solution in well-closed, glass-stoppered flasks, protected from light.

14 Perchloric Acid, 0.1 **N** *(1/10* **M***)* (Vol. I, p. 125)

15 Perchloric Acid, 0.02 **N** *(1/50* **M***)* (Vol. I, p. 125)

16 Potassium Bromate Solution, 0.1 **N** *(1/60* **M***)*

Each ml of the solution contains 2.7835 mg of $KBrO_3$.

Weigh in a 50 ml beaker accurately 2.7835 g of potassium bromate of analytical grade, when necessary, recrystallised in the way given in paragraph *20*, then rubbed to powder and dried at 80 to 100° for 3 to 4 hours. Rinse the substance weighed with 20° water directly from the beaker into a 1000 ml volumetric flask. Prior to that, lubricate the external surface of the mouth of the beaker with a thin layer of tap fat. When the dissolution has been completed, dilute the solution with water of 20° to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

Standardization of the solution

If necessary, check the titer of the solution in the way described in paragraph *20* for the 0.1 **N** potassium hydrogen iodate solution. The reaction between bromate and iodide can be promoted by dissolving about 10 mg of ammonium molybdate in the reaction mixture.

17 Potassium Bromate Solution, 0.01 **N** *(1/600* **M***)*

Each ml of the solution contains 0.27835 mg of $KBrO_3$.

(a) Dissolve in a volumetric flask an accurately weighed 0.2784 g of potassium bromate prepared according to paragraph *16*, in water of, 20° in the way specified in the mentioned paragraph, to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting 100.00 ml of 0.1 **N** potassium bromate solution, in a volumetric flask, with water of identical temperature, to produce 1000 ml of solution.

The titer of the solution prepared in this way is practically the same as that of the 0.1 **N** potassium bromate solution.

Check the titer of the solution, if necessary, in the way described in paragraph *20*, using a 0.01 **N** sodium thiosulphate solution. The reaction between bromate and iodide can be promoted by dissolving about 10 mg of ammonium molybdate in the reaction mixture.

18 Potassium Cyanide Solution, 0.02 **N** *(1/50* **M***)*

Each ml contains 1.302 mg of KCN.

Dissolve 0.13 g of potassium cyanide in a volumetric flask, in freshly boile and cooled water, to 100 ml.

Prepare the solution freshly. Establish the titer of the solution according to monograph 656 on *Tabletta hexamethylentetramini*, [c] in Vol. III, monograph No. 656.

19 Potassium Dichromate Solution, 0.1 **N** *(1/60* **M***)*

Each ml of the solution contains 4.9035 mg of $K_2Cr_2O_7$.

Recrystallise potassium dichromate, analytical grade, from water. Dry the powdered substance, at 100°. From the substance prepared in this way weigh accurately 4.9035 g into a 50 ml beaker. Transfer the substance weighed, with water of, 20° directly from the beaker into a 1000 ml volumetric flask. Prior to that, lubricate the external surface of the mouth of the beaker with a thin layer of tap lubricant. When the dissolution has been completed, dilute the solution with water of 20°, to produce 1000 ml of solution.

The titer of the solution prepared in this way is practically 1.000.

Standardization of the solution

To 20 ml of R-sulphuric acid in a 200 ml, glass-stoppered conical flask add 10.00 ml of 0.1 **N** potassium dichromate and 10 ml of water, 2 g of potassium hydrogen carbonate in large crystals. Loosely close the flask with its stopper. When the gas evolution ceased, add 1 g of potassium iodide, place the flask for 5 minutes in a dark place, then titrate the liquid with 0.1 **N** sodium thiosulphate solution, using **I**-starch solution as indicator. Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = the number of millilitres and T_a the titer of the sodium thiosulphate solution,

b = the number of millilitres of the potassium dichromate solution.

20 Potassium Hydrogen Iodate Solution, 0.1 **N**

Each ml of the solution contains 3.2495 mg of $KH(IO_3)_2$.

Recrystallise, if necessary, potassium hydrogen iodate of analytical grade from hot water. For that purpose, filter the hot saturated aqueous solution (1 + 4.13) through a folded paper filter, allow the filtrate to cool. Collect the precipitated powder-like substance on a glass filter or in a glass funnel equipped with a platinum cone, apply strong suction, and dry at first at room temperature, than at 80 to 100° for 3 to 4 hours. It is practical to preserve the purified substance in tight-necked small flasks stoppered with well-fitting cork stoppers.

Weigh in a 50 ml beaker accurately 3.2495 g of potassium hydrogen iodate, analytical grade, purified in the above-specified way, and, respectively, powdered and dried for 3 to 4 hours at 80 to 100°. Transfer the substance weighed, with water of 20°, from the beaker directly into a 1000 ml volumetric flask. Prior to that, lubricate the external surface of the mouth of the beaker with a thin layer of tap lubricant. When the dissolution has been completed, dilute the solution with water of 20° to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

Standardization of the solution

To about 40 ml of water in a glass-stoppered conical flask add 10.00 ml of 0.1 **N** potassium hydrogen iodate solution, 1 g of potassium iodide and 3 ml of R-sulphuric acid. After the elapse of 5 minutes, titrate the liberated iodine with 0.1 **N** sodium thiosulphate solution, using **I**-starch solution as indicator.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = the number of millilitres and T_a the titer of the 0.1 **N** sodium thiosulphate solution,

b = the number of millilitres of the 0.1 **N**, potassium hydrogen iodate solution.

21 Potassium Hydrogen Iodate Solution, 0.01 **N**

Each ml of the solution contains 0.32495 mg of $KH(IO_3)_2$.

(a) Dissolve in a volumetric flask, in water of 20°, an accurately weighed, 0.3250 g of potassium hydrogen iodate prepared in the way specified in the paragraph *20*, in the way described there, to produce 1000 ml of solution.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 100.00 ml of 0.1 **N** potassium hydrogen iodate solution with water of the same temperature, to 1000 ml.

The titer of the solution prepared in this way is practically the same as that of the 0.1 **N** potassium hydrogen iodate solution.

Check the titer of the solution if necessary, in the way specified in paragraph *20*, using 0.01 **N** sodium thiosulphate solution.

22 Potassium Hydrogen Iodate Solution, 0.005 **N** *(1/2400* **M***)*

Each ml of the solution contains 0.16248 mg of $KH(IO_3)_2$.

(a) Dissolve in a volumetric flask, in the way specified in paragraph *20*, 0.1625 g accurately weighed potassium hydrogen iodate prepared according to paragraph *20*, in water of 20°, to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 10.00 ml of 0.1 **N** potassium hydrogen iodate solution with water of the same temperature, to 200 ml.

The titer of the solution prepared in this way is practically the same as that of the 0.1 **N** potassium hydrogen iodate solution.

Check the titer of the solution, if necessary, in the way specified in paragraph *20* with the difference of using 0.005 **N** sodium thiosulphate for checking.

23 Potassium Hydroxide Solution in Alcohol, 0.5 **N** *(1/2* **M***)*

Each ml of the solution contains 28.052 mg of KOH, dissolved in 96 per cent, alcohol.

Dissolve in a volumetric flask 3.00 g of halogen-free R-potassium hydroxide, powdered in a porcelain mortar and weighed on a hand scales, in 96 per cent alcohol freshly distilled from over solid potassium hydroxide, to 100 ml.

Check the titer of the solution prior to each use by a blank test.

24 Potassium Hydroxide Solution, in Dehydrated Alcohol, 1.0 **N** *(1* **M***)*

Each ml of the solution contains 56.104 mg of KOH, dissolved in dehydrated alcohol.

Dissolve in a volumetric flask, in dehydrated alcohol 5.60 g of halogen-free R-potassium hydroxide, powdered in a porcelain mortar and weighed on a hand scales, to produce 100 ml of solution.

Check the titer of the solution in the way specified in paragraph *37*.

25 Potassium Hydroxide Solution in Propanol, 0.5 **N** *(1/2* **M***)*

Each ml of the solution contains 28.052 mg of KOH, dissolved in n-propanol.

Dissolve, by gentle heating, in a volumetric flask, 30.0 g of halogen-free R-potassium hydroxide, powdered in a porcelain mortar and weighed on a hand scales, in R-propanol freshly distilled from over solid potassium hydroxide. Dilute the solution on cooling with R-propanol to 1000 ml.

Establish the titer of the solution in each case by a blank test and check with 0.5 **N** hydrochloric acid, respectively.

26 Potassium Hydroxide Solution in Propanol, 0.1 **N** *(1/10* **M***)*

Each ml of the solution contains 5.6104 mg of KOH, dissolved in n-propanol.

Dilute in a volumetric flask 20.00 ml of 0.5 **N** solution of potassium hydroxide in propanol, with R-propanol freshly distilled over potassium hydroxide, to 100 ml.

Check the titer of the solution prior to each use by a blank test.

27 Potassium Permanganate Solution, 0.1 **N** *(1/50* **M***)*

Each ml of the solution contains 3.1605 mg of $KMnO_4$.

Dissolve in a 200 ml beaker, 3.2 g of potassium permanganate, analytical grade, weighed on a hand scales, in 50 ml of water under gentle heating. Transfer

the solution into a volumetric flask and on cooling, dilute with water, to 1000 ml. Filter the solution, if necessary, after the elapse of two days, through a glass filter previously washed with hydrogen peroxide-containing sulphuric acid and carefully rinsed with water.

