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PREPARATION AND LABELLING OF MONODISPERSE POLYSTYRENE- AND POLYSTYRENE- VINYLTOLUENE COPOLYMER LATICES

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ABSTRACT

The silicosis research in our laboratory require as test material a monodisperse aqueous suspension or aerosol radiolabelled with a gamma emitter which does not affect the living organism inconsistently with the experiment. The preparation of polystyrene and polystyrene- vinyltoluene copolymer latices elaborated for this purpose is described. The method is suitable for the production of monodisperse latices of 0,1-1,5 μm particle size. The PS latices were labelled with ^{51}Cr, incorporated in the form of ^{51}Cr acetylacetonate in the course of polymerization. The monodispersity of the products was established from electron microscopic pictures and particle size analyzer data. The ratio of the ^{51}Cr activity in the solid /polystyrene/ particles to that in the aqueous media of the suspension was measured both "in vitro" and "in vivo".

РЕЗЮМЕ

В нашей лаборатории для проведения исследований силикоза возникла необходимость получения меченых гамма-излучающим изотопом монодисперсных водных суспензий или аэрозолей с размером коллоидных частиц, которые после введения в живой организм не оказывают на него вредного воздействия. Разработан метод получения пригодных для этой цели латексов полистирола и полистирол-винилтолуол сополимера как для лабораторного получения, так и для получения в объеме полумикро. Размер монодисперсных частиц латексов, воспроизводимо получаемых путем эмульсионной полимеризации, 0,1-1,5 мк. Для мечения был использован ^{51}Cr, который в форме ^{51}Cr-ацетилацетоната вводился в частицы в процессе полимеризации. Электроскопические снимки, снятые с полученных продуктов, и анализ размера частиц подтверждают монодисперсность латексов. Пропорция распределения ^{51}Cr-ацетилацетоната между частицами полистирола и водной средой в суспензии и временная стабильность были определены исследованиями "in vitro" и "in vivo".

KIVONAT

A laboratóriumunkban folyó szilikózis-kutatáshoz szükség volt olyan, gamma-sugárzó izotóppal nyomjelzett, kolloid részecskeméretü monodiszperz vizes szuszpenzióra vagy aeroszolra, mely élő szervezetbe bejuttatva ott károsító hatást nem fejt ki. Kidolgoztuk az e célnak megfelelő polisztirol és polisztirol-viniltoluol kopolimer latexek laboratóriumi és félmikro térfogatban történő előállitási módját. Az emulziós polimerizációs eljárással reprodukálhatóan előállitott monodiszperz latexek szemcseméretе 0,1-1,5 μm között van. Nyomjelzésre ^{51}Cr-t választottunk, melyet ^{51}Cr -acetilacetonát formájában a polimerizáció folyamán épitettünk be a szemcsékbe. A termékekről készitett elektronmikroszkópos felvételek, valamint azok szemcseméret-analizise során kapott adatok bizonyitják a latexek monodiszperzitását. A ^{51}Cr -acetilacetonátnak a polisztirol szemcsék és a szuszpenzió vizes közege közötti megoszlási arányát, valamint annak időbeli stabilitását "in vitro" és "in vivo" vizsgálatokkal határoztuk meg.

INTRODUCTION

The experiments on animals for the study of the pathogenesis and diagnosis of silicosis, which are carried on in collaboration with the Korányi TBC and Pulmonology Institute, require special, non silicogen materials for the retention and clearance tests of the lungs of the animals treated with quartz powder. The test material must be a monodisperse aerosol of 0,1-1,5 /um particle size and a colloid suspension which does not affect the living organism inconsistently with the experiments. It must be, in addition, radiolabelled for the tracer technique employed in the experiments.

Monodisperse polystyrene /PS/ latices were chosen to start with, since this material had been already successfully used in similar biological experiments.

Several methods are available for the preparation of radiolabelled aerosols. The usual procedure is the vaporization of the combined solution of the test material and the radioisotope in e.g. an atomizer or a spinning disc generator. Upon evaporation of the solvent an aerosol product containing solid particles is obtained.

In the first set of experiments the labelled solid particles were introduced into the respiratory tract of the

animals in the form of an aqueous suspension. The test material had to be therefore so prepared that the labelling isotope should be fixedly bound to the solid particles of the suspension. For this reason the isotope was incorporated in the latex grains in the course of polymerization.

The radiolabelled product used for intratracheal injections in the form of an aqueous solution can be transformed by use of e.g. an atomizer to aerosol particles for the inhalation experiments.

