
# PREPARATION AND USE OF LYOPHILIZED "KITS": HUMAN SERUM ALBUMIN $^{99m}$Tc AND DERIVATIVES

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Labelled compounds
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# PREPARATION AND USE OF LYOPHILIZED KITS : HUMAN SERUM ALBUMIN $^{99m}Tc$ AND DERIVATIVES

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Constância Pagano G. da Silva[*]

## ABSTRACT

This paper presents the experimental data on preparation and conservation of human serum albumin (HSA) and its derivatives macro (MAA) and micro (MiAA) aggregates.

Lyophilized compounds are labelled with technetium 99m.

Results from labelling reactions obtained by means of ascending paper chrometography and absorption spectroscopy are described.

Results from electron and optical micrographs of MAA and MiAA, biological distribution and toxicity assay are also examined.

Maps obtained from placentography, pulmonary and liver scintilography and cardiac blood pool studies are shown by "Clinical Assay".

## 1 – INTRODUCTION

A number of methods dealing with the labelling of HSA with technetium 99m have been reported in the literature.

Some of them require the reduction of pertechnetate anion by iron-ascorbic acid mixture[10,9] stannous chloride[2] or electrolitic reduction[4].

The labelling process of HSA and its derivatives in kit form is established.

The kit proved to be simple and satisfactory for clinical use.

The stability of the kits permits its shipment to distant medical centers, so that the labelling process can be made whenever it is necessary.

## 2 – MATERIAL AND METHODS

### Materials

Solutions of sterile, pyrogen free human serum albumin (Behring), 0.1 and 0.2 normal hydrochloric acid (Merck), 0.1 and 0.2 normal sodium hydroxide and normal pysiological saline.

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Stannous chloride (Merck)

Sodium pertechnetate $^{99m}Tc$ (Mallinckrodt)

Ammonium pertechnetate $^{99}Tc$ (New England Nuclear)

Ketone (Merck)

**Equipment**

A precise water bath with continuous gentle agitation at a constant temperature of 78°C.

A chromatography apparatus for ascending paper chromatography.

An electron microscope with magnificant to 28000 X (Zeiss).

A scintillation counter facility for measuring radioactivity levels in albumin $^{99m}Tc$ and derivatives $^{99m}Tc$ samples.

A whole-body photoscanning equipment.

A Spectrophotometer (Perkin-Elmer).

Each kit is prepared in a special room of 4 $m^2$ provided of an anti-camera of 1.5 $m^2$.

This one has a germicide UV lamp and a cabinet to keep aprons, gloves and masks used by the operator.

The working room has a glove-box provided with an UV lamp and a nitrogen entrance.

A lyophilizator (De Virtis) is instaled in this room.

**Preparation of the HSA kit and its labelling with technetium-99m**

Stannous chloride is used as reducing agent of pertechnetate anion in presence of HSA.

Firstly stannous chloride is dissolved in 0.1 normal hydrochloric acid in nitrogen atmosphere.

A convenient part of this solution is poured into the HSA solution.

The solution is frozen to -40°C and lyophilized in nitrogen atmosphere.

To analyse the labelling process of HSA and its derivatives an adequate quantity of sodium pertechnetate $^{99m}Tc$ is added into the lyophilized sample.

Paper chromatography with ketone and saline as the solvent was used to distinguish and estimate the percentages of reduced technetium, pertechnetate anion and labelled human serum albumin.

**Preparation of MAA kit and its labelling with technetium-99m**

The MAA egregates were prepared according to the Taplin method[11] and the reducing agent dissolved in 0.1 normal hydrochloric acid was added.

The samples were immediately frozen at -40°C and lyophilized in nitrogen atmosphere.

The radiochemical quality control of the lyophilized sample labelled with technetium-99m was performed by ascending paper chromatography using ketone as solvent.

Studies to determine the organ distribution with the testing material were performed in rats, using 0.25 mg MAA/100 g body weight (up to 0.08 mCi) to determine pulmonary deposition. The security test, following international norms[6], was performed using mice (20 g) or rats (350 g).

Up to 0.05 ml of the material was injected to the first ones and up to 5.0 ml to the latters (conc. 7.5 mg MAA/ml).

After the injection the animals were observed during seven days, by registering any significant symptom or death.

