NRCP Research Journal
Vol. 3, No. 2, July-September, 1993

# THE DEVELOPMENT OF TECHNETIUM - 99m RADIOPHARMACEUTICALS: TECHNETIUM -99m PHYTATE

A.D. EUGENIO, E.M. DE JESUS, Ma.T.A. SALABIT
and L.F. MENDOZA
*Philippine Nuclear Research Institute*
*Diliman, 1101 Quezon City*

## ABSTRACT

***A kit for scintigraphy of the reticuloendothelial system has been prepared. It provides a predispensed sterile formulation for reconstitution with sterile $^{99m}$TC-pertechnetate solution. The resulting injection contains $^{99m}$TC labelled phytate. Each kit consists of 3 vials and each vial contains 10 mg phytate and up to 1 mg $SnCl_2$ $2H_2O$ in freeze-dried form.***

## INTRODUCTION

Although $^{99m}$Tc sulfur colloid is widely used for liver scanning it suffers from the disadvantage that its preparation is time-consuming and requires the use of trained personnel. Such a kit which avoids the drawbacks of $^{99m}$Tc sulfur colloid can be prepared from sodium phytate containing stannous chloride and $^{99m}$Tc pertechnetate solution.

In 1973, Subramanian, *et al.* first proposed and carried out the labelling of Sn phytate with $^{99m}$Tc and subsequently used this complex as a liver scintigraphic agent by allowing the administered $^{99m}$Tc-Sn-phytate to form the insoluble calcium salt in vivo. The biological distribution in the reticuloendothetial organs may be controlled by the phytate to stannous ion concentration ratio and total quantity of phytate injected. With a 5:1 ratio in mouse 85-90% of radioactivity localizes in liver at 15-30 minutes.

The preparation appears to be quite soluble in that it can be sterilized by passage through a 0.22 micron membrane filter although electron microscopy shows the presence of some several particles (0.1 - 0.2 microns in diameter).

The exact composition and structure of the complexes formed are not known with certainty. The sequence of steps in preparation

of $^{99m}Tc$ radiopharmaceuticals are: a) preparation of Sn(II) complex of the component b) reduction of $^{99m}TcO_4$ with Sn(II) complex with simultaneous binding of the reduced $^{99m}Tc$ to the liquid compound. Once the amount of this compound is fixed, the other conditions are carefully standardized to get optimum radiochemical purity and reproducibility.

Since at the moment no simple physico-chemical method is established for separating the various components arising out of these $^{99m}Tc$ complexing reactions, standardization is usually carried out using chromatographic methods in conjunction with biodistribution studies in laboratory animals.

The reaction conditions once standardized should be meticulously followed with appropriate quality control on all ingredients used including $^{99m}Tc$ pertechnetate.

In all $^{99m}Tc$ labelling procedures, $^{99m}Tc$ pertechnetate is reduced by an agent, followed by formation of stable chelate complexes with the ligand or by binding to suitable particles. The reduction of $^{99m}Tc$ pertechnetate is usually carried out using $SnCl_2.2H_2O$ ($Sn^{+2}$ ions) because of its high reduction efficiency, ease of handling and low toxicity.

The present paper reports the preparation and properties of $^{99m}Tc$-phytate. The composition and usage protocol for the resulting kit is described.

## MATERIALS

Sodium phytate (BDH, England), $SnCl_2.2H_2O$ (Ajax, Australia), Acetone (Merck), filtering system (Millipore), $^{99m}Tc$ generator (Amersham), Hydrochloric acid (Baker), Laminar flow hood with UV lamps, GM counter.

## METHODS

### Preparation of $^{99m}Tc$ phytate

The Stannous (II) phytate complex is prepared by adding a freshly prepared solution of sodium phytate (1.0 gram in 50 ml

Triple Distilled Water (TDW) to 100 mg of $SnCl_2.2H_2O$ dissolved in 50 ml dilute HCl to make the pH of the solution between 6.0 and 7.0. The resulting solution is passed through a millipore filter (0.22 micron). One-ml fractions of this solution were dispensed into borosilicate vials, and lyophylized if not used immediately. Lyophilized products are stable up to a maximum of 6 months at 2-4°C. The filtrate (1 mL), or saline-reconstituted lyophylized solution in vial, was made to react with 3 - 4 ml of $^{99m}$Tc pertechnetate in normal saline solution. After five to ten minutes, it is ready for injection.

## RADIOCHEMICAL PURITY OF $^{99m}$Tc-PHYTATE

The radiochemical purity of $^{99m}$c-phytate was determined by descending paper chromatography. Whatman #1 was cut into 2.5 cm-wide by 20 cm - long strips. The strips were lightly marked with pencil into 1 cm. sections and numbered successively. Five μL of solution was spotted onto the strips about 3 cm. from its upper edge. The upper edge of the strips, (about 1.5 cm) were dipped into the mobile phase in the chromatographic vessel previously saturated with acetone. After twenty minutes, the strips were taken out, the solvent fronts were marked, strips were allowed by dry in air. The paper strips were than cut into 1 cm sections and were separately counted in the GM counter. Results were compared with data from the Handbook of Radioactive Controls from Argentina.

## BIOLOGICAL DISTRIBUTION OF $_{99m}$Tc-PHYTATE

Biological distribution of the product was determined using white mice of approximately 30 gram body weight as experimental animals. Three mice were used for each run. Two tenth of $^{99m}$Tc-phytate in normal saline solution were injected intravenously into the tail veins of the mice. The animals were sacrificed after 30 minutes and the different organs, e.g. heart, liver, kidney and lungs were removed, weighed and counted separately using the GM counter.

