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by

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Joint Nuclear Research Centre
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The radioactive colloidal chromic phosphate does not hydrolyse and sediment.

The particle size of 50 per cent of colloid is about 200 Å and the other particles are condensed into aggregates.

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Preparation of Colloidal Chromic Phosphate (P^{32}) for Medical Use

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A method for preparation of radioactive colloidal chromic phosphate (1 mC P^{32}/mg $CrP^{32}O_4$) for medical use has been studied.

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PREPARATION DU PHOSPHATE (P^{32}) CHROMIQUE COLLOÏDAL POUR EMPLOI MEDICAL

Une méthode pour la préparation du phosphate chromique colloïdal radioactif (1 mC P^{32}/mg $CrP^{32}O_4$) pour usage médical a été étudiée.

Ce radiophosphate chromique colloïdal ne s'hydrolyse et ne sédimente pas.

La grosseur de 50 pour cent des particles du colloïde est de l'ordre de 200 Å et les autres particules sont condensées en agglomérés.

ПРИГОТОВЛЕНИЕ КОЛЛОИДНОГО ФОСФАТА (P^{32}) ХРОМА ДЛЯ УПОТРЕБЛЕНИЯ В МЕДИЦИНЕ

Изучался метод приготовления радиоактивного коллоидного фосфата хрома (1мкюри P^{32}/мгр $CrPO^{32}{}_4$) для употребления в медицине.

Радиоактивный коллоидный фосфат хрома не подвергается гидролизу и не осаждается.

Величина частиц 50% коллоида равна приблизительно 200 Å (Ангстрем), а другие частицы конденсируются в агрегаты.

DIE HERSTELLUNG VON KOLLOIDALEM CHROMPHOSPHAT (P^{32}) FÜR MEDIZINISCHE ZWECKE

Eine Methode zur Herstellung von radioaktivem kolloidalem Chromphosphat (1 mC P^{32}/mg $CrP^{32}O_4$) ist für den medizinischen Gebrauch studiert worden.

Das so erhaltene radioaktive, kolloidale Chromphosphat hydrolisiert und sedimentiert nicht.

Die Teilchengrösse von 5 prozent des Kolloids ist ungefahr 200 Å und die anderen Teilchen sind zu Aggregaten zusammengeballt.

INTRODUCTION

INTEREST is increasing in the use of colloids of radioactive materials in the therapy of neoplasms.

HAHN and co-workers[1] have used Au^{198} in colloidal form in interstitial, intraperitoneal and intravenous routes of administration.

As early as 1944 JONES and co-workers[2] demonstrated the use of P^{32} in a chemically inert colloidal form, chromic phosphate, for the irradiation of tissues of the reticuloendothelial system. This substance was also used interstitially in mice in therapy of mammary adenocarcinomata (1945) and then in treatment of patients afflicted with a wide variety of malignant tumours[3] with apparent good results.

Radioactive phosphorus P^{32} seems to have some advantages over radioactive gold Au^{198}.

Radioactive phosphorus P^{32}, in fact, is essentially a pure beta-emitter (E_{max} = 1·7 MeV); hence handling is simpler and safer than with radioactive gold (Au^{198} decays with

two beta spectra on the excited levels of 1·088 and 0·412 MeV of Hg^{198})[4].

Furthermore the half-life of P^{32} is 14·3 days while Au^{198} decays with a half-life of 2·69 days. Because of the longer half-life of P^{32}, storage is possible and considerably smaller doses than in the case of Au^{198}, in terms of millicuries, are equivalent in total ionizing radiation ultimately delivered.

These reasons indicate that colloidal chromic radiophosphate may displace Au^{198} colloids in radiotherapy.

Published methods of preparation of colloidal chromic radiophosphate can be divided in two main groups: precipitation methods and reduction methods.

The precipitation methods[5,6] are based on the original technique of JONES and co-workers[2]. This method consists in a precipitation reaction of insoluble chromic phosphate from a solution of alkaline phosphate and a soluble chromic salt (generally nitrate). The precipitate is then filtered, ignited, crushed, suspended and centrifuged. The results are not too satisfactory. The yield is rather low (about 25 per cent) and the operations require several days of work and elaborate equipment.

The reduction methods[7,8] are based on a reduction-oxidation reaction involving chromic acid, phosphoric acid and a reducing agent in absence, or presence of a protective colloid. The subsequent purification of the colloidal chromic phosphate obtained as above requires (ANGHILERI) flocculation, washing and suspension in isotonic solution or (CHEVALLIER and co-workers) elaborate technique based on the use of a lactose column.