The particle size distribution control was performed by optical or electron microscopy.

**Preparation of the MiAA kit and its labelling with technetium-99m**

The MiAA was prepared by the dilution of 25% HSA to 1 per cent with physiological saline.

The pH was adjusted to 10 by adding 0.2 N NaOH.

The solution was heated in a water bath to 78-80°C for 20-30 minutes. It was then cooled to room temperature and the pH lowered to 7.5 by adding 0.2 N HCl.

After the colloid preparation, stannous chloride in 0.1 N HCl was added in nitrogen atmosphere.

The samples were immediately frozen at -40°C and lyophilized.

Sterile and pyrogen free reagents, equipments and techniques were used in each preparation.

## 3 – RESULTS

In order to obtain the best conditions for the preparation of the kit and its labelling with technetium-99m, assays like variation in pH of labelling, protein mass, time of reaction and lyophilization conditions were carried out. Stability of the lyophilized products during storage was examined and quality control of the labelled products was performed.

### a) HSA $^{99m}$Tc Kit

– Influence of variation in pH:

The assays were carried out using 60 mg of HSA, 0.24 mg of stannous chloride and pH between 3 and 8.5. The reaction time was two hours in all cases.

The results indicated that the best labelling pH was 6.5, with a labelling yield of 85-95%. (Figure 1).

Figure 1 shows a great deal of the reduced technetium below pH 6.0 with a maximum in pH 5.0-5.5 which is in coincidence with the isoeletric point of the protein

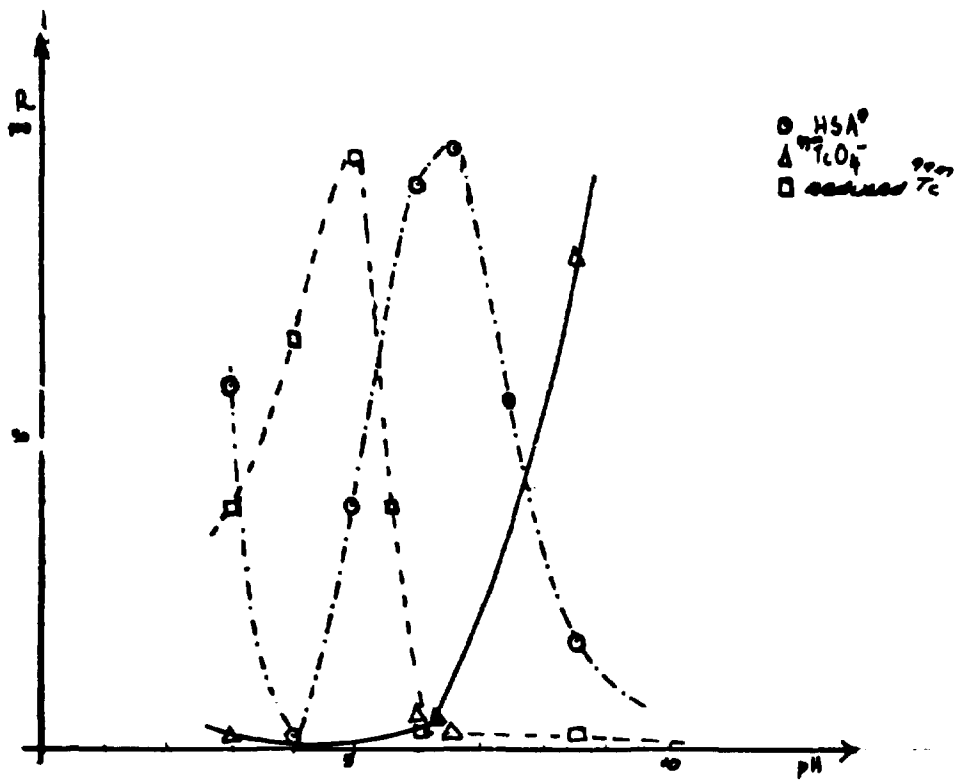


Figure 1 – Yield of Albumin Labelling as Function of pH

For pH higher than 6.5 the quantity of free pertechnetate increases, reaching 80 per cent in pH 8.