The preparation of 0,1-1,5 /um monodisperse PS and polystyrene- vinyltoluene copolymer /PS-PVT/ latices, the labelling of PS latices and the investigations of these products are described in this paper.

PREPARATION OF PS AND PS-PVT LATICES

Although stable, monodisperse PS latices are commercially available, for the radiolabelling the latices of different grain sizes had to be prepared at the laboratory.

The various procedures described in the literature /1-7/ did not yield products of adequate monodispersity and stability.

After several attempts a set of stable monodisperse products with particle sizes from 0,1 to 1,5 /um was prepared with good reproducibility from the following chemicals:

Styrene - Finom Vegyszer KTSZ, Hungary

Vinyltoluene - BDH, England

Anionic tenside NaDBS /sodium-dodecyl-benzene-sulphonate/ - BDH

Non-ionic tenside TRITON X-100 /octyl-phenol-decaethylene-glycol-ether/ - SERVA

Initiator $K_2S_2O_8$ - REANAL, Hungary

Emulsion polymerization technique was used. Grain sizes below 0,2 /um were obtained by direct /single step/ polymerization, for larger grain sizes seed polymerization was needed. Grain sizes above 0,7 /um could be produced only by the polymerization of vinyltoluene monomers on PS latex.

Table 1.lists the characteristic data of a few monodisperse latex products prepared in our laboratory.

Table 1.

The starting material containing all the components, except the initiator, was stirred for about 2 hours at room temperature, then heated to $70 \pm 2^{\circ}C$ before the addition of the freshly prepared initiator solution. The mixture was left to polymerize for 7 to 8 hours, filtered on paper filter and then was kept at 90-95°C for 2 hours for additional polymerization without being strirred to permit the residual persulphate to be decomposed. The final product was filtered on G3 glass filter.

Table 1.

Characteristic Data of Monodisperse Latex Products

	Latex							
	B	C	C_{1x}	C_1	C_2	C_3	C_5	C_6
Distilled water	220	220	96	93	67	82	52	65
Monomer mark +/	S	S	S	S	S	S	VT	VT
Monomer ml	44	44	21,7	22,2	14,7	17,0	12,0	14,0
Latex mark	-	-	C	C	C_1	C_2	C_4	C_5
Latex ml	-	-	60	60	45	60	50	48
1 % NaDBS, ml	15,0	2,65	5,5	6,5	4,65	2,6	1,5	2,5
18 % TRITON X-100, ml	13,0	8,15	0,7	0,7	1,55	1,7	0,75	1,0
0,1 N NaOH, ml	4,5	4,4	11,0	5,0	3,5	4,0	2,5	2,5
3 % $K_2S_2O_8$, ml	13,0	13,0	6,0	6,0	4,5	4,6	3,3	3,5
$\bar{D}$, /um	0,1281	0,2591	0,2994	0,3310	0,4758	0,5342	1,307	1,430
$\pm \sigma$, /um	0,0062	0,0138	0,0140	0,0127	0,0360	0,0093	0,196	0,079
$\pm \sigma_x$, /um	0,0003	0,0007	0,0005	0,0006	0,0016	0,0003	0,010	0,005

+/ S = styrene

VT = vinyltoluene

Seed polymerization is employed for the polymerization of an additional layer onto the latex formed by direct polymerization. It is important to let the PS particles pre-swell in the monomer used for the second layer formation so as to prevent the deterioration of the monodispersity if the grain size increases.

The polymerization of the vinyltoluene monomer on the PS latex takes 9 hours at 85°C.
Care must be taken of the relative concentrations of the components in each step. The ratio of the anionic to the nonionic tensides or their ratios to the total volume is allowed to vary in a relatively narrow range of values or else products of smaller grain size, the so called second generation or latices of poor stability are obtained /8/.

For an experimental run 20-30 ml of test solution with 1 mg/ml concentration is needed. Since the quantities prepared by the polymerization method described above are much higher, a new technique had to be developed for the preparation of volumes of a few milliliters, which lend themselves to radiolabelling.

To prepare these small quantities the polymerization was carried out in a double-walled glass flask of about 5 ml in volume. The mixture was agitated by shaking for 8 hours at a temperature of 76 ± 1°C.

The latex was purified by dialysis. To reduce the dialysis time the product was diluted to 1-2 % and purified in running tap water for 24 hours. This removed the emulgeator. To remove the electrolyte, it was subsequently dialyzed for 24 to 72 hours in distilled water. The latex obtained for 72 h dialysis /water exchanged 15 times/ was found to be 42 /uS by conductivity measurement.

After dialysis the latex was filtered again on G3 glass filter.

