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ABSTRACT

The aim of the present work was to deal primarily with a few essential trace elements and to obtain reliable results of adequate accuracy and precision for the analysis of biological samples. A few other than trace elements were determined by the nondestructive technique as they can be well evaluated from the gamma spectra. In the development of the method Bowen's kale was chosen as model material. To confirm the reliability of the method two samples were analysed proposed by the IAEA in the frame of an international comparative analysis series. The comparative analysis shows the present method to be reliable, the precision and accuracy are good.

АННОТАЦИЯ

Разработан метод активационного анализа, позволяющий проведение воспроизводимого высокоточного определения содержания следовых количеств элементов в биологических материалах. На основе гамма-спектра без разрушения образца были определены и несколько не следовых элементов. Рассмотрено, целесообразно ли применение метода активационного анализа для определения этих элементов. В качестве эталона при исследованиях служил образец саввойской капусты Бауэна. В целях подтверждения надежности метода приняли участие в международном сравнительном анализе, организованном Международным Агенством по атомной энергии. Полученные результаты показали, что этот метод является надежным, точным и воспроизводимым для многих элементов.

KIVONAT

Aktivációs analitikai eljárást dolgoztunk ki biológiai anyagok nyomelemeinek megfelelő pontossággal és precizitással történő meghatározására. Roncsolásmentes módszerrel néhány nem-nyomelemet is meghatároztunk, minthogy a gamma-spektrumból jól kiértékelhetők és megnéztük, hogy ezen elemek meghatározására az aktivációs analizis célszerüen használható-e vagy sem. A vizsgálatokhoz modell anyagként a Bowen-féle kelkáposzta mintát választottuk. A módszer megbizhatóságának bizonyitására résztvettünk a Nemzetközi Atomenergia Ügynökség által szervezett nemzetközi összehasonlitó vizsgálatsorozatban. Az eredmények azt mutatták, hogy az eljárás megbizható, pontossága és precizitása számos elemre megfelelő.

Introduction

The presence and concentration of several trace elements play an important role in the proper functioning of living organisms. Moreover it is known that the concentration of some types of trace elements changes if the functioning of the organism is anomalous or because of therapeutical interventions.

For this reason it is of interest to establish the so - called normal concentrations of trace elements in living organisms. A large amount of data has been already reported on the concentrations of different trace elements in a variety of biosamples /1/. Recent studies have shown that these concentrations may substantially vary not only from individual to individual but also within a given organ /2/. Further, it has been found that trace element concentrations may change with the conditions under with the samples are taken e.g. whether the blood is drawn on an empty stomach or not, before or after a repose of the patient /3, 4, 5/.

Trace elements can be divided into "essential" and "non-essential" species /6/. The "essential" types are always present in tissues and they are participating in the metabolic reactions of the organism. Such trace elements are cobalt, iodine, iron, manganese, molybdenum, selenium, zinc. Lately also chromium, fluorine, nickel, silicon and vanadium have been included. The data available on these elements are

difficult to interpret since their role in cell structure and in metabolic processes is little known.

Non-essential trace elements can be regarded as impurities originating from exogenic factors; they may accumulate or even squeeze out chemically similar essential elements.

It follows from above considerations that two points are of importance in the study of trace elements:

1. First, the essential elements have to be thoroughly studied in order to clarify their role as far as possible. Of course, if possible the determination of elements the function of which is still questionable is also useful as the thus obtained information may help in establishing new essential elements.

2. A high sensitivity trace analytical method of adequate accuracy is needed for the reliable detection of small changes in concentration. According to a large number of reports neutron activation analysis, which is known to be of high sensitivity, permits a generally satisfactory accuracy to be achieved if high resolution Ge/Li detectors and multichannel analyzers are applied. Some of the trace elements can be determined with the necessary accuracy and precision also by nondestructive activation analysis, though in certain cases only if the method is combined with an appropriate chemical separation. Above requirements are often ignored in reports on determinations by multielement activation analysis. The precision for some of the elements determined in this way is not better than $\pm$ 20-30%. Since such large deviations can be attributed also to a functional change, these data, though

useful, should be carefully pondered before making any inferences.

In the elaboration of the method to be described the aim was to deal primarily with some of the essential trace elements in order to obtain reliable results of adequate accuracy for the study of biosamples. In nondestructive measurements also some other than trace elements, which can be well evaluated from the gamma spectra, were determined to see whether or not activation analysis is useful method for their determination. Bowen's kale sample was chosen as model material since, according to reported investigations, it is an internationally adopted standard reference material for many elements.

Literature on Bowen's kale sample

Similarly to any analysis, that of biosamples requires a material of known composition which permits the analytical results to be checked by comparison. For biosamples no such a material has been available for a long time. An important event has been therefore the advent of Bowen's kale sample prepared to meet the following requirements to be satisfied by reference materials /7/:

1. better than $\pm$1% homogeneity in a sample of more than 1 g,

2. if dried under special conditions the loss in weight should be reproducible,

3. it should remain stable for years without decomposition or sedimentation,

4. it should be available in sufficient quantities /minimum 50 kgs/,

5. the main components should be carbon, hydrogen, nitrogen and oxygen,

6. for standard reference materials they should be successfully analysed by at least two or more analytical methods.

The kale sample was prepared by Bowen from 1.6 ton. of fresh leaves dried under special conditions to give 91 kg of powder /8/. Aliquots were sent to 29 laboratories using different methods of analysis. The results were sent to Bowen who calculated from these data a "grand mean" for each of the components and published the calculated values first in 1967 /9/. Since then the sample has been analysed by many more analysts as a consequence of which the "grand mean" values were sometimes modified. Table I shows these data for the most important elements and for those which have been most often analysed.