The results mentioned above indicate that the valence state of technetium and hence its chemical behaviour depends on the alcali or acidic medium. Results obtained by paper chromatography indicate that technetium reduced by stannous chloride labels albumin. This reduction is performed in absence of oxygen, as its presence and an acidic medium cause the formation of volatile $Tc_2O_7$ [3,1,5,8].

– Influence of reaction time:

Figure 2 shows that the reaction must be slow, about two hours, to obtain about 95% labeling yield and a stable labeled product.

Although the quantity of technetium as pertechnetate anion remains low, less than 5% for the present conditions (60 mg of HSA 0.24 mg of stannous chloride and pH 6.4), that of the reduced technetium is initially high, lowering before the first 90 minutes and reaching a minimum before two hours.

The labelled product remains stable for ten hours after labelling.

Even after 24 hours the labeling yield reaches 83%.

After this time the impurities are represented by reduced technetium (11%) and pertechnetate (about 6%).

– Influence of the albumin mass:

Figure 3 indicates that the labelling yield grows up with increasing albumin mass, and reaches a maximum at 60 mg.

The relation between reagent/reducing agent was always 250:1.

– Absorption spectroscopy:

The technetium is reduced by stannous chloride whenever it is added to lyophilized samples.

Absorption spectrum of the kit in water solution before its reaction, in presence of ammonium pertechnetate $^{99}Tc$ and sodium pertechnetate $^{99m}Tc$, pure albumin solved in water and pure ammonium pertechnetate $^{99}Tc$ are presented. The spectrum of pure albumin/stannous chloride are different (Figure 4).

– Stability of the lyophilized product during storage:

Stability until 45 days after preparation was examined.

The labelling yield was 90-95%, thus the same as that of the fresh ones.

– Standartization of the time of lyophilization:

Four hours is sufficient to obtain a lyophilized product of good quality, which dissolves easily by the addition of sodium pertechnetate in saline.