The method described in this work is a reduction method according to the technique of ANGHILERI. The reaction used involves chromic acid, sodium sulphite as reducing agent, phosphoric acid in presence of gelatin as protective colloid,

$$2H_2CrO_4 + 3Na_2SO_3 + 2H_3PO_4 \rightarrow 2CrPO_4 + 3Na_2SO_4 + 5H_2O.$$

The purification of the colloidal chromic radiophosphate is carried out by dialysis.

The yield is 80 per cent; the equipment and handling are very simple.

The colloidal chromic radiophosphate prepared by the method described in this paper is a green, clear suspension and has the following characteristics:

$CrPO_4$	3 mg/ml
gelatin	5·5 mg/ml
pH	6–7

EXPERIMENTAL

Materials

The following solutions are needed:

(1) Chromic acid solution: 10 mg/ml;

(2) Phosphoric acid solution: 10 mg/ml—(in this solution $H_3P^{32}O_4$ carrier-free is added according to the required specific activity);

(3) Sodium sulphite solution: 200 mg/ml;

(4) Gelatin solution: 20 per cent.

All these solutions are freshly (this is a very important point) prepared and sterilized at 112°C for half an hour.

Preparation

In order to obtain colloidal chromic radiophosphate, the operations are carried out as follows.

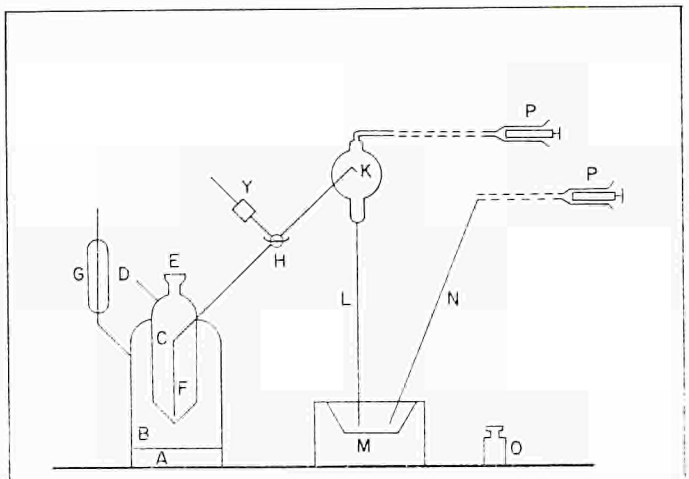
To 6 ml of chromic acid 4 ml of phosphoric acid (containing P^{32}) are added. When the mixture reaches the boiling point, 0·5 ml of gelatin, 6·5 ml of pyrogen-free water and 1 ml of sodium sulphite are added. The mixture is kept at the boiling point for 5 min.

All these operations are carried out while agitating by air bubbling through a capillary tube. The preparation requires 15 min. The reaction yield, tested by chromatographic analysis according to the EBEL technique[9], results 90 per cent.

The colloidal chromic phosphate has $R_f = 0$ and phosphoric ions $R_f = 0{\cdot}7 - 0{\cdot}8$, with Whatman No. 1 paper using ascending monodimensional method and as eluent: isopropyl alcohol (75 ml), water (25 ml), trichloroacetic acid (5 g), ammonia 22 Bé (0·3 ml), checked pH 1·5–2.

Purification and sterilization

The colloidal chromic phosphate containing about 10 per cent of free orthophosphoric ions is then dialysed overnight using cellulose membrane (C. ERBA, Milan).



A	Hot plate	H	Three way unlubricated stopcock
B	Hot bath	Y	Air filter
C	Reaction vessel	K	Reservoir
D	Reagent inlet	L	Delivery tube from reservoir
E	Connexion from C to vacuum	M	Dialyser
F	Capillary for air introduction and liquid transfer	N	Dispensing pipette
		O	Dispensing bottle
G	Condenser	P	Plungers

FIG. 1. Equipment used for the preparation of colloidal $CrP^{32}O_4$.

In this way the content of phosphoric ions is reduced to 1 per cent or less.

The colloid is then sterilized at 112°C for half an hour. In this way the content of phosphoric ions increases to 3–4 per cent.

The equipment used for this method of preparation and purification (Fig. 1) is placed in a glove box shielded by 12-mm Plexiglas walls.

DISCUSSION

The following characteristics were checked on the colloidal chromic radiophosphate prepared as above:

(a) Content in phosphoric ions,

(b) Chemical stability,

(c) Colloidal stability.

All these tests were made on samples containing P^{32} at tracer level.

(a) *Content in phosphoric ions*

The content in phosphoric ions was tested by chromatographic analysis[9] and was found equal to 1–4 per cent. The chromatographic analysis shows also the absence of condensed phosphate.