It is apparent from the table that the values obtained for some elements /e.g. chlorine, copper, sodium/ show a considerable variance in spite of the large number of analyses. Instrumental neutron activation analysis /INAA/ gives a poor result for potassium. Bowen mentiones that the values obtained by atomic absorption spectroscopy using flame technique are 5-30% higher than those obtained by other methods. It is possible that flame technique can be made responsible for errors in atomic absorption data. On the other hand, atomic absorption and spectrophotometric data are found to be of higher precision than INAA for magnesium and molybdenum. Atomic absorption and NAA give data of higher precision for zinc as compared with other methods.

Table I

Modifications in the values of the "grand mean" in the case of Bowen's kale sample /given in ug/g units/

Element	BOWEN[9x] different methods	BOWEN[10xx] different methods	BOWEN[7xxx] N.A.A.
Al	6.4-78.2	38.2 /6.4-88.4/	37.46±7.84 /7/
As	0.127-1.80	0.141 /0.11-0.22/	0.141±0.031 /18/
Ca	41400±2230 /41/	40850	40409±2544 /11/
Cd	1.0±0.1 /4/	0.80 /0.38-1.06/	0.76±0.192 /11/
Cl	3330±1060 /21/	3415 /2180-4450/	3711±368 /9/
Co	0.0562±0.0077 /22/	0.0581 /0.041-0.081/	0.0592±0.0103 /15/
Cr	0.331±0.155 /13/	0.308 /0.18-0.42/	0.356±0.127 /10/
Cu	4.81±0.735 /88/	4.99 /3.6-6.5/	4.679±0.644 /16/
Fe	119.5±19.5 /79/	118.3 /88-157/	117.3±16.2 /12/
K	24630±1218 /53/	24615 /20600-29300/	24248±1390 /5/
		INAA	20976±3701 /10/
Mg	1604±119 /43/	1572 /1350-1700/	1514±88 /6/
Mn	14.9±1.8 /83/	14.73 /12.6-18/	14.58±1.26 /21/
Mo	2.33±0.47 /44/	2.28 /1.5-3.1/	2.325±0.507 /12/
Na	2594±617 /52/	2506 /1220-3250/	2257±258 /12/
Rb	52.8±6.25	52.2 /49-57/	53.38±3.87 /7/
Sb	0.0653±0.0125 /6/	0.0689 /o.05-0.11/	0.0719±0.0173 /13/
Sc	0.00835±0.00074 /4/	0.00829	0.00779±0.00092/7/
Se	0.148±0.0137 /20/	0.121? /0.02-0.15/	0.1375±0.017 /10/
Sn	0.160±0.037 /4/	0.26 /0.16-0.36/	0.23±0.071 /7/
V	-	0.36 /0.33-0.41/	0.366±0.03 /5/
W	0.0605±0.00123 /8/	0.0605	0.06±0.0029 /3/
Zn	31.88±4.82 /77/	33.2 /30-38/	31.85±2.09 /23/

[x]results, standard deviation /number of analyses/

[xx]results /lower and upper limits/

[xxx]results, standard deviation /number of laboratory averages/

Before the description of the method developed in our laboratory a brief review is given of some reported methods utilizing Bowen's sample for their elaboration.

Girardi and his group /11/ analysed the kale sample using INAA after irradiations with either nuclear reactor or 14 MeV neutrons from a neutron generator. Using 3x3 in. NaI/Tl/ or 11 cm³ Ge/Li detector in combination with 512 channel analyzer 18 elements were determined by this method. Reactor irradiations were carried out for 1 minute and for 8 days with neutron fluxes of 2.10^{13} n/cm².s and 5.10^{12} n/cm².s, respectively. It is of interest to note that the ^{47}Ca activity was evaluated from the 1290 keV photopeak neglecting the contribution from the ^{59}Fe photopeak of the nearly same energy. For elements not detected in the gamma spectrum detection limits were evaluated by use of a computer program e. g. Cu<30, V<450 ppm.

X-ray fluorescence measurements made by the above authors showed this method to be suitable merely for fast nondestructive surveys when accuracy and precision are not so important.

Nadkarni and Ehmann /12/ determined nondestructively 15 elements and used additional chemical separation for gold and mercury. After appropriate cooling the measurements were made with 12 cm³ Ge/Li detector and 400 channel analyzer. Contrary to Girardi's group, both photopeaks of ^{59}Fe are utilized for evaluating iron while the interference of the 1296.1 keV photopeak of ^{47}Ca with the 1291.5 keV line of iron is considered negligible. It was found in our measurements that the interference between the above lines made it impossible to evaluate either of the elements from the composite peak. For this reason first the ^{59}Fe activity was evaluated from the 1098.6

keV photopeak area, then the ^{47}Ca activity was left to decay, thus also the second iron peak could be measured.

In a later paper Nadkarni and Morrison /13/ report the measurement of 36 elements in various biosamples. Out of these Orchard Leaves were used as standard while the others were regarded as "unknown". Each sample was twice irradiated. The short-lived /less then 15 hours/ radioisotopes were activated for 1-2 minutes with a neutron flux of 2.10^{12} n/cm².s, varying the activity measurements from 1 to 30 minutes. The long-lived species were activated for 80 hours with a neutron flux of 2.10^{13} n/cm².s. The gamma spectra of isotopes with half-lives from 26.4 hours to 4.53 days were measured for 1 hour, the others for 3-5 hours. The measurements were made in each case with 30 cm³ Ge/Li detector and 4096 channel analyzer. About 19 elements were determined after 2 min. activation. The other elements were determined after long irradiation with cooling times of 3-5 days, 2 weeks and 1 month. It is pointed out by the authors that not all the elements listed in the report can be analysed in every sample since the determination obviously depends on the concentration of the element and on the nature of the matrix. In the case of kale numerical values are reported for 26 elements. The accuracy of the measurements varies from ± 5 to 15%, except for elements present in very small amounts.

