In-vitro preparation of ^{113m}In (Ca)-phytate colloid for liver scintigraphy

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Summary

A method for preparation of in vitro radiocolloidal solution of ^{113m}In (Ca)-phytate is reported. The radiochemical purity was determined using paper chromatography and it was higher than 95% . Liver uptake in mice was high (87%) with low lung uptake. This radiopharmaceutical has, therefore, medical diagnostic specifications.

Key words : ^{113m}In (Ca) - phytate

INTRODUCTION

Radioisotope generator systems are very widely used in nuclear medicine for the production of short lived radioactive isotopes. One of these systems is the ^{113}Sn - ^{113m}In generator which has desirable characteristics for scintigraphy. The half life of the parent is 118 days (one can use the generator for several months) and that of the daughter is 1.7 hr and decays by emitting 393 KeV gamma rays. The mode of decay of ^{113m}In leads to lower radiation dose to the patient. Authors have prepared various ^{113m}In compounds for lung and liver scanning such as ^{113m}In Fe (OH) , ^{113m}In - phytate and ^{113m}In - colloids prepared by different methods (1,2,3,4) . It is the aim of this work to present a procedure for the preparation of ^{113m}In (Ca)-phytate as an in vitro radiocolloid for liver scanning.

EXPERIMENTAL AND RESULTS

Procedure

One hundred mg of sodium phytate were dissolved in dis. water and the pH was adjusted to 12.5 using 0.5-1 N NaOH . One ml portions were put into vials. The pH was brought down with diluted HCl and acidic ^{113m}In eluate was added to get a final pH equal to 8. The final volume was 5 ml containing sodium phytate in a concentration of 4 mg/ml . In another experiment the pH was brought down to 8 using phosphate buffer

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(PH = 6.8) instead of diluted HCl. ^{113m}In (Ca)-phytate was prepared in vitro by the reaction of ^{113m}In-phytate with calcium ions. 4 ml of ^{113m}In-phytate containing 20mg of sodium phytate were reacted with one ml portions containing 4 and 3.5 mg of CaCl whose PH was adjusted to 8.

Radiochemical analysis

The radiochemical analysis was carried out by ascending paper chromatography using paper strips (Whatman No. 3) treated with diluted HCl and dried. 85% methanol was used as a solvent. The paper strips were scanned, (thin layer scanner, Berthold, Germany) Cut and counted using the scintillation detector, Gammaszint, BF 5300 , Germany.

Gel chromatography column scanning (GCS) was applied in an attempt to determine ^{113m}In-phytate and unbound ^{113m}In (ionic and colloidal). Columns with inner diameter of 18 mm were filled to a hight of 25 cm with the swollen gel in 0.9% NaCl. The sample to be analysed was applied at the top of the column in a volume of 0.1 ml. The elution was carried out with 20-25ml of aqueous 0.9% NaCl (PH 6.8) , 0.9% NaCl in 0.04 N HCl (PH 1.5) , 1.2 mg and 4 mg of sodium phytate in 0.9% NaCl per each ml of the eluent. The gel chromatographic media applied were various sephadex G-types , sepharose 6B and Bio-gel p6. Fig.1 shows the GCS profile for ^{113m}In-phytate. The elution was carried out using 20 ml of the phytate solution (4 mg/ml) in saline. The sephadex type was G-50 fine.

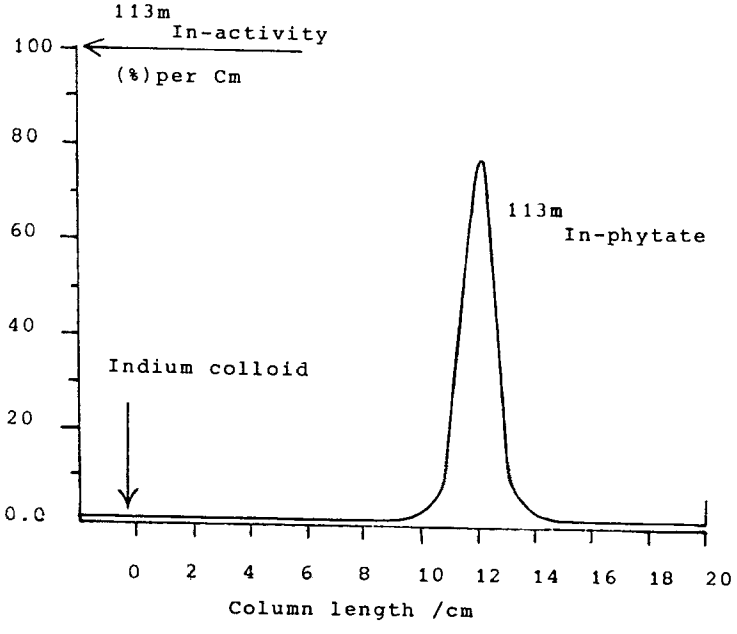

Fig.1. GCS PROFILE FOR ^{113m}In- PHYTATE.