(b) *Chemical stability*

The colloidal chromic radiophosphate shows satisfactory chemical stability. Hydrolysis was checked by chromatographic analysis[9]. The colloid kept at room temperature and at 40°C does not hydrolyse even after one month.

On the contrary the pH and the dilution have influence on the hydrolysis. The obtained data are reported in Tables 1 and 2. Tables 1(a) and 1(b) show that the hydrolysis is rather low for the pH of the human parts into which colloidal chromic radiophosphate can be injected[10].

Table 1. Influence of the pH

(a) Colloidal chromic radiophosphate kept at room temperature. Initial content of phosphoric ions = 1%.				(b) Colloidal chromic radiophosphate kept at 40°C. Initial content of phosphoric ions = 1%.			
pH	% of orthophosphoric ions			pH	% of orthophosphoric ions		
	After 1 day	After 2 weeks	After 4 weeks		After 1 day	After 2 weeks	After 4 weeks
4	6·5	9·5	11·5	4	14·5	52	52
5	3·5	6	6·5	5	9	40	40
6	2·2	3·2	3·5	6	6	6	6
7	2	3	3	7	6	6	6
8	2	4	4	8	6	6	6
9	3	4	4	9	6	6	6

Table 2. Influence of the dilution

(a) Colloidal chromic radiophosphate diluted with NaCl 0·9% and kept at room temperature. Initial content of phosphoric ions = 1%.				(b) Colloidal chromic radiophosphate diluted with NaCl 0·9% and kept at 40°C. Initial content of phosphoric ions = 1%.			
Dilution	% of orthophosphoric ions			Dilution	% of orthophosphoric ions		
	After 1 day	After 2 weeks	After 4 weeks		After 1 day	After 2 weeks	After 4 weeks
1:1	1	1·5	1·4	1:1	1·4	2·8	2·5
1:5	1·4	2·3	3	1:5	2·7	3·2	3·3
1:10	1·6	3	4·5	1:10	4	4·2	4·2

(c) *Colloidal stability*

Stability was checked for samples:

1—Kept at room temperature one month from preparation,

2—Kept at high and low temperature several hours,

3—Diluted with physiological solution (NaCl 0·9%),

4—Buffered from pH 4 to pH 9.

No flocculation was found in any sample.

COLLOIDAL CHROMIC PHOSPHATE WITH HIGH SPECIFIC ACTIVITY

Samples of colloidal chromic phosphate with high specific activity (3 mC/ml) were prepared as above.

Stability tests made on these samples show that also the colloidal chromic radiophosphate with high specific activity does not hydrolyse and sediment even after one month.

PARTICLE SIZE DETERMINATION

Great difficulties were found in the particle size determination by electronic microscope.

Colloidal chromic phosphate has, in fact, a remarkable tendency to condense into aggregates and its low mean atomic number makes a rather difficult task to obtain good micrographs.

Figure 2 shows the presence of aggregates and small diameter particles. By fractionated centrifuging the fraction containing aggregates was found equal to about 50 per cent. The remaining fraction has a diameter smaller than 200 Å.

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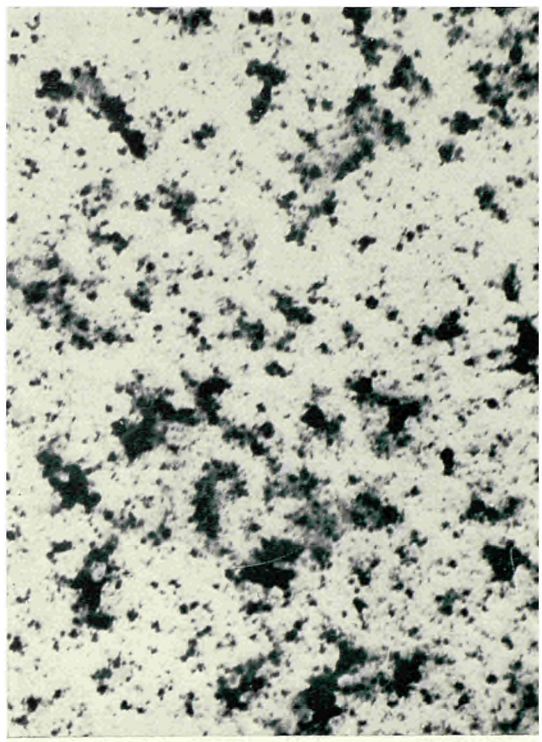

FIG. 2. Photograph at the electronic microscope of the colloidal chromic phosphate ($\times$72,000).

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