Plantin /14/ worked out a multielement chemical separation for the analysis of biosamples and checked the method by kale analysis. The samples were irradiated for 170 hours with a neutron flux of 2.10^{13} n/cm².s. After a cooling of merely 2 days the samples were destroyed in a mixture of conc. HNO_3

+ H_2O_2 then passed through a hydrated antimony-pentoxide /HAP/ column in an appropriately shielded procedure. Subsequently, the samples required no more radiation protection for their separation into several fractions by use of Dowex 1x8 and Dowex50 ion exchanger resins. According to this author chemical separation is still necessary in the case of biosamples contrary to the hope of activation analysts that these lenghty procedures would be eliminated with the advent of semiconductor detectors.

In Plantin's analyses multistandards were used, that is, known quantities of 6-7 elements were put into each ampoule. The gamma spectra were taken with 24 cm³ Ge/Li detector and 4096 channel analyzer using the 2000 channel range. The spectra were evaluated on computer. One of the major problems of ion exchange technique is that the same element may appear in several fractions in the case of multielement separation and according to the author this increases the error of the determination. This is so in the case of copper, selenium, antimony and chromium. Bromine cannot be fully removed by distillation, a few percent of this element appears in each fraction though without considerably interfering activity. It can however mask the 0.559 MeV photopeak of ^{76}As. Selenium is retained to 90% on the HAP column unless a carrier is used. However, with a carrier selenium enters different fractions. A large part of rubidium is also retained on the HAP column. Chromium appears in two fractions one of which contains ^{32}P which prevents the chromium to be measured without a large error. Plantin concludes from his observations that multielement activation analysis can hardly attain the accuracy and precision required

by a reliable analytical method. His experiments were inconvenienced also by the up to 10% gradient of the flux in the sample holder. Considering these difficulties the results were found to be satisfactory and numerical values could be given for 21 elements.

The aim of the now reported work was to find a comprehensive method of adequate accuracy and precision for the analysis of biosamples. We wanted to see which elements can be satisfactorily analysed by INAA and which of them can be more accurately determined by chemical separation or by some other method.

Experimental

The method was developed by use of Bowen's kale sample. In particular cases when some of the trace elements appear in biosamples in lower than usual concentration, the method was checked also by use of human serum sample.

Important nuclear data of the most investigated elements are listed in Table 2.

Most of the analyses were carried out nondestructively. When the instrumental technique proved to be impracticable or if a given element had to be more accurately determined for some reason, chemical separation of the type thought to be the most appropriate was carried out. Activity measurements were made with 45 cm^3 Ge/Li detector combined first with a 1024, later with a 4096 channel analyzer. The gamma spectra were evaluated on ICT-1905 computer by use of a suitable program /17/. Corrections for dead time, decay during the set of measurements and, if necessary, during the actual measuring time were calculated in the conventional way.

Table 2.

Important nuclear data on elements analysed in kale /15/

Element	Isotope	Nuclear reaction	E_γ/MeV/	$T_{1/2}$
Ca	^{47}Ca	$^{46}Ca/n,\gamma/^{47}Ca \xrightarrow{\beta^-} {}^{47}Sc$	0.160/^{47}Sc/ 0.4895 0.808 1.2969	4.7d→3.4d
Cd	^{115}Cd-^{115m}In	$^{114}Cd/n,\gamma/^{115}Cd \xrightarrow{\beta^-} {}^{115m}In$	0.3366/^{115m}In/	53h→4.5h
Cl	^{38}Cl	$^{37}Cl/n,\gamma/^{38}Cl$	1.6420 2.1668	37.3m
Co	^{60}Co	$^{59}Co/n,\gamma/^{60}Co$	1.1731 1.3324	5.26y
Cr	^{51}Cr	$^{50}Cr/n,\gamma/^{51}Cr$	0.3200	27.8d
Cu	^{64}Cu	$^{63}Cu/n,\gamma/^{64}Cu$	0.511 1.3455	12.8h
Fe	^{59}Fe	$^{58}Fe/n,\gamma/^{59}Fe$	1.0986 1.2915	45.1d
K	^{42}K	$^{41}K/n,\gamma/^{42}K$	1.5247	12.52h
Mg	^{27}Mg	$^{26}Mg/n,\gamma/^{27}Mg$	0.8440 1.0141	9.45m
Mn	^{56}Mn	$^{55}Mn/n,\gamma/^{56}Mn$	0.8469 1.8107	2.58h
Mo	^{99}Mo-^{99m}Tc	$^{98}Mo/n,\gamma/^{99}Mo \xrightarrow{\beta^-} {}^{99m}Tc$	0.1405	66.0h→6.04h
Na	^{24}Na	$^{23}Na/n,\gamma/^{24}Na$	1.3684 2.7536	15.0h
Rb	^{86}Rb	$^{85}Rb/n,\gamma/^{86}Rb$	1.0766	18.7d
Sb	^{124}Sb	$^{123}Sb/n,\gamma/^{124}Sb$	0.6026 1.6907	60.0d
Sc	^{46}Sc	$^{45}Sc/n,\gamma/^{46}Sc$	0.8894 1.1203	83.9d
V	^{52}V	$^{51}V/n,\gamma/^{52}V$	1.4344	3.76m
Zn	^{65}Zn	$^{64}Zn/n,\gamma/^{65}Zn$	1.1154	245.0d

Nondestructive measurements. Samples of about 0.1 g were irradiated after careful weighing. Two irradiations were carried out - if necessary the same sample can be used. The fast, 1 min. irradiation was carried out by use of a pneumatic tube system and of a neutron flux of 2.10^{13} n/cm².s. The long, 115 h. irradiations were made in another vertical channel of the reactor where the neutron flux varied from $3\text{-}4.10^{13}$ n/cm².s. depending on the sample position.