Standardization of the solution

To a mixture of 10 ml of R-sulphuric acid and 40 ml of water in a glass-stoppered conical flask add 10.00 ml of 0.1 **N** potassium permanganate solution and 1 g of potassium iodide. Allow the mixture to stand for 5 minutes, and titrate the liberated iodine with 0.1 **N** sodium thiosulphate, using **I**-starch solution as indicator.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = the number of millilitres and T_a the titer of the 0.1 **N** sodium thiosulphate solution,

b = the number of millilitres of the 0.1 **N** potassium permanganate solution.

Check periodically the titer of the 0.1 **N** potassium permanganate in solution if necessary, preserve the solution in a glass-stoppered flask purified with hydrogen peroxide-containing sulphuric acid, protected from light and dust.

28 Potassium Permanganate Solution, 0.01 **N** *(1/500* **M***)*

Each ml of the solution contains 0.31605 mg of $KMnO_4$.

Dilute 10.00 ml of 0.1 **N** potassium permanganate solution in a volumetric flask, with water, to 100 ml.

Establish the titer of the 0.01 **N** potassium permanganate solution in each case in a blank test. Preserve the solution in a glass-stoppered flask cleaned with hydrogen peroxide-containing sulphuric acid, protected from light and dust.

29 Potassium Thiocyanate Solution, 0.1 **N** *(1/10* **M***)*

Each ml of the solution contains 9.7180 mg of KSCN.

Dissolve 10.0 g of potassium tiocyanate in a volumetric flask, in water, to 1000 ml.

Standardization of the solution

Transfer 10.00 ml of 0.1 **N** silver nitrate solution into a 100 ml conical flask, and add 40 ml of water, 5 ml of R-nitric acid and 1 ml of **I**-iron(III) nitrate solution. Titrate the mixture with the 0.1 **N** potassium thiocyanate solution to be tested.

Calculate the titer of the solution by the formula:

$$T = \frac{a}{b} \times T_a$$

where a = the number of millilitres and T_a the titer of the 0.1 **N** silver nitrate solution,

b = the number of millilitres of the 0.1 **N** potassium thiocyanate solution.

The titer of the solution can also be determined in the way specified under *32* (see below).

Instead of 0.1 **N** potassium thiocyanate solution, also 0.1 **N** ammonium thiocyanate solution may be used.

30 Potassium Thiocyanate Solution, 0.01 **N** *(1/100* **M***)*

Each ml of the solution contains 0.97180 mg of KSCN.

Dilute 100.00 ml of 0.1 **N** potassium thiocyanate solution in a volumetric flask, with water to 1000 ml. Check the titer of the solution in the way specified in paragraph *29*, using 0.01 **N** silver nitrate solution, or establish the titer in each case by a blank test.

31 Silicotungstic Acid Solution, 0.05 **N**

Each millilitre of the solution contains 41.3854 mg of silicotungstic acid ($SiO_2 \cdot 12WO_3 \cdot 26H_2O$).

Dissolve 41.38 g of silicotungstic acid, in a volumetric flask, weighed on a hand scales, in freshly boiled and cooled water, to 1000 ml. If the liquid is not completely clear, filter through a sintered glass filter G4.

Preserve the solution in a well-closing, glass-stoppered flask, protected from light.

Standardization of the solution

Dissolve, in 10 ml of water, about 0.020 g of thiamine hydrochloride accurately weighed, checked for its content of active substance, according to paragraph [*j*] of monograph 356 on *Thiaminum hydrochloricum* in Vol. II. Titrate the solution, in the presence of 4 drops of **I**-methanyl yellow solution as indicator, with 0.05 **N** silicotungstic acid solution, until a violet-pinkish-colour is produced.

Calculate the titer of the solution by the formula

$$T = \frac{a \times g \times 10}{8.4315 \times b}$$

where a = percentage of active substance content of thiamine hydrochloride,
g = weight (in grams) of the applied thiamine hydrochloride, and
b = number of millilitres of 0.05 **N** silicotungstic acid consumed in the titration
(lg 8.4315 = .92591)

32 Silver Nitrate Solution, 0.1 **N** *(1/10* **M***)*

Each ml of the solution contains 16.989 mg of $AgNO_3$.

Powder in a clean porcelain mortar, and dry for one hour at 80° about 20 g of silver nitrate, analytical grade, or if necessary, adequately recrystallised from water acidifed with some nitric acid. During drying, periodically stir the eventually baked powder, with the aid of a clean glass rod and allow to cool in a desiccator. Dissolve 16.989 g, accurately weighed, in a 50 ml beaker, in 20 ml of water, and transfer the solution with 20 ml portions of water, into a 1000 ml volumetric flask. Lubricate previously a thin layer of tap lubricant on the external surface of the mouth of the beaker, and pour the solution and the washing water, respectively, into the flask without using any funnel. Adjust the temperature of the solution to 20°, and add water of 20°, to produce 1000 ml of solution. The titer of the solution prepared in this way is practically 1.000.

Standardization of the solution

It is practical to combine the eventually required checking of the titer of the 0.1 **N** silver nitrate solution with the determination and checking, respectively, of the titer of the 0.1 **N** potassium thiocyanate solution. For that purpose, add 20 ml of water, 5 ml of R-nitric acid and 1 ml of **I**-iron(III) nitrate solution to 10.00 ml of the 0.1 **N** silver nitrate solution to be checked in a 100 ml flask. Titrate the mixture with the 0.1 **N** potassium thiocyanate to be checked. Denote by a the number of millilitres, multiplied by 2.5, of 0.1 **N** potassium thiocyanate consumed in the titration. To 25.00 ml of 0.1 **N** silver nitrate solution to be checked add in a 200 ml flask, 5 ml of R-nitric acid and 20 ml of water. Dissolve 2 g of R-potassium nitrate in the solution, add 20.00 ml of 0.1 **N** hydrochloric acid and keep the reaction mixture for 3 minutes in vigorous boiling. Titrate the cooled solution with 0.1 **N** potassium thiocyanate solution, using 1 ml of **I**-iron(III) nitrate solution as indicator. Denote by b the amount of solution consumed in the second titration.

Calculate the titer (T_t) of the 0.1 **N** potassium thiocyanate solution by the formula

$$T_t = \frac{20}{a\left(1 - \frac{b}{a}\right)}$$

Calculate at the same time the titer of the 0.1 **N** silver nitrate solution (T_s) by the formula

$$T_s = \frac{20}{25\left(1 - \frac{b}{a}\right)}$$

If the titer of the applied 0.1 **N** hydrochloric acid is other than 1.000 then the values obtained by the above specified formulas must be multiplied by that factor. Instead of 0.1 **N** hydrochloric acid also a 0.1 **N** sodium chloride solution prepared from sodium chloride of analytical grade may be used.

Store the solution procted from light and dust.

33 Silver Nitrate Solution, 0.01 **N** *(1/100* **M***)*

Each ml of the solution contains 1.6989 mg of $AgNO_3$.

(a) Dissolve in a volumetric flask, in water at 20°, an accurately weighed 1.6989 g of silver nitrate prepared in the way specified in paragraph *32*, in the mode described there, to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 50.00 ml of 0.1 **N** silver nitrate solution with water of the same temperature, to produce 500 ml of solution. The titer of the solution prepared this way is practically the same as that of the 0.1 **N** silver nitrate solution.

Check the titer of the solution, if necessary, in the way specified in paragraph *32*, using 0.01 **N** hydrochloric acid and 0.01 **N** potassium thiocyanate solution.

Preserve the solution protected from light and dust.

34 Sodium Edetate Solution, 0.05 **M**

Each ml of the solution contains 18.613 mg of disodium edetate.

Dissolve 18.6130 g of disodium edetate of analytical grade, accurately weighed, in a volumetric flask, in water of 20°, to 1000 ml. (If the substance does not dissolve clearly in the first portions of water, add a few drops of R-sodium hydroxide solution to the opalescent liquid, and complete the volume only when the liquid has become completely clear.)

The titer of the solution prepared in this way is practically 1.000.

Check the titer of the solution, if necessary, as follows.

In the titration of calcium and magnesium salts, use the titer of 0.05 **M** Sodium Edetate Solution standardized against calcium chloride, while in the titration of aluminium, bismuth, zinc, lead and iron salts, use the titer standardized against zinc-sulphate.

Standardization of the solution against calcium chloride

Dilute 10.00 ml of 0.05 **M** calcium chloride solution in a 200 ml conical flask with water to about 100 ml. Add 2 ml of R-sodium hydroxide solution and 200 mg of **I**-murexide. Titrate the pink solution with the Sodium Edetate solution to be checked, until a violet blue colour is produced.

Calculate the titer of the solution by the formula:

$$T = \frac{b}{a} \times T_b$$

where a = number of millilitres of 0.05 **M** Sodium Edetate solution,
b = number of millilitres and
T_b = the titer of 0.05 **M** balcium chloride solution.

Standardization of the solution against zinc sulphate

Dilute 10.00 ml of 0 .05 **M** zinc sulphate solution in a 100 ml conical flask with 30 ml of water, and add 10 to 20 mg of **I**-methyl thymol blue. Add to the solution dropwise a 20 per cent solution of hexamethylenetetramine until the colour turns just to vivid blue. Titrate the prepared solution subsequently with the 0.05 **N** Sodium Edetate solution to be checked, until a yellow colour is produced.

Calculate the titer of the solution by the formula:

$$T = \frac{b}{a}$$

where a = number of millilitres of 0.05 **M** Sodium Edetate solution,
b = number of millilitres of 0.05 **M** zinc sulphate solution.

35 Sodium Edetate Solution, 0.01 **M**

Each ml of the solution contains 3.7226 mg of sodium edetate.