RADIOLABELLING OF PS LATICES

Choice of the isotope used for labelling

The radioisotope which is suitable for radiolabelling the latices was chosen to meet the following requirements:

- emitter of gamma rays with energies from 0,1 to 2,0 MeV,
- half-life not much shorter than the time taken by the measurements on the experimental animals /about 60 days/
- a chemical form insoluble in water but well soluble in styrene and PS to enable the stable incorporation of the radioisotope into the PS particles during the polymerization.

^{51}Cr /E_γ = 0,325 MeV, $T_{1/2}$ = 27,8 d/ was found to be suitable since Cr^{+++} forms with acetylacetone a compound well soluble in apolar solvents and it is quickly eliminated from the living organism.

Preparation of Chrome /III/ acetylacetonate /Cr/aca/$_3$/

The aqueous solution of 0,5 % $CrCl_3.6H_2O$ containing ^{51}Cr was adjusted to pH = 6,5-7,0 with 0,1 N NaOH solution. Acetylacetone was then added in a quantity which could be dissolved in the aqueous solution. After 2-3 hours the solution was shaken with benzene and after separation of the two phases the benzene phase containing the Cr/aca/$_3$ was left to evaporate the solvent at room temperature. The yield of the ^{51}Cr-acetylacetonate, determined by activity measurement was found to be 70 %.

^{51}Cr labelling of PS latices

^{51}Cr/aca/$_3$ was dissolved in the styrene monomer. The attempts at direct polymerization from the radiolabelled monomer proved to be of poor reproducibility, some times polydisperse products were obtained.

Labelled monodisperse latexes were obtained only by introducing the radiolabelled monomer using the method of seed polymerization.

In Table 2. the characteristic data of radiolabelled PS latices with two different particle sizes produced by seed polymerization are listed.

Table 2.

The ^{51}Cr activity of the labelled latices varied from 3 to 30 /uCi/mg of dry material.

Table 2.

Characteristic Data of Monodisperse Latex Products Labelled with ^{51}Cr-acetylacetonate

	Latex	
	C_1-$^{51}Cr/aca/_3$	C_2-$^{51}Cr/aca/_3$
Monomer mark [x]	S	S
Monomer ml	0,44	0,40
Latex mark [x]	C	C_1
Latex ml	1,20	1,20
Tenside solution, ml	2,00 [+/]	1,80 [++/]
0,1 N NaOH, ml	0,10	0,20
2 % $K_2S_2O_8$, ml	0,20	0,20
$\bar{D}$, /um	0,2990	0,4744
$\pm \sigma$, /um	0,0158	0,0323
$\pm \sigma_x$, /um	0,0008	0,0015

[x] see Table 1.

[+/] 6,5 ml 1 % NaDBS
0,70 ml 18 % TRITON X-100
67 ml distilled water

[++/] 5,8 ml 1 % NaDBS
2,1 ml 18 % TRITON X-100
79 ml distilled water

EXPERIMENTAL INVESTIGATION OF THE LATICES

The grain size distribution of the PS and PS-PVT latices was determined from the pictures taken with TESLA BS 242 E type electron microscope. The latex samples were deposited on carbon films by use of ultrason generator vaporizer. Fig. 1 shows some pictures of the PS, PS-PVT and ^{51}Cr labelled PS latices taken with electron microscope.

Fig. 1

The grain size distribution curves were plotted from the data obtained with OPTON TGZ 3 particle size distribution analyzer. The nominal average grain size $\overline{D}$, the standard deviation σ and the medium standard deviation σ_x evaluated from the analyzer data are given in Tables 1 and 2.

It is apparent from the pictures and from the data in the tables that the present method is suitable for the preparation of monodisperse latices with particle sizes from 0,1 to 1,5 /um.

The stability of the stored products kept at 4-5°C was checked by taking from time to time electronmikroscopic pictures of the latices. No appreciable deterioration of the grain size distribution was observed on the products stored for 1-2 years.