The gamma spectra taken on the samples irradiated for 1 min. permitted sodium, chlorine, potassium, manganese, magnesium and vanadium to be quantitatively determined. The activities were measured 3 minutes after termination of the irradiation. The sample was then left to cool for about 3 hours enough for the decay of the ^{27}Mg activity. The gamma spectrum taken after cooling permitted to evaluate the photopeak of ^{56}Mn. The photopeaks of the two activities could not be separated in the first spectrum therefore their different half-lives had to be utilized in this way. Fig.1 shows the gamma spectrum of a sample irradiated for 1 min.

The long-irradiated samples were carbonized at 200°C before irradiation to avoid the explosion of ampoules in the WWR-S type reactor where the channel temperature can reach 3-400°C. The samples which burned in the ampoules had to be removed with 10% nitric acid. The gamma spectra were then taken on samples dried in glass vessels of identical sizes.

The intense bremsstrahlung of ^{32}P considerably interfered with the measurements up to energies of 0.7-0.8 MeV. Filters of various sizes /10 mm thick plexiglass, 7 mm thick aluminium or 5.5 mm thick copper/ were applied for its suppression. The

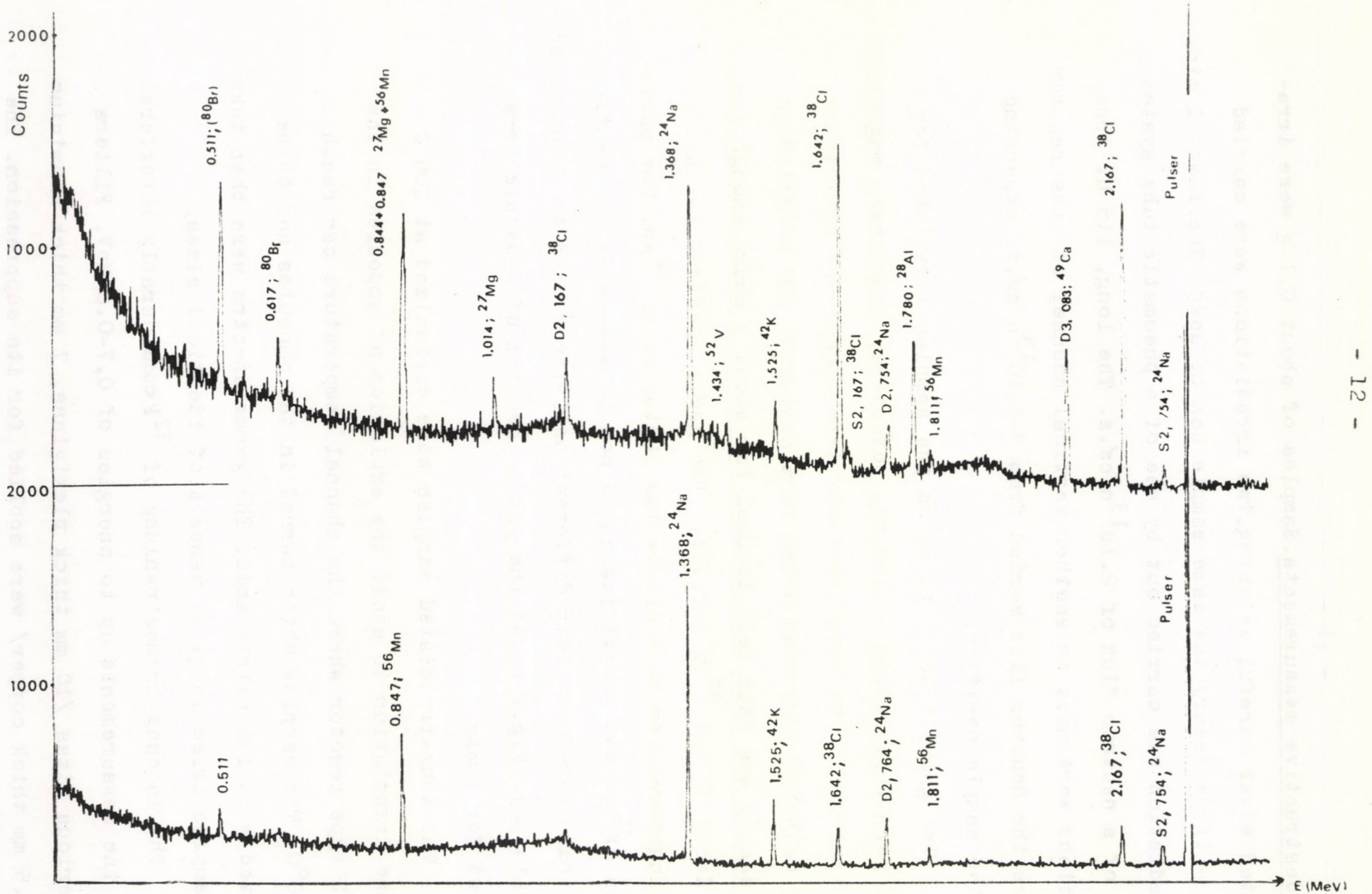

Fig.1. Gamma-spectrum of a kale sample counted after irradiation for 1 min and cooling 3 min and 2 hours, respectively.