(a) Dissolve 3.7226 g of sodium edetate accurately weighed in a volumetric flask in 20° water, to 1000 ml.

The titer of the solution produced in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 200.00 ml of 0.05 **M** sodium edetate solution (the titer of which has been checked prior to dilution) with 20° water, to produce 1000 ml of solution. The titer of the solution

prepared in this way is practically the same as that of the 0.05 **M** sodium edetate solution.

Standardization of the solution

Check the titer of the 0.01 **M** sodium edetate solution, if necessary, in the way specified under *34*, though with the use of a 0.01 **M** calcium chloride and 0.01 **M** zinc sulphate solution, respectively, prepared from 0.05 **M** calcium chloride and 0.05 **M** zinc sulphate solution, respectively, by an accurate dilution.

36 Sodium Edetate Solution, 0.005 **M**

Each ml contains 1.8613 mg of sodium edetate.

(a) Dissolve 1.8613 g of sodium edetate accurately weighed in a volumetric flask in 20° water, to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 100.00 ml of 0.05 **M** sodium edetate solution (the titer of which has been checked prior to dilution) with water of 20°, to produce 1000 ml of solution. The titer of the solution prepared in this way is practically the same as that of the 0.05 **M** sodium edetate solution.

Standardization of the solution

Check the titer of the 0.005 **M** sodium edetate solution, if necessary, in the way specified under *34*, though with the use of 0.005 **M** calcium chloride and 0.005 **M** zinc sulphate solution, respectively, prepared from 0.05 **M** calcium chloride and 0.05 **M** zinc sulphate solution, respectively, by accurate dilution.

37 Sodium Hydroxide Solution, 1 **N** *(1* **M***)*

Each ml of the solution contains 40.005 mg of NaOH.

Preparation of approximately 1.0 **N** *sodium hydroxide solution*

Dissolve 100 g of sodium hydroxide in a 2 l flask, in 1800 g of water and add a lime pulp prepared from 50 g of ignited lime and 200 g of water. Seal the flask with a two-hole rubber stopper, equipped with a suction pipe and a trap filled with coarse granular soda lime. On shaking, allow the liquid to sediment. The clear supernatant over the sediment is the stock solution of sodium hydroxide.

Standardization of the solution

Titrate 10.00 ml of 1.0 **N** hydrochloric acid with the stock solution of sodium hydroxide, using **I**-methyl red solution as indicator. On approaching the end point of titration, add some coarse pumice powder in the flask, and remove carbon dioxide from the liquid by boiling. Complete the titration in the cooled liquid.

Repeat the titration without boiling, using **I**-phenolphthalein solution as indicator. The difference between the results of both titrations must not exceed 0.04 ml.

Calculate the titer of the solution separately for the mixture containing **I**-methyl red solution and for that containing **I**-phenolphthalein solution. Use the mean value of the two data.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = number of millilitres and T_a the titer of 1.0 **N** hydrochloric acid, and
b = number of millilitres of 1.0 **N** sodium hydroxide solution.

If the titer of the sodium hydroxide solution is essentially higher than 1.0, dilute the solution with freshly boiled water cooled to 20°, until the titer is approximately 1.0.

Frequently check the titer of the solution.

Store the solution in flasks stoppered with cork stopper coated with melted paraffin.

38 Sodium Hydroxide Solution, 0.1 **N** *(1/10* **M***)*

Each ml of the solution contains 4.005 mg of NaOH.

(a) By a diluting buret, measure into a 1000 ml volumetric flask, $\frac{100}{T_b}$ ml of 1.0 **N** sodium hydroxide of 20° and of a titer T_b, and dilute it with water freshly boiled and cooled to 20°, to 1000 ml. Check the titer of 1.0 **N** sodium hydroxide solution prior to dilution.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting in a volumetric flask, 100.00 ml of 1.0 **N** sodium hydroxide, the titer of which was checked prior to dilution, with freshly boiled water cooled to the temperature of the sodium hydroxide solution, to 1000 ml.

The titer of the solution prepared in this way is practically the same as that of the 1.0 **N** sodium hydroxide.

Standardization of the solution

Determine the titer of the solution in the way specified in paragraph *37*, with the difference that 10.00 ml of 0.1 **N** chrochloric acid is used in the determination.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = number of millilitres and T_a the titer of 0.1 **N** hydrochloric acid, and
b = number of millilitres of 0.1 **N** sodium hydroxide solution.

Frequently check the titer of the solution.

39 Sodium Hydroxide Solution, 0.02 **N** *(1/50* **M***)*

Each ml of the solution contains 0.8001 mg of NaOH.

Dilute 200.00 ml of 0.1 **N** sodium hydroxide solution in a volumetric flask, with freshly boiled and cooled water, to 1000 ml.

Check the titer of the solution in the presence of **I**-methyl red solution as indicator, in the way described in paragraph *37*, using, however, 0.02 **N** sulphuric acid.

40 Sodium Hydroxide Solution, 0.01 **N** *(1/100* **M***)*

Each ml of the solution contains 0.40005 mg of NaOH.

Dilute 10.00 ml of 0.1 **N** sodium hydroxide in a volumetric flask, with freshly boiled and cooled water to produce 100 ml.

Check the titer of the solution in the presence of **I**-methyl red solution as indicator, in the way described in paragraph *37*, using, however, 0.01 **N** hydrochloric acid.

41 Sodium Thiosulphate Solution, 0.1 **N** *(1/10* **M***)*

Each ml of the solution contains 24.8206 mg of $Na_2S_2O_3 \cdot 5H_2O$.

Dissolve in a volumetric flask 25.5 g of sodium thiosulphate, weighed on a hand scales, in about 500 ml of freshly boiled and cooled water. Add 10 ml of isobutanol as a preserving agent, and freshly boiled and cooled water, to produce 1000 ml of solution.

Standardization of the solution

Pipet 10.00 ml of 0.1 **N** potassium hydrogen iodate solution in a glass-stoppered conical flask, and add 1 g of potassium iodide and 2 ml of R-sulphuric acid. Loosely seal the flask with its moistened stopper. Allow the mixture to stand for 5 minutes, and titrate it with 0.1 **N** sodium thiosulphate solution, using **I**-starch solution as indicator.

Calculate the titer of the solution by the formula

$$T = \frac{a}{b} \times T_a$$

where a = number of millilitres and T_a the titer of the 0.1 **N** potassium hydrogen iodate solution, and

b = number of millilitres of 0.1 **N** sodium thiosulphate solution.

Check frequently the titer of the solution.

Preserve the solution in flasks previously cleaned with hydrogen peroxide—containing sulphuric acid.

42 Sodium Thiosulphate Solution, 0.01 **N** *(1/100* **M***)*

Each ml of the solution contains 2.48206 mg of $Na_2S_2O_3 \cdot 5H_2O$.

Transfer 100.00 ml of 0.1 **N** sodium thiosulphate solution, measured by the aid of a volumetric flask, into a 1000 ml volumetric flask. Add 10 ml of isobutanol and freshly boiled and cooled water, to produce 1000 ml of solution.

Standardization of the solution

Pipet 10.00 ml of a 0.01 **N** potassium hydrogen carbonate solution, in a glass-stoppered conical flask. Add 5 ml R-sulphuric acid and 0.5 g potassium hydrogen carbonate reduced to coarse powder previously, and loosely close the flask with its stopper. Mix the reaction mixture at intervals by mild swinging of the flask, and dissolve after the lapse of 20 minutes 0.5 g potassium iodide in the liquid and seal the flask tightly with its stopper. After 5 minutes standing titrate the liquid with 0.01 **N** sodium thiosulphate solution using **I**-starch solution as the indicator.

Check frequently the titer of the solution.

Preserve the solution as specified in paragraph *41*.

43 Sodium Thiosulphate Solution, 0.005 **N** *(1/200* **M***)*

Each ml of the solution contains 1.24103 mg of $Na_2S_2O_3 \cdot 5H_2O$.

Titrate 10.00 ml of 0.1 **N** potassium hydrogen iodate solution in a 100 ml conical flask, in the way described in paragraph *41*. Transfer into a volumetric

flask from the same burette, the same amount of 0.1 **N** sodium thiosulphate as that consumed in the titration, and dilute to 200 ml with freshly boiled water cooled to 20°.

The titer of the solution prepared in this way is practically 1.000.

Check the titer of the solution in each case in the way specified in paragraph *42*, with the difference that use 0.005 **N** potassium hydrogen iodate solution. Prefer the use of freshly prepared solutions.

44 Sulphuric Acid, 1.0 **N** *(1/2* **M***)*

Each ml of the solution contains 49.041 mg of H_2SO_4.

Preparation of approximately 1.0 **N** sulphuric sulphuric acid stock solution.

Weigh 100.00 g of concentrated R-sulphuric acid, purified according to Vol. I, p. 297, on a counter balance into a 100 ml beaker. Pour the acid cautiously into a 2000 ml volumetric flask containing about 1000 ml of water. Rinse the beaker with water, make up the volume of the solution with water. Preserve this *stock solution* in flasks stoppered with cork stoppers saturated with melted paraffin and seal with paraffin.