**b) MAA $^{99m}Tc$ Kit**

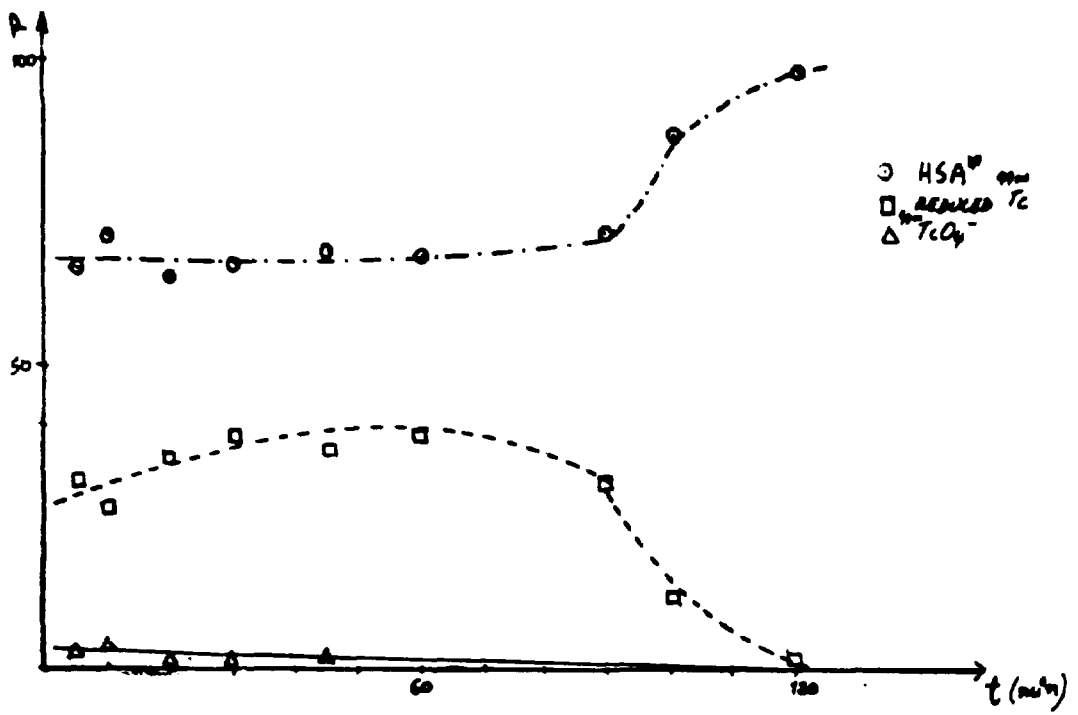


Figure 2 – Yield of Albumin Labelling as Function of Reaction Time

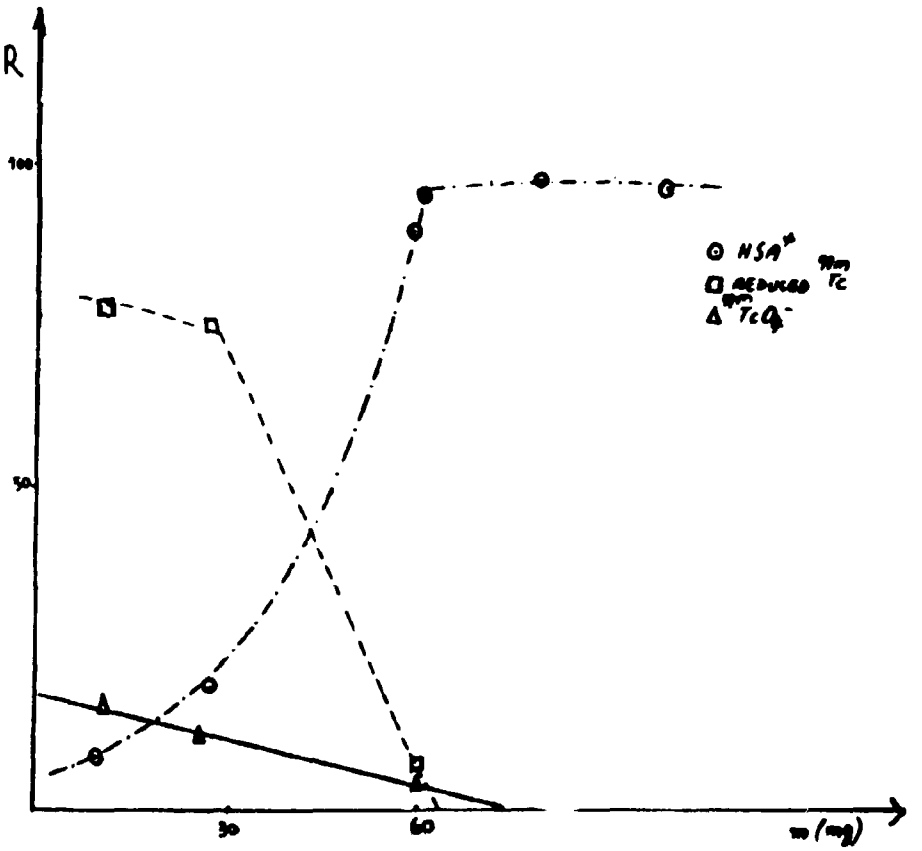


Figure 3 - Yield of Albumin Labelling as Function of Protein Mass

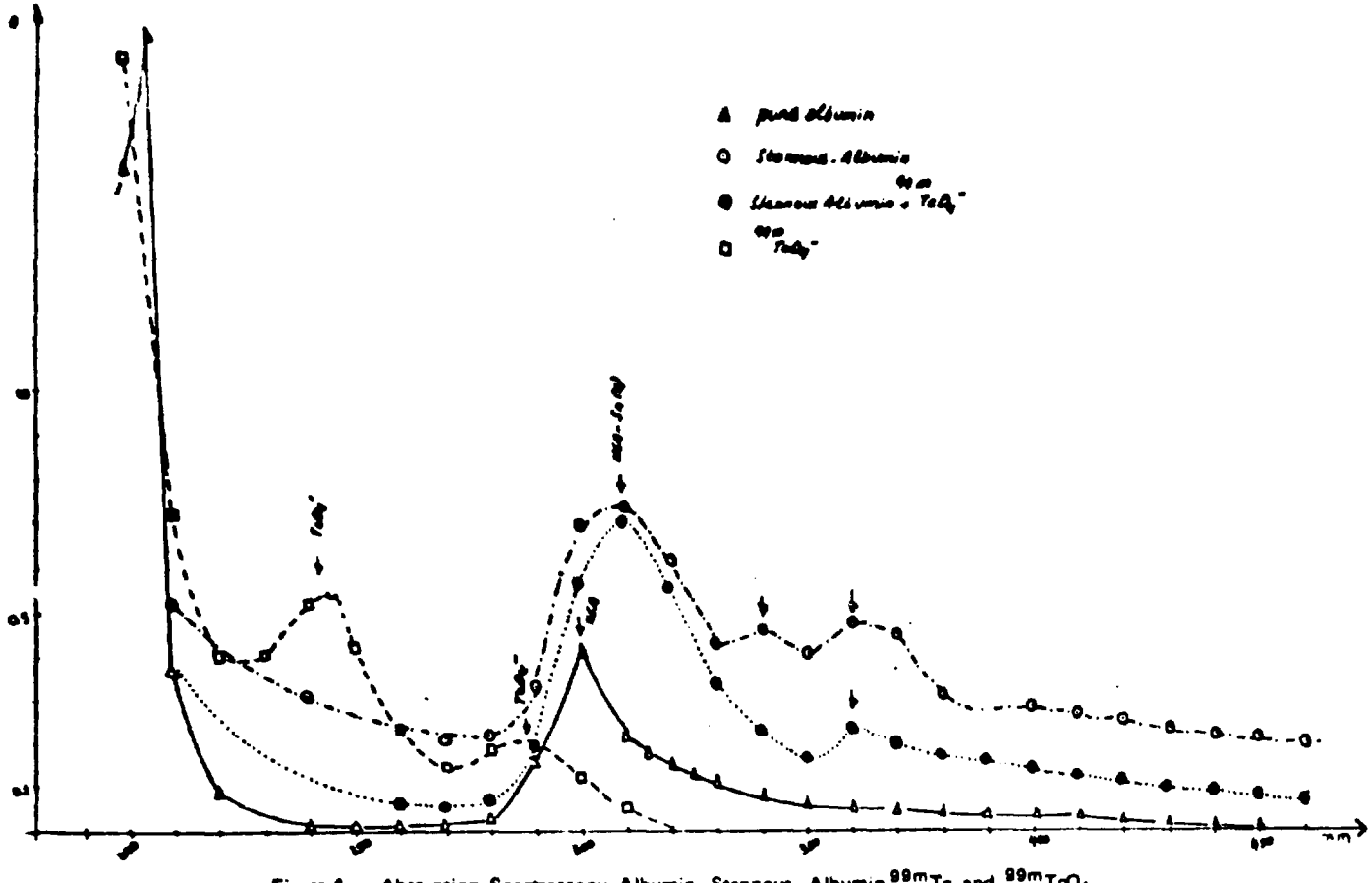

Figure 4 — Absorption Spectroscopy Albumin, Stannous, Albumin-$^{99m}$Tc and $^{99m}TcO_4$

- Influence of the MAA mass:

Experiments carried out with the lyophilized material (MAA/stannous chloride 250:1) indicate that whitin the range 30-60 mg of MAA the labelling yield is high.

Ascending paper chromatography (Whatman Nº I) using ketone as solvent was used to study the labelling yield (Figure 5).

- Influence of the time of reaction:

The time required for the reaction between MAA/stannous chloride and technetium 99m is very short, less than five minutes.

The labelled product is stable for at least 10 hours after its preparation (Figure 6).

- Biological control:

Biological distribution control, in a significant number of lyophilized flasks, indicates that at least 90-95 per cent of the injected dose is deposited in the lungs after the first 15 minutes post injection.

Laboratory rats with 300 g body weight and a dose of 20 $\mu$Ci were used in the experiments.

- Stability control during storage:

Repeated tests for free $^{99m}Tc$ in water suspension aggregates and observation through an optical (OM) and electronic microscope (EM) show the following results:

Table I

| Storage period | Labelling yield % | Observations |
|---|---|---|
| 1 day | 100 | Good dissolution<br>Particles 80–115 $\mu$ (OM)<br>(Photo 1) |
| 4 days | 100 | Idem |
| 1 month | 100 | Good dissolution.<br>Particles 25–110 $\mu$ (OM and EM)<br>(Photo 2) |
| 2 months | 80 | Bad dissolution.<br>Particles 20–0,5 $\mu$ (EM) |
| 6 months | 50 | Bad dissolution.<br>Most of the particles<br>0.4–0.7 $\mu$ and agglomerates (EM)<br>(Photo 3) |
| 7 months | 41 | Bad dissolution.<br>Decomposition of aggregates.<br>Particles 0.3–0.5 $\mu$ |