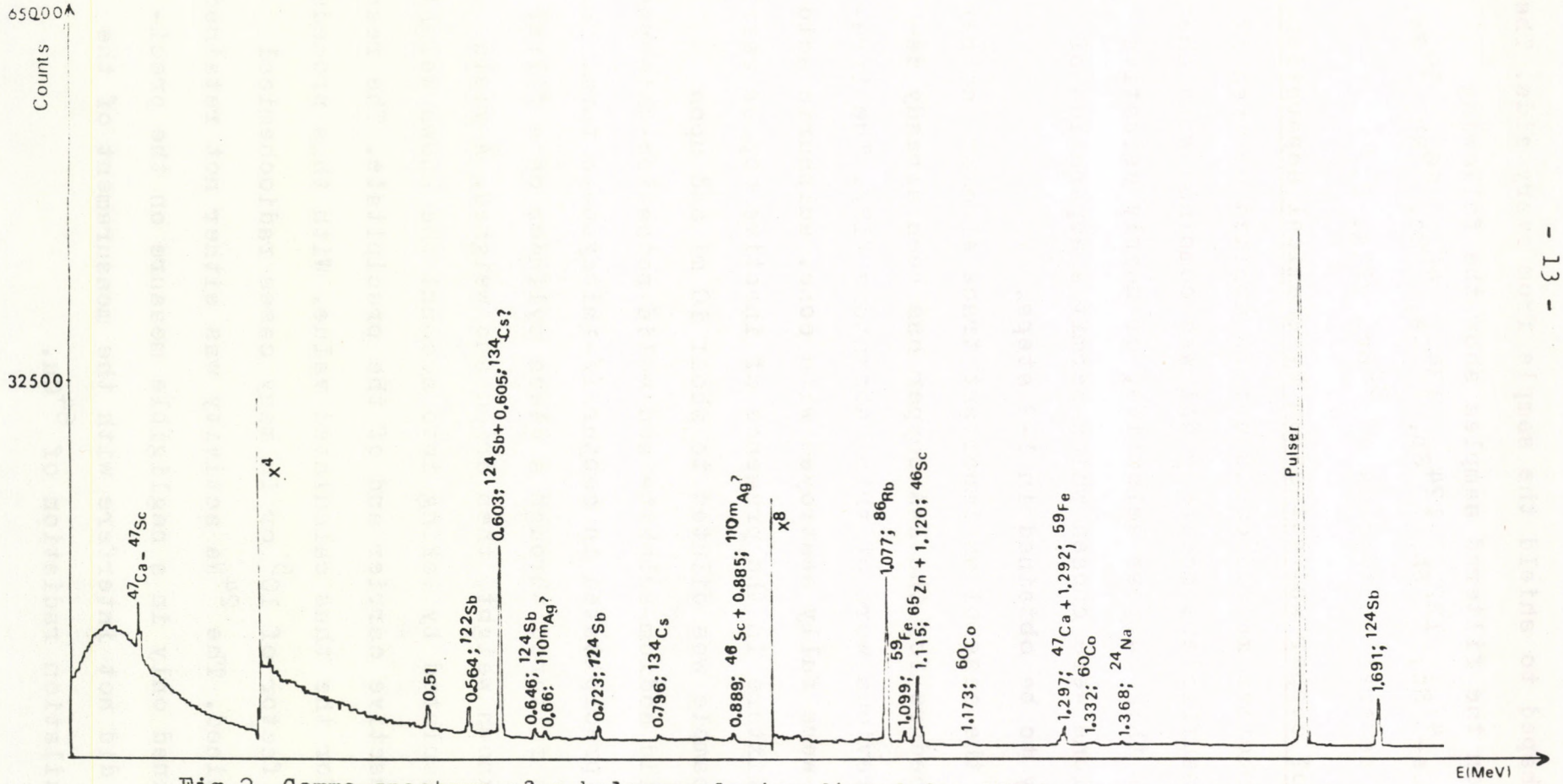

Fig.2. Gamma-spectrum of a kale sample irradiated for 100 hours and counted after 10 days cooling.

filters were shaped to shield the sample from every side. The spectra taken on the filtered samples show the following activities: ^{47}Ca-^{47}Sc, ^{122}Sb, ^{124}Sb, ^{134}Cs, ^{46}Sc, ^{86}Rb, ^{59}Fe, ^{65}Zn, ^{60}Co and several photopeaks of ^{82}Br. /Fig. 2./

Determination of copper and potassium by chemical separation.

If the nondestructive method could not be applied or when it proved to be insufficient accuracy NAA was combined with chemical separation. For this purpose selective, or nearly selective chemical reactions were chosen which permit a separation of adequate purity to be obtained in 1-2 steps.

This was the case of an important trace element, copper. A suitable procedure for the NAA copper has been already developed in a previous work of this laboratory /18/. The irradiated samples were fully destroyed with conc. sulphuric acid - nitric acid mixture in the presence of inactive copper carrier. The destroyed sample was diluted to about 10 ml and upon addition of solid sodium-sulphite and solid potassium-thiocyanate the copper was precipitated in copper/I/-thiocyanate form. The precipitate was filtered through a glass cylinder on a filter paper disk of known weight, then dried and weighed. A yield percent was calculated by taking into account the known weights of the added inactive carrier and of the precipitate. The result was corrected for the thus calculated value. With this procedure a purification factor of 10^5 or in many cases radiochemical purity was obtained. The ^{24}Na activity was either not retained at all or retained only in a negligible measure on the precipitate thus it did not interfere with the measurement of the 0.511 MeV annihilation radiation of ^{64}Cu.

In Fig. 3. a gamma-spectrum of copper/I/-thiocyanate precipitated from a kale sample is shown.

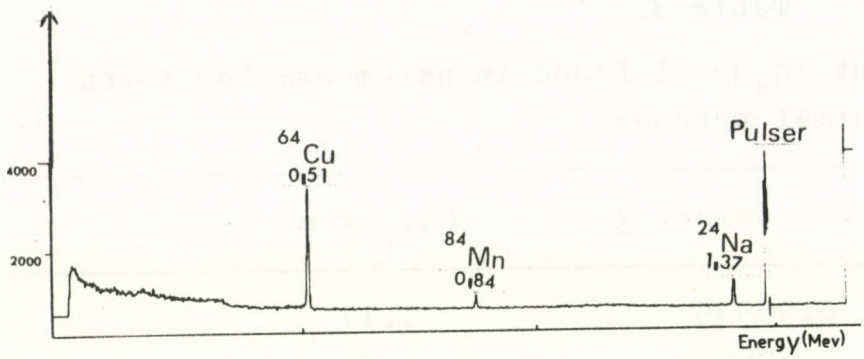

Fig. 3.