Determination of the sulphuric acid content of the sulphuric acid stock solution

Recrystallise, if necessary, potassium hydrogen carbonate of analytical grade from water saturated with carbon dioxide, powder the dried crystals, then allow them to dry in a desiccator over freshly ignited sodium sulphate, in a carbon dioxide atmosphere. During drying, stir the potassium hydrogen carbonate powder several times with a clean glass rod. From the substance prepared in this way, weigh 10 g accurately in a 50 ml beaker, and rinse with 100 ml of water into a 300 ml conical flask. In a dry weighing buret (Fig. 31), pour about 120 g of the sulphuric acid stock solution, and weigh the filled weighing buret to mg accuracy, then titrate the solution of potassium hydrogen carbonate with the stock solution, using **I**-methyl red solution as indicator. Prior to the end point of the titration, remove carbon dioxide from the solution by boiling. In order to pretent retarded boiling, add some pumice to the flask. Complete titration in the cooled liquid. On completing the titration, slightly evacuate the air in the buret, by suction, slightly open the tap of the buret, and allow the liquid in the outflow pipe of the buret to be sucked back into the buret. Prior to weighing the buret, compensate the pressure of air inside the burette and outside of it by an adequate turn of the tap. Establish again the weight of the buret to mg accuracy.

FIG. 31

Calculate the weight of the sulphuric acid stock solution (K) required for the preparation of 1000 ml of 1.0 **N** sulphuric acid by the formula

$$K = 100.114 \times \frac{a}{b}$$

where b = weight (in grams) of sulphuric acid stock solution consumed in the titration, and

a = weight (in grams) of potassium hydrogen carbonate weighed in for the titration

(lg 100.114 = .00049).

Determine the sulphuric acid content of the stock solution in at least three parallel runs. Calculate the K values from these determinations and take the mean value. The single K values must not deviate from the mean by more than 0.1 per cent.

On the label of the flask note the weight (K) of the sulphuric acid stock solution required for preparing 1000 ml of 1.0 **N** sulphuric acid and the date of determination. When the stock solution of sulphuric acid is stored in a flask carefully sealed with paraffin coat, repeated checking is necessary only in exceptional case.

Preparation of the exact 1.0 **N** *sulphuric acid*

Transfer into an adequate flask, the amount accurately calculated for 1 litre (K) with 10 mg accuracy, of sulphuric acid, stock solution of known K value, carefully shaken previously. Smear a thin layer of tap lubricant on the external surface of the mouth of the flask, then rinse its contents with water of 20°, into a 1000 ml volumetric flask. Stir the contents of the volumetric flask by swinging, and add water of 20° to produce 1000 ml of solution.

The titer of the solution prepared in this way is practically 1.000.

Standardization of 1.0 **N** *sulphuric acid*

Check the titer of the solution if necessary, as follows. Dissolve in 50 ml of water in a 100 ml conical flask, a 1.00 g portion of potassium hydrogen carbonate accurately weighed, processed in the previously specified way. Titrate the solution with the sulphuric acid solution to be checked against **I**-methyl red solution as indicator, using a 12 ml buret. Near to the end point of titration, remove carbon dioxide from the solution by boiling, and complete titration in the cooled liquid.

Calculate the titer (T) of the solution by the formula

$$T = \frac{g \times 1000}{b \times 100.114},$$

where g = weight (in grams) of potassium hydrogen carbonate, and
b = amount (in ml) of 1.0 **N** sulphuric acid to be checked, consumed in the titration
(lg 100.114 = .00049).

45 Sulphuric Acid, 0.1 **N** *(1/20* **M***)*

Each ml of the solution contains 4.9041 mg of H_2SO_4.

(a) Measure in an adequate flask, with 10 mg accuracy, an amount corresponding to one tenth of the value K of the sulphuric acid stock solution of known K value, carefully shaken, and dilute accurately in the way prescribed for the preparation of 1.0 **N** sulphuric acid, to produce 1000 ml of solution.

The titer of the solution prepared in that way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 100.00 ml of 1.0 **N** sulphuric acid, the titer of which has been checked prior to dilution, with water of 20°, to 1000 ml.

The titer of the solution prepared in this way is practically the same as that of 1.0 **N** sulphuric acid.

Standardization of 0.1 **N** *sulphuric acid*

Check the titer in the way specified in paragraph *44*, with the following difference. Dissolve the prescribed amount of potassium hydrogen carbonate in a volumetric flask, in water of 20°, to produce 100 ml of solution, and titrate 10.00 ml portions of this solution with the 0.1 **N** sulphuric acid to be checked.

Calculate the titer of the solution by the formula

$$T = \frac{g \times 1000}{10.0114 \times b},$$

where g = weight (in grams) of potassium hydrogen carbonate dissolved in 100 ml of water, and

b = amount (in ml) of 0.1 **N** sulphuric acid consumed in the titration (lg 10.0114 = .00049).

46 Sulphuric Acid, 0.02 **N** *(1/100* **M***)*

Each ml of the solution contains 0.98082 mg of H_2SO_4.

(a) Measure in an adequate, glass-stoppered flask, with mg accuracy, an amount corresponding to 1/50 of the value K, of the sulphuric acid stock solution according to paragraph *44*, of known K value, shaken carefully, and dilute it, accurately in the way prescribed for the preparation of 1.0 **N** sulphuric acid, to 1000 ml.

The titer of the solution prepared in this way is practically 1.000.

(b) Another method consists in diluting, in a volumetric flask, 200.00 ml of 0.1 **N** sulphuric acid the titer of which has been checked prior to dilution with water of 20°, to 1000 ml.

The titer of the solution prepared in this way is practically the same as that of 0.1 **N** sulphuric acid.

Standardization of 0.02 **N** *sulphuric acid*

Check the titer of 0.02 **N** sulphuric acid in the way specified in paragraph *44*, with the difference of weighing accurately 0.20 g of potassium hydrogen carbonate, and dissolve in water, to 100 ml in a volumetric flask. Titrate 10.00 ml portions of this solution with the 0.02 **N** sulphuric acid to be checked.

Calculate the titer of the solution by the formula

$$T = \frac{g \times 100}{2.00228 \times b},$$

where g = weight (in grams) of potassium hydrogen carbonate dissolved in 100 ml of water, and

b = volume (in ml) of 0.02 **N** sulphuric acid consumed in the titration (lg 2.00228 = .30152).

47 Zinc Sulphate Solution, 0.05 **M**

Each ml of the solution contains 8.072 mg of $ZnSO_4$ or 14.378 mg of $ZnSO_4 \cdot 7H_2O$.

To about 4 g of granulated zinc, analytical grade, weighed on a hand scales add, in a small crystallisation dish, 10 ml of water and 10 ml of R-sulphuric acid. After a few minutes of vivid gas evolution, decant the liquid off the zinc,

dry the residue, rinse it several times with water, then with some R-alcohol. Transfer the zinc thus pretreated, onto a watch-glass, and dry for an hour at 100°. Into a 200 ml digestion flask fixed obliquely in a stand add 3.2690 g of the zinc prepared in the above-described way, and accurately weighed. Add in small portions a total of 25 ml of 50 per cent sulphuric acid to the zinc, and cautiously heat the contents of the flask over a small flame, until all zinc dissolves. If the zinc particles are rather small, add sulphuric acid in small doses, very cautiously, and heat the contents of the flask also very cautiously, and only if necessary. After the complete dissolution of all zinc, dilute the cooled sulphuric acid solution with water, rinse the liquid into a 100 ml volumetric flask, and dilute it with water to 100 ml.

The titer of the solution thus prepared is 1.000.

VI INDICATORS

For the preparation of indicator solutions use freshly boiled and cooled water, alcohol distilled from over potassium hydroxide or another solvent prescribed in the specifications. When diluting solid indicators as diluting agent use potassium nitrate of analytical grade, previously dried for 2 hours at 105°.

It is advisable to preserve the indicator solutions in adequate dropping flasks, and the diluted solid indicators in well-closed, glass-stoppered flasks, protected from light.

On the label of the flasks note the name of indicator, its concentration and the pH values of the colour change.

Indicator solutions for aqueous solutions are denoted by **I**, those for non-aqueous titrations with perchloric acid by **I***.

Perform titrations in daylight if titration is to be continued until attaining a fixed tint or if the colour of the indicator turns to blue or to green during titration.

SOLID INDICATORS AND INDICATOR SOLUTIONS

I-Benzylorange Solution

Dissolve 0.010 g of benzyl orange (Vol. I, p. 273) in a volumetric flask, in water, to 100 ml.

The pH values of colour change are: 1.9 to 3.3 (*from red to yellow*).

*-Brillant Green Solution

Dissolve 0.05 g of brilliant green (Vol. II, monograph No. 371) in a volumetric flask, in dehydrated acetic acid, to 100 ml.

Colour change: *from green to yellow.*

I-Bromocresol Green Solution

Triturate in a mortar, preferably of agate, 0.040 g of bromocresol green (Vol. I, p. 274) with 0.58 ml of 0.1 **N** sodium hydroxide solution, then dissolve the mixture in a volumetric flask in water, to 100 ml.

The pH values of the colour change are: 4.0 to 5.6 (*from yellow to blue*).

I-Bromophenol Blue Solution

Triturate in a mortar, preferably of agate, 0.040 g of bromophenol blue (Vol. I, p. 274) with 0.60 ml of 0.1 **N** sodium hydroxide solution then dissolve the mixture in a volumetric flask in water, to 100 ml.

The pH values of the colour change are: 3.0 to 4.6 (*from yellow to bluish violet*).

I-Bromothymol Blue Solution

Triturate in a mortar, preferably of agate, 0.040 g of bromothymol blue (Vol. I, p. 281) in 0.64 ml of 0.1 **N** sodium hydroxide solution, then dissolve the mixture in a volumetric flask in water, to produce 100 ml of solution.

The pH values of the colour change are: 6.0 to 7.6 (*from yellow to blue*).

I-Diphenylamine Solution

Dissolve 0.50 g of diphenylamine in 50 ml of 80 per cent sulphuric acid.