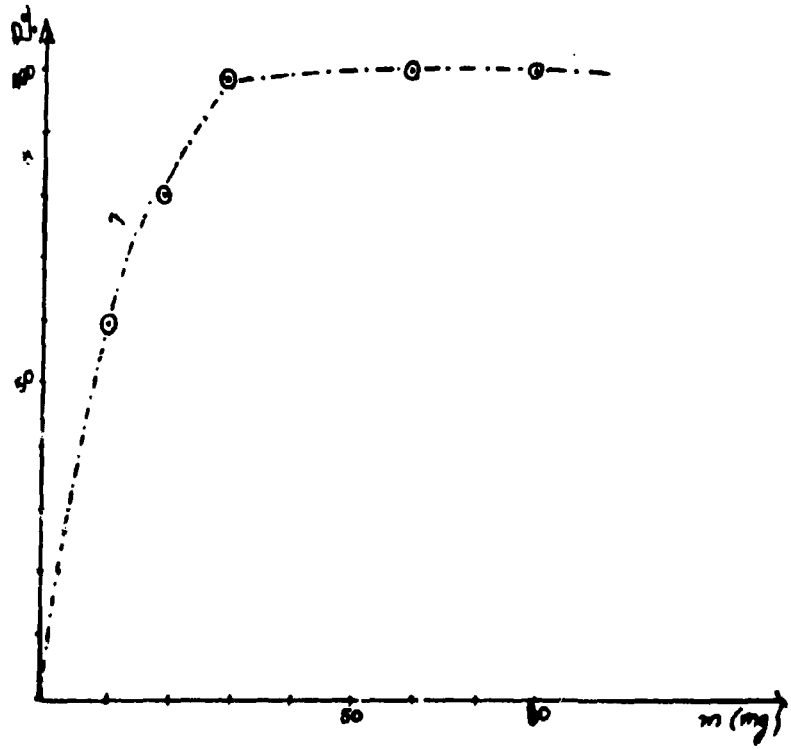


Figure 5 – Yield of MAA Labelling as Function of Protein Mass

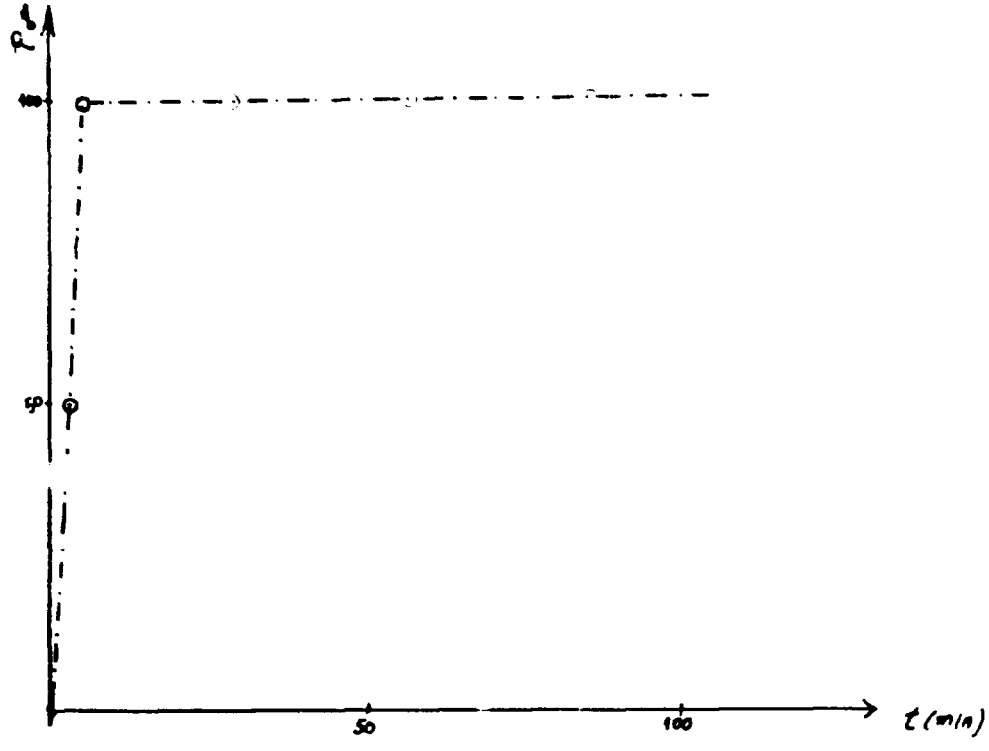


**Figure 6** – Yield of MAA Labelling as Function of Reaction Time

c) MiAA $^{99m}$Tc Kit

– Influence of the MiAA mass:

Keeping the relation MiAA/stannous chloride on 250:1 it was observed that the maximum labelling yield is obtained with 30 mg MiAA (Figure 7).

– Influence of the reaction time:

The labeling of MiAA is reached by adding sodium pertechnetate to the lyophilized kit and is completed in a few seconds.

Results from paper ascending chromatography using ketone or saline as solvent indicate the absence of free pertechnetate and albumin, giving 100 per cent labelled colloid.

– Biological control:

The biological distribution control was performed with a significant number of flasks, using laboratory rats, 300 g body weight (0.025 mg MiAA/Kg, 0.025 μCi).

The flasks are sent to the users whenever 85-90 per cent of the aggregate deposites on the liver after 6 min following the intravenous injection.