Gamma-spectrum of copper/I/-thiocyanate precipitated from a kale sample

This procedure was thought to be suitable for the determination of lower copper concentrations as well. There is a frequent call for the determination of copper in blood or in serum. That is why the method was checked on human serum. The normal serum samples were provided by the Gynecological Clinic of Szeged. Each sample was taken from a different person. 0.5 ml of serum were analysed and also weighed. In Table 3 the results obtained for 11 samples of serum are listed.

As apparent from Table 3, the precision of the results is satisfactory and the data compare well with the reported values, /e. g. Kasperek et al. /3/ report the value of serum -

copper as 1.04±0.145 /ug/ml. In Bowen's compiled NAA data obtained from different laboratories show on the average 1.10 /ug/ml±22.5% for copper/. Based on our results it seems that the copper concentration in human sera does not show appreciable variations.

Table 3.

Copper content in /ug/ml found in serum samples taken from normal persons

Serial No.	wet weight, g	Cu, /ug/ml
20.	0.50936	1.17
22.	0.50870	1.00
27.	0.50271	1.07
56.	0.50623	1.17
63.	0.50513	1.07
71.	0.50468	1.19
73.	0.50051	1.08
76.	0.50559	1.01
81.	0.50061	1.00
86.	0.50288	1.17
94.	0.50235	1.12
	mean: 1.096±0.07	

As it will be seen from the comparative table /Table 5/ the accuracy of the potassium determination by INAA is good /a large number of analyses were performed/ but the precision of the data is poor, the standard deviation is more than ±10%. According to Bowen's data /7/ the nondestructive determination of potassium shows large inaccuracies. It is not always possible to make a large number of analyses because of the given small quantity of the sample. This problem had to be faced in this laboratory while investigating the electrolyte equilibrium in inner ear liquids and only individual samples of minute quantity

were available. To improve the precision of the method, NAA was combined with chemical separation for the determination of potassium /19/. Three separation methods were tried: single, double precipitation and selective retention. It was found that sodium can be determined from the aliquot of the filtrate without further chemical handling after the removal of the potassium. The samples were irradiated for 10 hours with a neutron flux of 2.10^{13} n/cm².s. The sodium and potassium standards were irradiated under the same conditions. After irradiation the samples were destroyed in a mixture of nitric acid and hydrogen peroxide then evaporated to dryness. The nitric acid was removed when boiled three times with hydrochloric acid.

Potassium was precipitated with 3% aqueous potassium-tetraphenyl borate. The pH of the sample solution varied between 4 and 6 in the presence of 10 mg inactive potassium carrier and 3 mg sodium holdback carrier. The reaction with the potassium ions takes place according to the formula

$$\left[B(C_6H_5)_4\right]^- + K^+ = K\left[B(C_6H_5)_4\right]$$

The white precipitate was left standing for a short time, then filtered. The activity was measured with 3x3 in. NaI/Tl detector combined with 256 channel analyzer. The potassium tetraphenyl borate contained also a considerable amount of ^{24}Na activity which interfered with the determination of potassium. The gamma spectrum could be evaluated after a second precipitation of potassium from the first precipitate dissolved in aceton. The second precipitate was found to be free from sodium but the procedure was lengthy.

For selective retention again potassium tetraphenyl borate precipitate was chosen. It was freshly prepared with 50%

tetraphenyl borate ions in excess, centrifuged and washed three times in water. The precipitate was suspended in water and the sample previously destroyed as described before as well as taken up in water and having the necessary pH added. First of all the amount of the inactive precipitate needed for the possible complete retention has to be determined which was carried out by the addition of solutions containing about 10^4 cpm radioactive potassium to precipitates of 50, 100, 200, 300, 400 and 450 mgs. Since the solubility of the potassium tetraphenyl borate was found to be the lowest between pH 4 and pH 6 /20/ the sample was adjusted to pH=5. The mixture has been shaken mechanically for 15 mins and the precipitates filtered by identical sizes of G4 filters followed by washing with 50-60 ml distilled water. The percentages retention is given in Table 4.

Table 4.

Variation of potassium retention with the quantity of the precipitate

precipitate, mg	retention, %
50	15
100	39.3
200	87.5
300	96.0
350	99.2
400	99.5
450	100

Varying the pH and the shaking time we have found, that the extent of the retention is unchanged between pH 2 -7, therefore in order to avoid the retention of other radioactive ions a slightly acidic /pH 3 - 5/ medium has been chosen. Shaking times of 5, 10 or 30 mins did not affect the retention

either and due to the strong foaming a slow stirring of 5 mins has been applied and a 99.5-100% retention with a precipitate of 400 mgs achieved.

The main object of the separation was the elimination of sodium interference. Experiments were made therefore in the presence of 2.10^4, 5.10^4 and 2.10^5 cpm of sodium. No sodium was retained by the precipitate, nor was the presence of phosphate ions which impede the retention of potassium ions detected.

A calibration curve plotted for potassium in the range from 2 to 10 /ug of potassium /about these quantities had to be determined in the study of inner ear liquids/ is shown in Fig. 4.

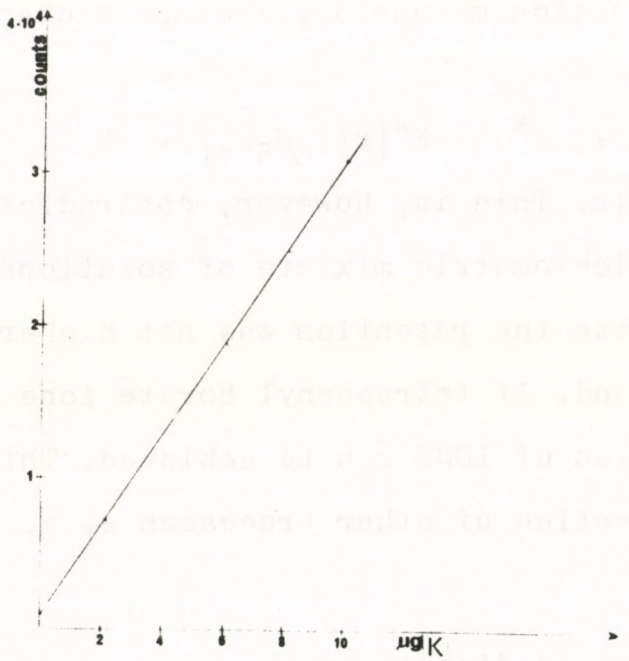

Fig.4. Calibration curve in the range from 2 to 10 /ug of potassium.