Preserve the solution protected from light.

I-Eriochrome Black-T

Triturate carefully 0.05 g of eriochrome black-T (Vol. I, p. 283) with 5 g of potassium nitrate.

I-p-Ethoxychrysoidine Solution

Dissolve 0.20 g of p-ethoxychrysoidine hydrochloride (Vol. I, p. 284) in a volumetric flask, in 96 per cent alcohol, to 100 ml.

The pH values of the colour change are: 3.5 to 5.5 (*from red to yellow*).

I-Ferroin Solution

Solution of trio-o-phenanthroline-iron(II) sulphate

Dissolve 1.76 g of o-phenanthroline hydrochloride (Vol. I, p. 298) and 0.70 g of crystalline iron(II) sulphate in a volumetric flask, in water, to 100 ml.

I*-Gentian Violet Solution

Dissolve 0.10 g of gentian violet (Vol. II, monograph No. 231) in a volumetric flask in glacial acetic acid, to 100 ml.

Colour change: *from violet blue to green.*

I-Iron(III) Nitrate Solution

Dissolve 10 g of crystalline iron(III) nitrate (Vol. I, p. 289) in a volumetric flask, in 10 ml of 50 per cent nitric acid and in enough water, to produce 100 ml of solution.

I*-Malachite green Solution

Dissolve 0.50 g of malachite green (Vol. I, p. 292) in a volumetric flask, in dehydrated acetic acid, to produce 100 ml of solution.

Colour change: *from green to yellow.*

I-Methanyl Yellow Solution

Dissolve 0.20 g of methanyl yellow (Vol. I, p. 294) in a volumetric flask, in water, to 100 ml.

I*-Methylene Blue Solution

Dissolve 0.10 g of methylene blue (Vol. II, monograph No. 225) in a volumetric flask, in dehydrated acetic acid, to 100 ml.

I-Methyl Orange Solution

Dissolve 0.10 g of methylorange (Vol. I, p. 294), in a volumetric flask, in water, to 100 ml.

The pH values of the colour change are: 3.1 to 4.4 (*from red to yellow*).

I-Methyl Red Solution

Titurate 0.05 g of methyl red (Vol. I, page 294) with some 96 per cent alcohol, then dissolve the mixture in a volumetric flask in 96 per cent alcohol, to produce 100 ml of solution.

The pH value of the colour change are: 4.4 to 6.3 (*from red to yellow*).

I-Methyl Red-Methylene Blue Solution

To 2 volume of **I**-methyl red solution add one volume of a 0.05 per cent alcoholic solution of methylene blue (Vol. I, p. 294).

Colour change: *from violet to green.*

I-Methyl Thymol Blue

Titurate 0.05 g of methyl thymol blue (Vol. I, p. 294) with 5 g of potassium nitrate.

I-Murexide

Triturate 0.05 g of murexide (Vol. I, p. 295) with 5 g of potassium nitrate.

I*-α-Naphtholbenzein Solution

Dissolve 0.50 g of α-naphtholbenzein (Vol. I, p. 295) in a volumetric flask in dehydrated acetic acid, to 100 ml.

Colour change: *from yellow to green.*

I-Phenolphthalein Solution

Dissolve 1.00 g of phenolphthalein (Vol. II, monograph No. 298) in a volumetric flask, in R-alcohol, to 100 ml.

The pH values of the colour change are: 8.2 to 10.0 (*from colourless to red*).

I-Phenol Red Solution

Triturate in a mortar, preferably of agate, 0.020 g of phenol red (Vol. I, p. 298) with 0.57 ml of 0.1 **N** sodium hydroxide solution, then dissolve the mixture in a volumetric flask in water, to 100 ml.

The pH values of the colour change are: 6.8 to 8.4 (*from yellow to red*).

I-Potassium Chromate Solution

R-Potassium Chromate Solution (Vol. I, p. 302).

I-Pyridylazonaphthol Solution

Dissolve 0.10 g of pyridylazonaphthol in a volumetric flask, in 96 per cent alcohol, to 100 ml.

I-Starch Solution

Dissolve 0.1 g of salicylic acid in a 300 ml conical flask, in 100 ml of water, under vigorous boiling. To the hot liquid add in small portions the well-shaken, milk-like mixture of 1 g of potato starch (Vol. III, monograph No. 381) and 20 ml of water. Continue boiling under periodical replacement of the evaporated water, until starch has been completely dissolved and a slightly opalescent liquid has been formed. Filter the cooled solution, if necessary, through a paper filter.

I*-Sudan III Solution

Dissolve 0.50 g of Sudan III (Vol. I, p. 317) in a volumetric flask, in dehydrated acetic acid, to 100 ml.

Colour change: *from orange red to blue.*

I-Thymol Blue Solution

Triturate in a mortar, preferably of agate, 0.040 g of thymol blue (Vol. I, p. 321) with 0.86 ml of 0.1 **N** sodium hydroxide solution, then dissolve the mixture in a volumetric flask in water, to 100 ml.

The pH values of the colour change are:-1.2 to 2.8 (*from red to yellow*), 8.0 to 9.6 *(from yellow to blue).*

I-Thymolphthalein Solution

Dissolve 0.10 g of thymolphthalein (Vol. I, p. 321) in a volumetric flask, in R-alcohol, to 100 ml.

The pH values of the colour change are: 9.3 to 10.5 (*from colourless to blue*).

I-Tropeolin Solution

Dissolve 0.010 g of tropeoline 00 (Vol. I, p. 322) in a volumetric flask, in water, to 100 ml.

The pH values of the colour change are: 1.3 to 3.2 (*from red to yellow*).

I*-Tropeolin Solution

Dissolve 0.20 g of tropeoline 00 (Vol. I, p. 322) in a volumetric flask, in dehydrated acetic acid, to 100 ml.

Colour change: *from yellow to red.*

I-Variamin Acetate Solution

Triturate in a small porcelain mortar 0.20 g of variamine blue (Vol. I, p. 323) with a few crystals of ascorbic acid, then rinse the mixture into a separatory funnel with four 5 ml portions of water. Add 5 ml of 1.0 **N** sodium hydroxide solution, and extract with 20 ml of benzene. Repeat the extraction with two successive 5 ml portions of benzene. Filter the benzene phases into another separatory funnel, through filter paper washed previously with benzene, and extract the liquid with 20 ml of 20 per cent acetic acid. Filter the aqueous phase through filter paper moistened with water. The indicator solution prepared in this way has a blue colour which becomes deeper in a few hours.

The solution is suitable for use for 3 weeks!

INDICATOR PAPERS

Congo Paper

Non-glossy, hygroscopic paper strips stained red with a 0.1 per cent alcoholic solution of congo red.

The pH values of the colour change are: 2.5 to 4.0 (*from violet blue to red*).

Dilute 1 ml of 0.1 **N** hydrochloric acid with water to 100 ml. Congo paper dipped in this about 0.001 **N** hydrochloric acid must turn violet blue. Dilute 10 ml of about 0.001 **N** hydrochloric acid with water to 100 ml. Congo paper dipped in the about 0.0001 **N** hydrochloric acid prepared in this way must retain its red colour.

Curcuma Paper

Non-glossy, hygroscopic paper strips stained yellow with curcuma tincture.

The pH values of the colour change are: 7.5 to 9.5 (*from yellow to brown*).

Litmus Paper, Blue

Non-glossy, hygroscopic paper strips stained blue with a slightly alkaline, 1 per cent solution of azolithmine.

The pH values of the colour change are: 5.0 to 8.0 (*from blue to red*).

Immerse in about 0.0002 **N** hydrochloric acid (prepared by adding 250 ml of freshly boiled and cooled water to 0.5 ml of 0.1 **N** hydrochloric acid) a strip of blue litmus paper and stir the liquid by shaking. The blue litmus paper must turn red in one minute.

Litmus Paper, Red

Non-glossy, hygroscopic paper strips stained pink with a slightly acidic, 1 per cent solution of azolithmine.

The pH values of the colour change are: 5.0 to 8.0 (*from red to blue*).

Immerse in about 0.00025 **N** sodium hydroxide solution (prepared by adding 200 ml of freshly boiled and cooled water to 0.5 ml of 0.1 **N** sodium hydroxide) a strip of red litmus paper and stir the liquid by shaking. The colour of red litmus paper must turn blue in one minute.

Starch-Potassium Iodide-Paper

Triturate 0.25 g of starch with 5 ml of water, and pour the homogenous pulp to 90 ml of water heated to boiling. Allow the mixture to boil for one minute, and filter after cooling. To the filtrate add the solution of 0.4 g of potassium iodide in 5 ml of water. Immerse strips of filter paper in the solution obtained in this way. Dry the strips at room temperature, protected from light and dust.

Place one drop of R-sulphuric acid on starch potassium iodide paper. No colouration must be produced.

Dilute 5 drops of a freshly prepared 1 per cent solution of sodium nitrite with water, to obtain 50 ml of solution. Shake a 5 ml portion of this solution with 2 drops of R-sulphuric acid. Place one drop of this mixture on starch potassium iodide-paper. A blue colour must be produced in 5 minutes.

VII BUFFER SOLUTIONS

Prepare the buffer solutions from the stock solutions made with freshly boiled and cooled water and with buffer substances marketed particularly for that purpose and very carefully checked. Follow the instructions given in Table 35 (Vol. I, p. 360). Weigh the primary substances on analytical balance, and adjust the volume of the solution at 20.0°.