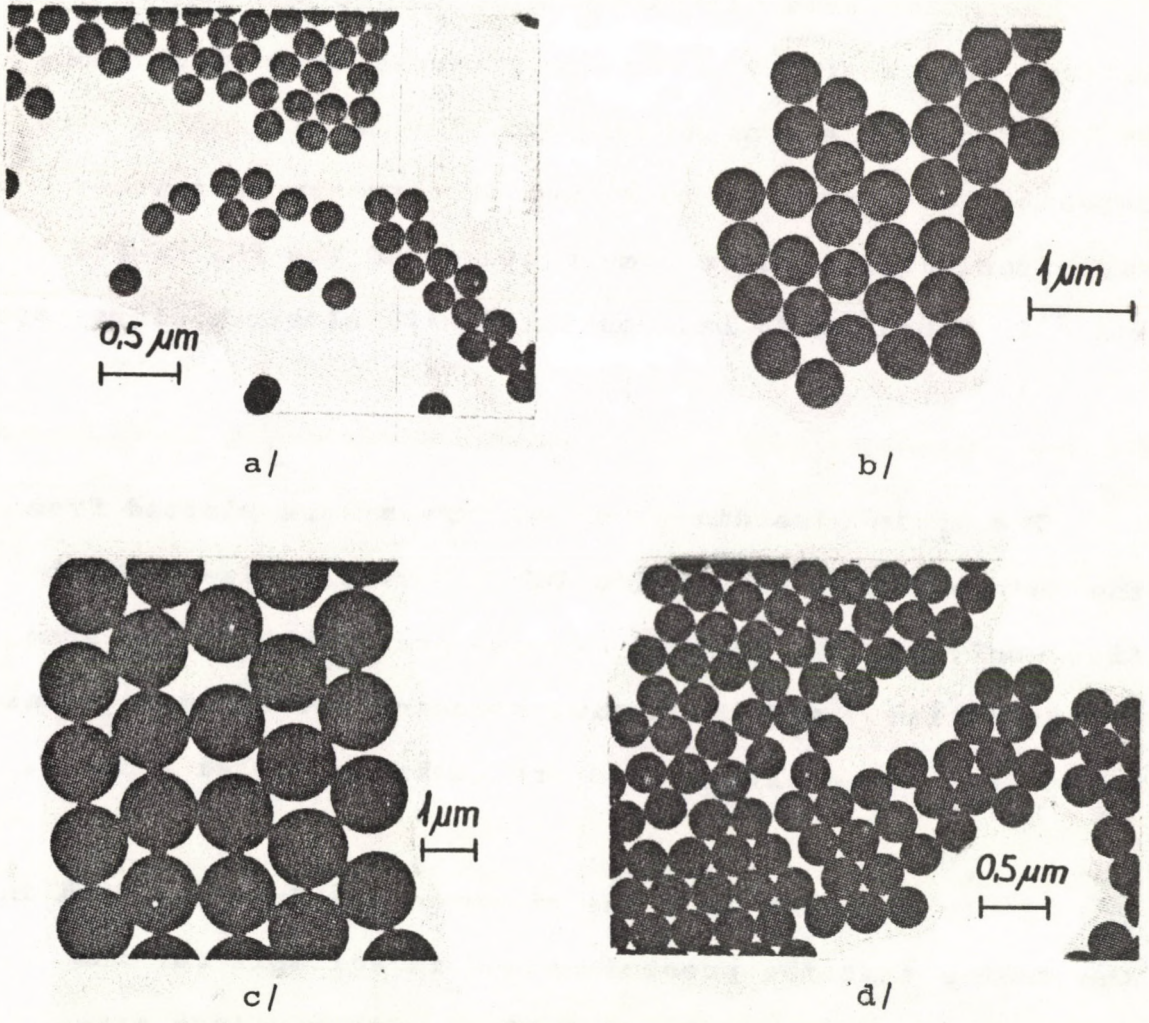

Fig. 1

Some electron microscopic pictures of PS, PS-PVT and ^{51}Cr labelled PS latices

a,b = PS latices
c = PS-PVT latex
d = ^{51}Cr labelled PS latex

The ratio of the $^{51}Cr/aca/_3$ in the aqueous medium to that in the labelled PS solid particles of the latex was determined "in vitro" and "in vivo" by the following measurements. Immediately after the dialysis and after a few weeks of storage a sample of about 1 ml of the latex was precipitated with NaCl solution and the precipitate filtered on filter paper.

The separate activity measurements of the filtrate and the PS precipitate on the filter performed with well type NaI/Tl/ detector showed that both immediately after dialysis and after storage more than 99 % of the starting activity was measured on the precipitate.

The stability of the $^{51}Cr/aca/_3$-PS combination was checked also "in vivo" by measurements after intraperitonial injection of the product into rats. Latex is known to be practically retained by the organism upon intraperitonial injection, while free $Cr/aca/_3$ is quickly discharged /9/. The results were in excellent agreement with the "in vitro" data, that is the ratio of the discharged to the retained ^{51}Cr activity was measured as 1:99. This proves that ^{51}Cr is kept in the PS grains even in the living organism.

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