Following the filtration of the potassium tetraphenyl borate precipitate we can determine the sodium content from the aliquot of the filtrate without further chemical treatment.

The determination method was elaborated by use of human serum samples, available in any quantity. As a comparative method flame photometry was chosen which is extensively used in clinical practice for sodium and potassium determination. Data of an average measurement show the following:

Activation analysis		Flame photometry		percentual deviation	
Na^+	K^+	Na^+	K^+	Na^+	K^+
mequ.		mequ.			
135.4	4.6	142	4.7	-4.7	-2.3

Because of the small volume /2-5 µl/ of the samples flame photometry is impracticable for the analysis of the inner ear liquid of guinea pigs.

As to the retention mechanism, isotope exchange of the form

$$K[B(C_6H_5)_4] + K^* \rightarrow K^*[B(C_6H_5)_4] + K$$

seems to be plausible. This is, however, contradicted by the fact that for a stoichiometric mixture of solutions when preparing the precipitate the retention was not higher than 70 - 80%. On the other hand, if tetraphenyl borate ions are added in excess, a retention of 100% can be achieved. This implies the possible intervention of other processes e. g. coprecipitation.

Experimental results and discussion

The elements chosen for investigation were determined in 16 - 18 analyses each. The average values and their standard deviations are compared in a tabulated form with Bowen's activation analytical averages and with results of other

Table V

Values given in ppm on elements in Bowen's kale sample as reported by different authors

Elements	Bowen[x] /7/	Girardi /11/	Nadkarni, Ehmann /12/	Nadkarni, Morrison /13/	Plantin /14/	M.Sankar Das[xxx] /16/	Present work	Deviation from Bowen %
Ca	40409±2544	40000±350	-	44300	47000	41000±2450	41773±6635	+ 3,4
Cl	3711±368	3000±100	-	3600	-	3360	3750±121	+ 1,1
Co	0.0592±0.0103	0.060±0.003	0.054±0.009	0.044	0.041	0.065±0.014	0.082±0.011	+39
Cu	4.679±0.644	<30	-	-	-	4.87±0.044	4.56±0.21	- 2.5
Fe	117.3±16.2	113±1	122±4.87	103	123	117±5	118.1±14.5	+ 0.7
K	24248±1390 20976±3701 INNA[xx]	-	-	24400	-	23600±2300	24031±2602	- 0.9
Mg	1514±88	1605±65	-	1600	-	1500	1333±112	-12
Mn	14.58±1.26	17.48±0.6	-	14.8	-	14.7±1.7	14.0±0.96	- 4.0
Na	2257±258	2495±10	-	1700	-	-	2260±112	+ 0.1
Rb	53.38±3.87	57.5±1.5	-	59.6	50	-	50.2±2.45	- 6.0
Sb	0.0719±0.0173	<0.2	0.11±0.02	0.07	0.15	-	0.20±0.06	+178
Sc	0.00779±0.00092	0.0077±0.0001	0.008±0.0005	0.006	0.0070	-	0.12±0.002	+54
V	0.366±0.03	<450	-	0.37	-	0.337; 0.378	0.45±0.085	+23
Zn	31.85±2.09	37±3	30.4±1.3	31.7	32	31.4±3.2	31.5±1.9	- 1.1

[x] Activation analytical data

[xx] Average obtained by instrumental analysis

[xxx] Values obtained from the averages of at least 10 other than his own laboratory. For the listed elements M.S.D. proposes the kale sample as a standard reference material. For Mg and Cl the kale sample is proposed as reference material for V the thinks it to be suitable for comparative measurements

authors /Table 5./.

The reason why neutron activation analytical data were chosen from Bowen's compilation of averages is that the great differences in the value of some elements due to the differences between the methods which cannot be taken into consideration would unduly complicate the calculation of the grand mean taken over all methods /11 procedures/ and that the data used by Bowen are taken from about 1000 contributions out of which about half give results obtained by activation analysis.

Considering the table following inferences can be made:

1./ For some of the essential trace elements, such as copper, iron, manganese, rubidium, zinc, molybdenum, the accuracy and precision of the present method are satisfactory.

2./ Since a large number of analyses was made, the accuracy of the method seems to be adequate for some other than trace elements such as calcium, potassium and sodium. The variance is good for sodium but higher than $\pm$10% for the other two elements. In the case of large quantities a better accuracy can be obtained by e.g. flame photometry or atomic absorption spectroscopy, thus the latter techniques are more reliable for these elements in this case. Potassium can be accurately determined if NAA is combined with chemical separation as described. This technique is justified if potassium is present as a trace element.

3./ The data reported on antimony show its determihation by NAA to be problematical. The high values obtained in the present measurement suggested that it would be of interest to check the purity of the quartz ampoules. Empty ampoules were irradiated 1, 2, 3 times, than washed with the acidic mixture

used for the dissolution of the samples. It was found that all the ampoules of various origins contained antimony which is non-reproducibly removed from the ampoules during the acidic washing. For this reason the analysis of antimony in biosamples was not continued in the present study. It may be noted that also other elements than antimony were analysed in the ampoules. though some impurities could be identified their quantities were negligible in the analysis of biosamples.