STOCK SOLUTIONS

Borax Stock Solution, 0.05 M

Borax used for the preparation of buffer solution ($Na_2B_4O_7 \cdot 10H_2O$; Mol. Wt. 381.44) must contain not less than 99.9 and not more than 100.1 per cent of $Na_2B_4O_7 \cdot 10H_2O$. In order to check the substance, dry about 2 g, accurately weighed, at 105° for 3 hours, then cautiously ignite it. The weight loss must be not less than 47.2 and not more than 47.3 per cent.

Each mg of residue on ignition ($Na_2B_4O_7$) is equivalent to 1.895 mg (lg .27764) of $Na_2B_4O_7 \cdot 10H_2O$.

Preparation

Dissolve 19.072 g of borax at 20°, in a volumetric flask, in freshly boiled and cooled water, to 1000 ml.

Borax Stock Solution, 0.01 M

Check the borax serving for the preparation of the solution in the way specified in the preceding paragraph.

Preparation

Dissolve 3.8144 g of borax in a volumetric flask, in freshly boiled and cooled water at 20°, to 1000 ml.

Calcium Hydroxide Stock Solution, Saturated

Calcium oxide (CaO; Mol. Wt. 56.08) serving as primary material must contain not less than 88 per cent of CaO. Check the substance as follows.

Slake with some boiled-out water, 1.00 g of calcium oxide, weighed with 0.01 g accuracy. Rinse the slaked lime with freshly boiled and cooled water into a 100 ml volumetric flask, and dilute it with water, to 100 ml. Shake the stoppered flask for a few minutes.

Dilute a 10.00 ml portion of the well-shaken liquid, in a volumetric flask, with boiled and cooled water, to 100 ml. Titrate a 10.00 ml portion of the diluted solution with 0.1 **N** hydrochloric acid.

Each ml of 0.1 **N** hydrochloric acid is equivalent to 2.804 mg (lg .44778) of CaO.

Preparation

Shake 2 g of ignited lime with small portions of about 10 g of distilled water. Rinse the pulp with 50 ml of water into a beaker, shake up, and allow the mixture to sediment for a few hours. Decant and discard the supernatant clear liquid. To the residue add 100 g of distilled water, agitate, and use the clear supernatant until the $Ca(OH)_2$ content of the checked solution ranges between 0.14 and 0.16 per cent.

Each ml of 0.1 **N** hydrochloric acid is equivalent to 3.705 mg (lg .56877) of $Ca(OH)_2$.

Citric Acid Stock Solution, 0.1 M

Citric acid ($C_6H_8O_7 \cdot H_2O$; Mol. Wt. 210.14) serving as primary material must contain not less than 99.5 and not more than 100.2 per cent of $C_6H_8O_7 \cdot H_2O$. Check the substance as follows.

I. Dry about 1 g of citric acid, accurately weighed, at first at 50° for 3 hours, then at 105° to constant weight. The loss on drying must be not less than 8.1 and not more than 9.1 per cent.

II. In a volumetric flask dissolve 0.35 g of citric acid, accurately weighed, in freshly boiled and cooled water, to produce 100 ml of solution. Titrate a 20.00 ml portion of the solution with 0.1 **N** sodium hydroxide, using 2 to 3 drops of **I**-phenolphthalein solution as indicator.

Each ml of 0.1 **N** sodium hydroxide is equivalent to 7.005 mg (lg .84538) of $C_6H_8O_7 \cdot H_2O$.

Preparation

Dissolve 21.014 g of citric acid in a volumetric flask, at 20°, in freshly boiled and cooled water, to 1000 ml.

To the solution add a few grains of mercury(II) iodide as preserving agent.

The pH of a mixture of 4.00 ml of 0.2 **M** phosphate stock solution and 6.00 ml of 0.1 **M** citric acid stock solution must be 4.1 at 20°.

Hydrochloric Acid Stock Solution, 0.1 M

The titer of 0.1 **M** hydrochloric acid must range between 0.990 and 1.010, using **I**-methyl red solution for the determination.

The pH of a mixture of 6.00 ml of 0.05 **M** borax stock solution and 4.00 ml of 0.1 **M** hydrochloric acid stock solution must be 8.3 at 20°.

Potassium Hydrogen Phthalate Stock Solution, 0.05 M

Dry potassium hydrogen phthalate serving as primary material (Vol. I, p. 303) at 120° for 2 hours.

Preparation

Dissolve 10.2110 g of dried potassium hydrogen phthalate in a volumetric flask, at 20° in freshly boiled and cooled water, to 1000 ml.

Potassium Hydrogen Tartrate Stock Solution, Saturated

Potassium hydrogen tartrate ($C_4H_5O_6K$; Mol. Wt. 188.18) serving as primary material must contain at least 99.50 per cent of $C_4H_5O_6K$. Check the substance as follows.

Dissolve about 0.2 g of potassium hydrogen tartrate, accurately weighed, in 30 ml of freshly boiled water, if necessary, under gentle heating. Titrate the solution with 0.1 **N** sodium hydroxide, using 5 drops of **I**-phenolphthalein solution as indicator.

Each ml of 0.1 **N** sodium hydroxide is equivalent to 18.82 mg (lg .27456) of potassium hydrogen tartrate ($C_4H_5O_6K$).

Preparation

To 1000 ml of water, add 7 g of powdered potassium hydrogen tartrate. Boil the liquid under stirring with a glass rod, for 5 minutes, then cool under frequent shaking and allow the formed crystals to sediment. Use the clear supernatant as the reagent.

Potassium Sodium Phosphate Stock Solution, 0.025 M

Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$; Mol. Wt. 178.01) serving as primary material must contain not less than 99.9 and not more than 100.2 per cent of $Na_2HPO_4 \cdot 2H_2O$. Check the substance in the way specified at the 0.2 **M** stock solution of sodium phosphate.

Potassium dihydrogen phosphate (KH_2PO_4; Mol. Wt. 136.09) serving as primary material must contain not less than 99.8 per cent of KH_2PO_4. Check the substance as follows.

I. Dry about 5 g of finely powdered potassium dihydrogen phosphate, accurately weighed in vacuo, at 20 to 30 torr at 100°, for 24 hours. The weight loss must not exceed 0.2 per cent.

II. Ignite in a platinum crucible about 5 g of finely powdered potassium dihydrogen phosphate, accurately weighed. The weight loss must not be less than 13.1 and more than 13.3 per cent.

Preparation

Dissolve 3.40 g of potassium dihydrogen phosphate and 4.45 g of disodium hydrogen phosphate in freshly boiled and cooled water, at 20°, to 1000 ml.

Potassium Tetraoxalate Stock Solution, 0.05 M

Potassium tetraoxalate ($C_4H_3O_8K \cdot 2H_2O$; Mol. Wt. 254.20) serving as primary material must contain not less than 99.8 and not more than 100.1 per cent of $C_4H_3O_8K \cdot 2H_2O$. Check the substance as follows.

I. Dry at 130° for 2 hours about 1 g of potassium tetraoxalate, accurately weighed. The loss on drying must be not less than 14.1 and not more than 14.3 per cent.

II. Dissolve about 0.25 g of potassium tetraoxalate, accurately weighed, in 30 ml of water. Add 8 ml of concentrated R-sulphuric acid, and on heating the mixture to about 60°, titrate with 0.1 **N** potassium permanganate solution.

Each ml of 0.1 **N** potassium permanganate solution is equivalent to 6.353 mg (lg .80298) of $C_4H_3O_8K \cdot 2H_2O$.

Preparation

Dissolve 12.71 g of potassium tetraoxalate in a volumetric flask in freshly boiled and cooled water, to 1000 ml.

Sodium Hydroxide Stock Solution, Free of Carbonate, 0.1 M

The titer of 0.1 **M** sodium hydroxide solution must range between 0.990 and 1.010, when using **I**-phenolphthalein solution as indicator.

The pH of a mixture of 5.50 ml of 0.05 **M** borax stock solution and 4.50 ml of 0.1 **M** sodium hydroxide stock solution at 20° must be 10.3.

Sodium Phosphate Stock Solution, 0.2 M

Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$; Mol. Wt. 178.01) serving as primary material for preparing the solution must contain not less than 99.9 and not more than 100.2 per cent of $Na_2HPO_4 \cdot 2H_2O$. Check the substance as follows.

Dry in a platinum crucible at 110° for 3 hours about 1 g, accurately weighed, then ignite.

Each mg of the residue on ignition is equivalent to 1.339 mg (lg .12667) of $Na_2HPO_4 \cdot 2H_2O$.

Preparation

Dissolve 35.602 g of disodium hydrogen phosphate dihydrate in a volumetric flask, at 20°, in freshly boiled and cooled water, to 1000 ml. To the solution add a few drops of metallic mercury as preserving agent.

TABLE 35

pH	Composition of buffer solution, ml				
	0.2 **M** sodium phosphate stock solution	0.05 **M** borax stock solution	0.1 **M** citric acid stock solution	0.1 **M** hydrochloric acid stock solution	0.1 **M** sodium hydroxide stock solution
2.2	2.00	—	98.00	—	—
2.5	8.50	—	91.50	—	—
3.0	20.60	—	79.40	—	—
3.5	30.40	—	69.60	—	—
4.0	38.60	—	61.40	—	—
4.5	45.50	—	54.50	—	—
5.0	51.50	—	48.50	—	—
5.5	56.90	—	43.10	—	—
6.0	63.20	—	36.80	—	—
6.5	71.00	—	29.00	—	—
7.0	82.40	—	17.60	—	—
7.5	92.30	—	7.70	—	—
8.0	97.30	—	2.70	—	—
8.5	—	64.50	—	35.50	—
9.0	—	85.00	—	15.00	—
9.5	—	80.00	—	—	20.00
10.0	—	59.60	—	—	40.40
10.5	—	53.10	—	—	46.90
11.0	—	50.20	—	—	49.80

From the stock solutions prepare the buffer solutions listed in Table 35. They may be kept on stock according to necessity.