Biodistribution

The organ distribution of ^{113m}In (Ca) - phytate prepared in vivo and in vitro was determined using about 16 gm white mice. Aliquots of 0.1 ml were injected into the tail veins of 3 mice. The animals were killed with diethylether at certain time intervals. Radioactivity in the lungs, liver , spleen, kidneys and remaining carcass (minus the tail to avoid potential errors from infiltration of the injection) was determined and the per cent of injected dose calculated assuming that equivalent doses were injected. The results of the organ distsibution are shown in Table 1.
Blood clearance rates of ^{113m}In (Ca) - phytate prepared in vitro and in vivo were performed in newzeland albino rabbits (4 rabbits) weighing about 4 kg by injection of about 200 uCi into ear veins of the rabbits. One ml blood samples were taken from the animal at different time intervals (2 to 60 min) and their actvity measured using a well type gamma scintillation detector. The results are shown in Fig. 2.

Table 1. The organ distribution of ^{113m}In (Ca) phytate in mice 30 min post intravenous injections.

Organ	% of injected dose			
	A	B	C	D
Liver	75.0	80.8	84.9	87.7
Lungs	4.5	3.7	2.3	2.4
Spleen	6.0	1.8	4.0	3.9
Kidneys	0.23	2.5	1.8	0.7

A, B : The agent was prepared in vivo.
The PH was brought down using diluted
HCl (A) and phosphate buffer (B).
C, D : The agent was prepared in vitro using 4 mg/ml (C) and 3.5 mg/ml of CaCl solution (D) .

DISCUSSION

It has been mentioned by Subramanian et al that the human blood system contains an average of 100 mg of calcium per one litre (5). The ^{113m}In-phytate injected into the blood system reacts with calcium ions and is converted into a colloidal solution which accumulates in the liver. The same thing proceeds with ^{99m}Tc-phytate. Since Ca plays an important role in the control of many activities of the body it is essential that the extracellular free Ca concentration is regulated within narrow limits. The deficiency of calcium ions in the blood is not fully investigated in the literature. It is not, therefore, advisable to apply in vivo formed radiocolloids for liver scanning even if the same quality of liver scans are obtained using radiocolloids prepared in vitro or in vivo.

The procedure applied for the preparation of in vitro ^{113m}In (Ca) - phytate gave rise to high liver uptake (87%) and low lung uptake (2.4%). In fact all known liver scanning agents such as ^{113m}In-radiocolloid (4) , ^{99m}Tc-phytate (6) and ^{99m}Tc-sulphur colloid (7) lead to liver uptake between 85 to 90 % of the injected dose.

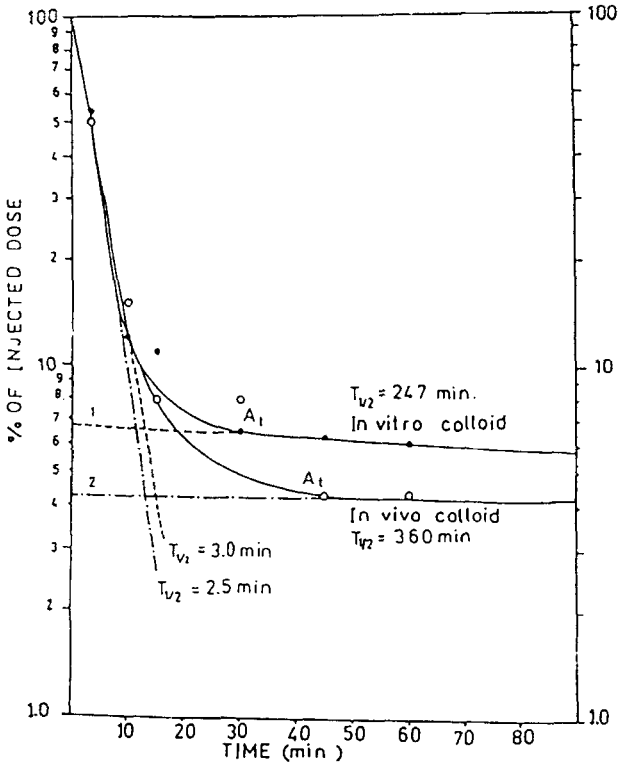

Fig.2 - Blood disappearance curves of ^{113m}In-phytate in rabbits (average results from 2 rabbits) over a period of 1 hr post injection using in vitro colloidal solution (●—●) or in vivo colloidal solution (○—○) of ^{113m}In-phytate. Two components of decay are isolated.

A; $T_{½}$ = 2.5 min fast, 247 min slow.

B; $T_{½}$ = 3.0 min fast , 360 min slow.

The radiochemical yield determined by paper chromatography was higher that 95% . The labelling yield of ^{113m}In-phytate was measured by the GCS method. The ^{113m}In colloid and the In-phytate were very well separated using Sephadex G-50 fine and phytate dissolved in 0.9% saline as eluent (Fig.1) . At all experimental conditions the colloidal ^{113m}In remains at the top of the column.

The blood clearance data of ^{113m}In radiocolloids prepared in vitro and in vivo in rabbits showed that the biological half-times of the fast components were 3 and 2.5 min and those of the slow components were 247 and 360 min respectively (Fig.2). It is evident that the major fractions of both colloids are released from the blood within a short time. The phytate kits in non-lyophilized and lyophilized form were stable at least for more than one months.

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