4./ A higher than expected value was obtained for scandium. This can be explained by the fact that the computer evaluation of the 0.8894 MeV photopeak of ^{46}Sc is interfered with by the Compton edge of ^{65}Zn and that the 1.1203 MeV photopeak of the former cannot be gamma-spectrometrically separated from the much more intense 1.1154 MeV photopeak of ^{65}Zn. It is possible to separate the latter two photopeaks by a computer program but the error for scandium is large. The tabulated value was calculated from the results obtained from both photopeaks. Only minute quantities of scandium are usually present in biosamples nor has been its role understood as yet. Nevertheless, its analysis was continued since also considerable quantities were found in some biosamples and in these cases also the results were much more reliable.

5./ Biomaterials contain only trace amounts of cobalt. Computer evaluation of gamma spectra involves large errors. The present result seems to be high. Becker and his group /21/ report values for 9 elements analysed in kale. Some of their elements agree with ours. For cobalt their value averaged over 12 measurements is 0.076 ± 0.008, thus close to the present result. The authors /21/ suggest that Bowen's grand mean could be too low for this element.

6./ The analysis of magnesium and vanadium from the gamma spectra of the samples irradiated for 1 min. showed that NAA seems to be useful only for a fast surveying measurement of these elements in biosamples. For magnesium a much lower than Bowen's value was obtained. The photopeak area showed appreciable variations in the evaluation of some samples inducing a large error into the calculation. As apparent from Fig. 1. the small and uncertain photopeak of vanadium is difficult to evaluate.

It follows from the above discussion of the results that the now presented method is useful and reliable for the analysis of the essential trace elements and also for the determination of some less important elements.

To confirm the reliability of this method, we took part in the international comparative analysis series organized by the IAEA in 1972/73. Two of the proposed samples were chosen: dried potato powder which is easy to handle and a sample of animal bones heated to 400°C, then pulverized. The purpose of the international comparative measurements was to check the precision of the analytical methods used in different laboratories and to establish in some cases also the accuracy of the method. Table 6 shows the results obtained for some components of the analysed samples along with the data evaluated by the IAEA /22/.

The analyses were made by different laboratories each using its own method, mostly NAA and atomic absorption spectroscopy. The table lists the averages and the standard deviations from the average. The standard error /S.E./ was calculated by the IAEA using the formula

$$S.E. = \frac{S.D.}{n}$$

where n is the number of accepted laboratory averages. The

Table 6

Comparison of analytical results on some components of potato powder and animal bones

	Dried potato				Animal bone			
Element	IAEA	S.E.	Present work	S.E.	IAEA	S.E.	Present work	S.E.
Zn ppm	11.9±1.3	0.3	10.9±0.75	0.23	183±12	3.3	178±.610	1.9
Co ppb	20.5±6.4	2.4	24.2±2.52	0.79	0.463±0.16 ppm	0.062	0.72±0.0047	0.014
Fe ppm	18.6±4.7	1.1	20±3.45	1.0	1.52±0.36 mg/g	0.11	2.10±0.088	0.027
Cr ppm	/0.25/ median	-		0.17	683±241	91	908±40.6	16
Mn ppm	2.42±0.53	0.13	2.4±0.33	0.12	-	-	-	-
Rb ppm	6.10±0.54	0.27	5.55±0.318	0.097	-	-	3.8±0.8	-
Na mg/g	1.740±0.29	0.170	1.97±0.040	0.02	-	-	13.3±2.07	0.84
K mg/g	-	-	9.8±1.19	0.39	-	-	-	-
Cu ppm	4.18±1.1	0.24	4.56 /4 parallel/		6.83±2.3	0.82	6.35 /2 parallel/	-

tabulated data contain more then the real digits because the evaluation was made in IAEA by computer without rounding off. For some elements /e.g. zinc, manganese, rubidium/ the results were close to one another while for some others /e.g. chromium and iron in bones/ the results show great differences. It is possible that the bone powder was contaminated with chromium during grinding.

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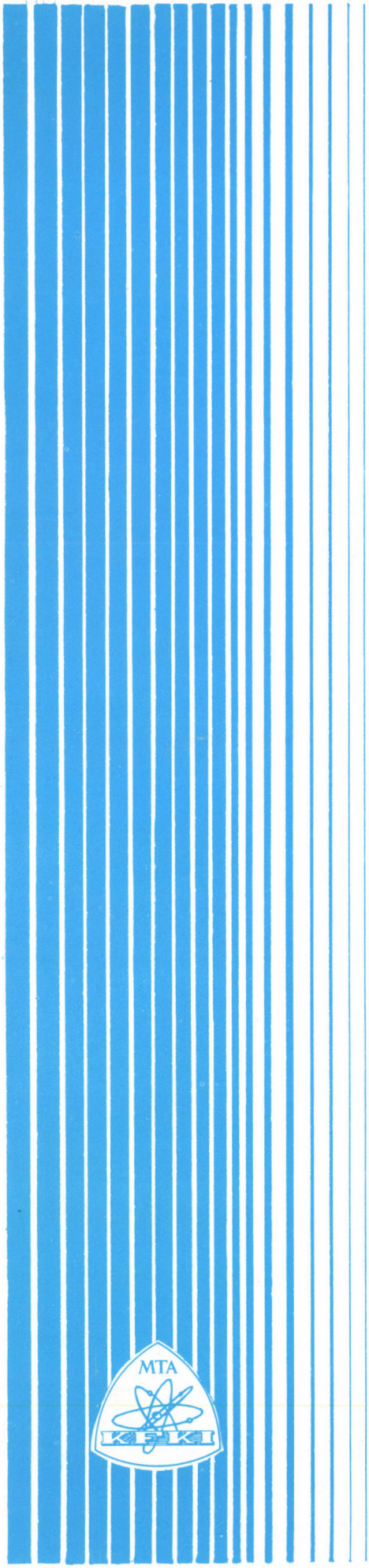

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