Store the buffer solutions in glass-stoppered flasks of resistent glass, and periodically check the pH value of the solutions.

VIII REAGENTS AND STAINS FOR USE IN THE CLINICAL LABORATORY

Filter the prepared solutions — if permitted by the nature of the substance and if seems necessary — through a dry paper filter until clear.

(A) REAGENTS FOR THE EXAMINATION OF URINE

(a) REAGENTS FOR PROTEIN

Acetate Buffer-Solution

Dissolve 118 g of crystalline sodium acetate and 60 g of concentrated acetic acid in water in a volumetric flask to 1000 ml. The solution is a clear fluid with a faint odour of acetic acid. Its hydrogen-exponent pH = 4.7.

Acetic Acid, 1 per cent

Dissolve 1.0 g of concentrated acetic acid in water in a volumetric flask to 100 ml. The solution is a clear fluid with a mild odour of acetic acid.

Nitric Acid, 25 per cent

Dilute 38 g of 66 per cent nitric acid with water in a volumetric flask to produce 100 ml. A clear colourless, strongly acidic fluid results.

Sulphosalicylic Acid Solution, 20 per cent

Dissolve 20 g of sulposalicylic acid in a volumetric flask in water to produce 100 ml. A clear, colourless fluid results.

Esbach's Solution

Dissolve 1.0 g of picric acid and 2 g of citric acid in water in a volumetric flask to 100 ml. A clear, yellow-coloured fluid results.

Potassium-Iron(II) Cyanide Solution, 5 per cent

Dissolve 5.0 g of potassium-iron(II) cyanide in a volumetric flask in water to 100 ml. A clear yellow-coloured fluids results.

(b) REAGENT FOR PUS

Alkali Lye Solution, 20 per cent

Dissolve 20 g of potassium or sodium hydroxide in a volumetric flask in water to 100 ml. A clear colourless caustic lye results.

(c) REAGENTS FOR SUGARS

Sodium Hydroxide Solution, 15 per cent

Dissolve 15 g of sodium hydroxide in water in a volumetric flask to 100 ml. A clear, colourless, caustic lye results.

Fehling's Solution I

Dissolve 36.64 g of crystalline copper sulphate in water in a volumetric flask to 500 ml. A clear, blue coloured fluid results.

Fehling's Solution II

Dissolve 173 g potassium sodium tartrate and 50 g of sodium hydroxide in water in a volumetric flask to produce 500 ml. A clear, colourless, basic fluid results.

Nylander's Solution

Dissolve 4.0 g of potassium sodium tartarate and 10 g of sodium hydroxide in a volumetric flask in 50 ml of water. Add to the fluid 2.0 g of basic bismuth nitrate and after dissolution, dilute with water to 100 ml. After sedimentation, filter the fluid on glass wool — if necessary. A clear, colourless, basic fluid should result.

Benedict's Solution

Dissolve 17.3 g of tertiary potassium or sodium citrate and 27 g of crystalline sodium carbonate in 70 ml of water in a volumetric flask. Dissolve in a smaller flask 1.73 g of crystalline copper sulphate in 15 ml of water. Add the latter solution with constant stirring, in small portions slowly to the first solution and fill up with water to 100 ml. A clear, blue-coloured, basic fluid results.

Solutions for Hagedorn-Jensen's Determination of Blood Sugar

(cf. 5.71, Vol. I, p. 160)

d) REAGENTS FOR PENTOSES

Iron(III) Chloride Solution, 10 per cent (g/v)

Dilute 20 g of 50 per cent iron(III) chloride solution in a volumetric flask with water to 100 ml. A clear, brownish-red-coloured solution results.

Bial's Solution

Dissolve 0.30 g of resorcin in 50 ml of 30 per cent hydrochloric acid in a volumetric flask and after mixing with 5 drops of a 10 per cent iron(III) chloride solution, dilute with 30 per cent hydrochloric acid to 100 ml. A clear light-coloured, strongly acidic fluid results.

(e) REAGENT FOR FRUCTOSE

Seliwanoff's Solution

Dissolve 0.05 g of resorcin in a volumetric flask in 18 per cent of hydrochloric acid to 100 ml. A clear, colourless or a faintly yellow fluid results. Preserve protected from light.

(f) REAGENTS FOR ACETONE

Sodium Nitroprusside Solution

Dissolve one part per weight of sodium nitroprusside in 4 parts per weight of water. Use each time a freshly prepared solution.

Sodium Hydroxide Solution

15 per cent

Acetic Acid, Concentrated

not less then 96 per cent

Ammonia Solution

10 per cent

Rothera's Mixture

Prepare a powder mixture from 1 g of sodium nitroprusside, 20 g of ammonium sulphate and 20 g of dried sodium carbonate. Preserve in a dry place in a glass-stoppered wide mouth glass bottle.

(g) REAGENT FOR ACETOACETIC ACID

Iron(III) Chloride Solution

50 per cent (g/v)

(h) REAGENTS FOR BILE PIGMENTS

Fouchet's Solution

Dissolve 25 g of trichloroacetic acid and 10 ml of an aqueous, 10 per cent iron(III) chloride solution in a volumetric flask to 100 ml. A clear, yellow coloured, acidic fluid is produced.

Gmelin's Solution

Dissolve for each 5 ml of a 50 per cent nitric acid 0.01 to 0.02 g of sodium nitrate or add a drop of fuming nitric acid. Prepare the reagent directly before use.

Lugol's Solution with 5 per cent of Iodine

Dissolve 10 g of potassium iodide in a volumetric flask in 10 ml of water. Add to the fluid 5 g of finely powdered iodine. After dissolution of the latter, dilute the liquid with water to 100 ml. A clear, dark-brown-coloured liquid results.

Iodine Solution, Alcoholic, 1 per cent

Dissolve 1 g of powdered iodine in alcohol 96 per cent, in a volumetric flask, to 100 ml. A clear dark-brown-coloured, alcoholic fluid results.

(i) REAGENT FOR UROBILINOGEN

Ehrlich's Solution

Dissolve 2.0 g of dimethylaminobenzaldehyde in a volumetric flask in 20 per cent hydrochloric acid to 100 ml. A clear yellow coloured, strongly acid fluid results.

(j) REAGENTS FOR INDICAN

Obermayer's Solution

Dilute 4 g of a 50 per cent iron(III) chloride solution in a volumetric flask, with concentrated hydrochlorid acid, 36 per cent, to 100 ml. A light yellow-coloured, strongly acidic liquid results.

Lead Acetate Solution, 25 per cent

Dissolve 25 g of crystalline lead-acetate in 75 g of freshly boiled water. After cooling filter through a paper filter.

Chloroform

(Vol. I, p. 277)

(k) REAGENT FOR BARBITURIC ACID

Millons's Solution

(Vol. I, p. 294)

(l) REAGENTS FOR HEMOGLOBIN

Acetic Acid, 30 per cent

Dilute 30 g of concentrated acetic acid in a volumetric flask to 100 ml.

Benzidine

(Vol. I, p. 273)

Hydrogen Peroxide, 3 per cent g/g

(Vol. I, p. 287)

Aminophenazone Solution, 5 per cent, Alcoholic

Dissolve 5.0 g of aminophenazone in alcohol, 96 per cent, in a volumetric flask to 100 ml. A clear, colourless, alcoholic fluid results.

Acetic Acid, 50 per cent

Dilute 50 g of concentrated acetic acid with water to 100 ml.

(B) REAGENTS FOR THE EXAMINATION OF GASTRIC FLUID

Dimethylaminoazobenzene Solution, 0.5 per cent

Dissolve 0.5 g of p-dimethylaminoazobenzene in a volumetric flask in alcohol 90 per cent, to 100 ml. A clear, yellow-coloured, alcoholic fluid results.

Phenolphthalein Solution, 1 per cent Alcoholic
Töpfer's Solution

(Vol. I, p. 354)

Dissolve 0.25 g of p-dimethylaminobenzene and 2.0 g of phenolphthalein in a volumetric flask in alcohol, 90 per cent, to 100 ml. A clear, yellow coloured, alcoholic fluid results.

Sodium Hydroxide Solution, 0.1 N

Hydrochloric Acid, 0.1 N

(C) REAGENTS FOR THE EXAMINATION OF CEREBROSPINAL FLUID

REAGENTS FOR PROTEIN

Nonne-Appelt's Solution

Dissolve 85 g of ammonium sulphate in 100 ml hot water. After 1 to 2 days, separate the saturated solution from the precipitated crystals by filtration. Neutralize the solution if necessary with ammonia solution.

Pándy's Solution

Shake 10 g of liquefied phenol in a 200 ml flask with 100 ml of tepid water Keep the mixture for several days in a dark place at room temperature and shake it often. Decant the clear solution saturated with phenol from the separated, lower, oil-like layer. A clear, light rose-coloured fluid results. Preserve in a dark place, protected from light.

Gold-Sol

Previous titration: to 100 ml of water add 1 ml 1 per cent potassium oxalate solution and 1 ml 1 per cent gold chloride solution. Titrate after addition of 2 drops of I-phenolphthalein solution, with 2 per cent solution of dried potassium carbonate until a permanent red colour is obtained.