## TOXICITY TEST

The toxicity test was done by injecting 10 experimental and 10 control mice each time with the product in doses of 0.3, 0.4 and 0.5 ml and monitoring their survival one day after. The mice were

subjected to a minimum of 60μCi in 1.5 mg of phytate for the 0.3 ml dose and a maximum of 300μCi in 2.5 mg phytate for the 0.5 ml dose.

### STABILITY TEST

In-vitro stability was determined by conducting descending paper chromatography on the product.

### STANNOUS (II) DETERMINATION

Stannous (II) content of random vials was determined by spectrophotometric assay at 460 nm. using molybdate and thiocyanate solutions.

## RESULTS AND DISCUSSION

Chromatographic results done on the product are presented in Tables I and II. Although Whatman #1 was used instead of ITLC S.G. the results were identical with the Handbook of Radiochemical Controls (HRC). An average of 98.38% ± 0.89 was obtained for Rf = 0 of $^{99m}$Tc phytate colloid. IAEA TECDOC 649 requires that radioactivity corresponding to $^{99m}$Tc phytate should not be less than 95% of total radioactivity.

For biological distribution, IAEA TECDOC 649 requires that total radioactivity in the liver and spleen should not be less than 80% and should be less than 5% in the lungs. The total concentration of $^{99m}$Tc phytate in the liver and spleen ranges from 80-86% and from 0.1 to 3.2% in the lungs.

For toxicity testing, HRC requires that the whole lot of animals must be alive twenty four hours after injection of the product. Table IV shows all the animals survived one day after the test.

From Table V it can be seen that the $^{99m}$Tc phytate was still potent in terms of activity and as a chelate after its first half-life was gone. Thus, the preparation was stable six hours after it was prepared. From TECDOC 649 the radioactive formulation was recommended to be used as early as possible, and not later than 4 hours after preparation due to the short half-life of $^{99m}$Tc ($t_{1/2}$ = 6 hrs).

Random sampling of the unlabelled preparations showed stannous chloride content to vary from 85% to 95% of the expected value which is 1 mg. TECDOC 649 requires that the average value of stannous chloride should not be less than 80% of the expected value. Determination of the Sn (II) content of the unlabelled preparation was determined to make sure that the stannous remains in its reduced state and was not oxidized by any dissolved oxygen in the solutions.

## CONCLUSION

Critical tests performed on the product passed all the quality control procedures embodied in the Handbook for Radioactive Controls from Argentina and those from IAEA TECDOC 649. After passing sterility tests and pyrogenicity required by BFAD the product maybe released for public use.

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## ACKNOWLEDGMENT

The study was made possible through a grant given by the National Research Council of the Philippines (NRCP Research No. D-112).

The authors thank Ms. Rizalina A. Galler for doing the Sn (II) determination and the data entry.

**Table 1. Chromatogram Results of Products vs. HR Control.**

| | HR CONTROLS | PRODUCT |
|---|---|---|
| Solid Support | ITLC S.G. | WHATMAN #1 |
| Solvent | Acetone | Acetone |
| Time | 5 minutes | 25 minutes |
| Rf Values | | |
| $^{99m}TcO_4$ | 1.0 | 1.0 |
| $^{99m}$Tc Phytate | 0.0 | 0.0 (98.38% ± 0.89) |

**Table 2. Summary of Radiochemical Runs by Paper Chromatography.**

| Trial | Runs | | | Average |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| 1 | 99.6 | 99.0 | 98.6 | 99.1 |
| 2 | 97.8 | 98.9 | 97.4 | 98.0 |
| 3 | 99.3 | 98.2 | 97.3 | 98.3 |
| 4 | 98.9 | 98.6 | 97.3 | 98.3 |
| 5 | 95.6 | 99.5 | 99.6 | 98.2 |
| 6 | 96.0 | 97.0 | 99.6 | 97.5 |
| 7 | 98.7 | 96.8 | 94.9 | 96.8 |
| 8 | 97.5 | 99.0 | 96.0 | 97.5 |
| 9 | 99.8 | 99.3 | 99.4 | 99.5 |
| 10 | 99.9 | 99.5 | 99.9 | 99.8 |
| 11 | 99.5 | 98.6 | 99.5 | 99.2 |

Rf Values of all runs of $^{99m}$TC-Phytate = 0

**Table 3. Biological Runs of $^{99m}$Tc-Phytate.**

| Trial # | %Act. in Liver | %Act. in Spleen | % Total Activity (in Liver & Spleen) | %Act. in Lungs |
|---|---|---|---|---|
| Trial 1 | 82.7 | 1.98 | 84.9 | 0.1 |
| Trial 2 | 84.7 | 1.50 | 86.2 | 0.3 |
| Trial 3 | 78.0 | 2.70 | 80.7 | 3.2 |
| Trial 4 | 57.8 | 21.9 | 79.7 | 2.6 |

**Table 4. Toxicity Test of $^{99m}$Tc-Phytate.**

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | |
| | Exp. | Control | Exp. | Control | Exp. | Control |
| Dose (mL) | 0.3 | 0.3 | 0.4 | 0.4 | 0.5 | 0.5 |
| No. of Mice Injected | 10 | 10 | 10 | 10 | 10 | 10 |
| No. of Mice alive after 24 hrs. | 10 | 10 | 10 | 10 | 10 | 10 |

**Table 5. In-Vitro Stability Test of $^{99m}$Tc-Phytate.**

| TIME | %Total | | | AVERAGE (%) ± S.D. |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| 10:00 AM | 98.0 | 95.47 | 99.82 | 97.76 ± 1.78 |
| 12:00 PM | 99.42 | 98.82 | 99.83 | 99.36 ± 0.41 |
| 2:30 PM | 98.67 | 97.38 | 99.22 | 98.42 ± 0.77 |
| 4:15 PM | 98.40 | 98.17 | 99.12 | 98.56 ± 0.40 |