Preparation: to 100 ml of water add 1 ml of a 1 per cent potassium oxalate solution and a quantity of a 2 per cent potassium carbonate solution determined in the previous titration. Boil the mixture, remove the flame, than add dropwise 1 ml 1 per cent gold chloride solution. Heat again, until the solution attains a Burgundian red colour.

(D) REAGENTS FOR THE EXAMINATION OF BLOOD

Hayem's Solution for the Counting of Red Blood Corpuscles

Dissolve 0.25 g of mercury(II) chloride, 0.5 g of sodium chloride and 2.5 g of crystalline sodium sulphate in a volumetric flask in water to 100 ml. A clear, colourless fluid results.

Türk's Solution for the Counting of White Blood Cells

Dilute 1.0 g concentrated acetic acid and 1.0 ml 1 per cent aqueous gentian violet solution in a volumetric flask to 100 ml. A clear, bluish violet coloured fluid results.

Szmuk's Solutions for the Staining of Blood Corpuscles

1 Phosphate Buffer

Dissolve 2.298 g of disodium hydrogen phosphate-hydrate and 0.998 g of potassium dihydrogenphosphate in a volumetric flask to 1000 ml. A colourless, clear fluid results.

2 Methylene Blue Solution

Dissolve 1.0 g of methyelene blue and 0.5 g of sodium hydrogencarbonate in a volumetric flask in water to 100 ml. Heat the solution in a water bath for one hour. On the following day, filter the solution and complete to 500 ml with phosphate buffer. A blue-coloured, clear fluid results.

3 Eosin Solution

Dissolve in the mixture of 500 ml of phosphate buffer and 25 ml of aceton 0.2 g of water-soluble yellow eosin (tetrabromfluorescein sodium). A clear, yellow-coloured fluid results.

May–Grünwald's Solution

Mix equal parts of a 1.2 per cent aqueous eosin solution and of 1 per cent aqueous methylene blue solution. After 24 hours filter off the precipitate, wash with a small amount of water and dry at a temperature under 100°. Transfer to a volumetric flask a 0.25 g portion of the precipitate with small portions of aceton-free methanol. Fill up with methanol to 100 ml. After 24 hours standing, filter on a paper filter if necessary.

Giemsa's Solution

Powder carefully equal weight portions of methylene blue and of methylene azure (dimethylamino-thiazonium chloride). (This mixture is designated as azure "II"). Mix 1.2 per cent aqueous eosin solution with equal amount of 1 per cent aqueous methylene azure solution. Proceed further as described for May–Grünwald's solution. Triturate carefully the so prepared methylene azure eosin with an equal part per weight of methylene blue eosin. This mixture is designated as "azure II eosin".

Dissolve 3 g of azure II eosin and 0.8 g of azure II in 250 g of neutral glycerin at a temperature not exceeding 60°. Mix the cooled solution with 250 g of aceton-free methanol. Filter after 24 hours the fluid on a paper filter if necessary.

Biuret Reagent

Dissolve 1.6 g of crystalline copper(II) sulphate and 6.4 g of crystalline potassium sodium tartarate in a volumetric flask in 500 ml of hot water. After cooling, add 320 ml of 10 per cent sodium hydroxide solution and fill up with water to 1000 ml.

Thymol Solution

Boil 1.38 g of barbital, 1.03 g of barbitalsodium and 3 g of thymol with 500 ml water in a 1 litre flask, shake thoroughly and cool. Spread over the surface of the liquid a small amount of crystalline thymol and shake it again. Allow to stand overnight and after shaking filter. The hydrogen exponent of the liquid, pH = 7.8.

Drabkin's Solution

Dissolve 1 g of sodium hydrogen carbonate, 0.1 g of dried potassium carbonate, 0.05 g of potassium-cyanide and 0.20 g of potassium iron(III) cyanide with water in a volumetric flask to 1000 ml.

Diazo-I Reagent

Dissolve 0.5 g of sulfanilic acid in a volumetric flask with 5 g of concentrated hydrochloric acid and dilute with water to 100 ml.

Diazo-II Reagent

Dissolve 0.5 g of sodium nitrite in a volumetric flask with water to 100 ml.

Magenta Solution, 0.02 per cent

(Synonym: Diamond-fuchin solution)

Dissolve 0.02 g of magenta in a volumetric flask in water to 100 ml.

Picric Acid Solution, Saturated

Shake several times 1.5 g of picric acid in a glass stoppered flask with 100 ml of water for 3 days. Use the saturated supernatant decanted from the undissolved crystals.

(E) STAIN SOLUTIONS FOR BACTERIOLOGY

Prepare first from the dyes saturated alcoholic stock solutions. For that purpose, allow to stand 1 weight portion of dye with 10 weight portion of alcohol, 96 per cent, for a few days and shake several times. If the dyes were entirely dissolved, add as much more dye into the liquid that a small amount should remain undissolved.

Prepare from the saturated stock solutions thus obtained the diluted dye solutions suitable for staining. Filter one and a half amount of the necessary quantity of the prescribed stock solution through a dry paper filter. Return the excess of stock solution to the original stock.

Methylene Blue Solution

Dilute 5.0 ml of methylene blue stock-solution with 100 ml water. A clear, dark blue coloured fluid results.

Löffler's Methylene Blue Solution

Dilute 30 ml of methylene blue stock-solution with 100 ml of a 0.01 per cent potassium hydroxide solution prepared with freshly boiled and cooled water. A clear, blue-coloured fluid results.

Toluidine Blue Solution

Dissolve 0.5 g of toluidine blue in the mixture of 10 ml alcohol, 90 per cent, and 10 ml water. Mix the dye solution with 50 ml of a 5 per cent aqueous phenol solution. Filter after two days thorough paper filter. A blue, faintly phenol-scented, clear fluid results.

Magenta Solution

(Synonym: Fuchsin Solution)

Dilute 5.0 ml of magenta stock solution with 100 ml water. A clear, red-coloured fluid results.

Magenta Solution with Phenol, Diluted

(Synonym: Carbol Fuchsin Solution, Diluted)

Dilute 1.0 ml of magenta stock solution with 100 ml of a 0.5 per cent phenol solution. A clear, vivid red-coloured, phenol scented fluid results.

Solutions for Gram Staining

1 Carbol Gentianviolet Solution

Dilute 10 ml of gentianviolet stock solution with 100 ml of a 5 per cent phenol solution. A clear, dark violet-coloured fluid results.

2 Lugol's Solution (with 0.33 per cent iodine)

Dissolve 2.0 g of potassium iodide in 2 ml of water. Spread into the liquid 1.0 g of powdered iodine. After dissolution, fill up with water to produce 300 ml. A clear yellow-brown coloured fluid results.

3 Alcohol, 90 per cent v/v

4 Carbol Fuchsin, diluted Solution

See above (p. 368)

Solutions for Ziehl-Neelsen's Staining

(for the detection of Mycobacterium tuberculosis)

1 Magenta Solution with Phenol

(Synonym: Carbol-Fuchsin Solution)
Dilute 10 ml of magenta stock-solution with 100 ml of 5 per cent phenol solution. A dark red phenol-scented clear fluid results.

2 Hydrochloric Acid, Alcoholic Solution

Dilute 3.0 ml of hydrochlorid acid, 25 per cent, in a volumetric flask with alcohol, 96 per cent, to 100 ml. A clear, alcohol-scented acid fluid results.

3 Methylene Blue Solution

Dilute 5.0 ml of methylene blue stock solution with water to 100 ml. A clear blue coloured solution results.

Ebner's I-Solution

To 50 ml of saturated aqueous sodium chloride solution add 50 ml of water and 2 ml of 25 per cent hydrochloric acid solution.

Ebner's II Solution

Dissolve 0.5 g of potassium chloride in 5 g of hydrochlorid acid, 10 per cent, and 15.5 g of water, and add 80 g of alcohol, 96 per cent, to the solution.

Solution for Neisser's Staining

(for detection of Corynebacterium diphtheriae)

1 Methylene Blue Solution with Acetic Acid

Dissolve 0.3 g of methylene blue in a mixture of 6.0 ml of 96 per cent alcohol, 15.0 ml of concentrated acetic acid and 300 ml of water. Filter the liquid through dry paper filter. A clear, blue-coloured, slightly acetic acid-scented fluid results.

2 Crystal Violet Solution

Dissolve 1.0 g of crystal violet in a mixture of 10 ml of alcohol, 96 per cent, and 300 ml of water, and filter through a dry paper filter. A clear blue-coloured flud results.

Prior to staining mix 2 volumes of solution *1* and 1 volume of solution *2* and usie this mixture for staining.

3 Chrysoidine Solution

Dissolve 2.0 g of chrysoidine in 300 ml of hot water. After cooling, filter through a dry paper filter. A clear, yellow coloured fluid results.

Lyubinskii's Pyoctanin Solution

(for detection of Corynebacterium dyphtheriae)

Dissolve 0.25 g of pyoctanine (Methylrosaniline chloride, Gentianaviolet, Vol. II, monograph No. 230) in a volumetric flask in 5 per cent acetic acid to 100 ml. Filter through a dry paper filter. A clear, violet coloured, acetic acid scented liquid results.

Manson's Solution (for Wright's germ count)

Dissolve in 100 ml water in a flask while boiling 5.0 g of borax and 2 g of methylene blue (Hoechst). After cooling, filter through a paper filter. A clear, dark blue-coloured, slightly alkaline liquid results. Prepare a tenfold dilution with water prior to use.

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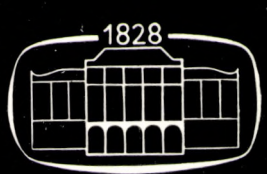
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