– Stability under storage:

Significative samples of each batch were diluted in pure distilled water and then examined by electronmicroscopy, giving the following results:

**Table II**

| Storage period | Labelling yield % | Observations |
|---|---|---|
| up to 1 month | 100 | Good dissolution. Particles 0.8–2.0 μ (EM), definite form. (Photo 4) |
| 2 months | 70 | Good dissolution. Particles 0.5–1.0 μ (EM) |
| 3 months | 60 | Good dissolution. Particles 0.1–0.4 μ (EM) |
| 6 months | 50 | Bad dissolution. Particles 0.1–0.4 μ (EM), without definite form. (Photo 5) |

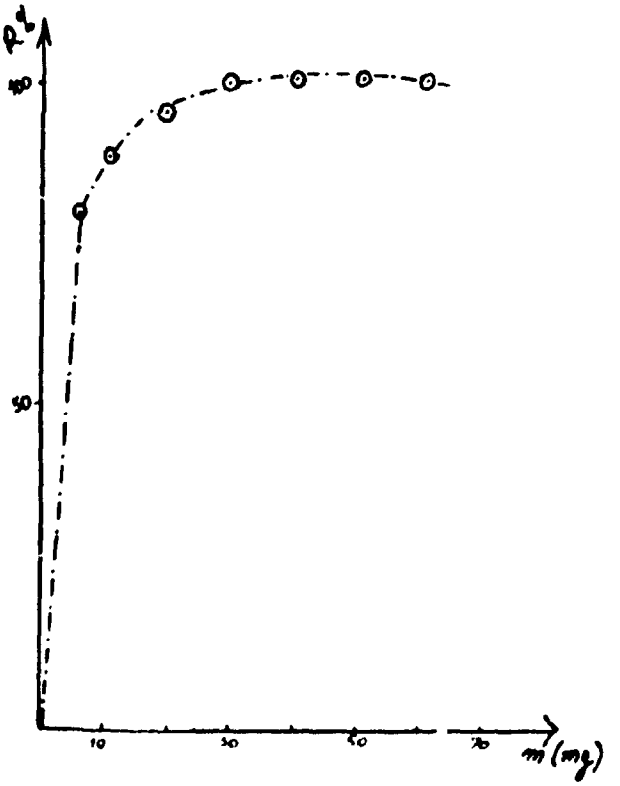


Figure 7 – Yield of MiAA Labelling as Function of MiAA Mass

d) Clinical Assays

HSA $^{99m}$Tc kit was used for placentography and for cardiac blood pool studies. Ten patients were studied by placentography. The scans (frontal and lateral views) were initiated 10 min. later and performed with a $\gamma$ camera with whole body scanning system. Placenta could be precisely located in all cases (Figure 8). Three patients were studied by cardiac blood pool. The dose used was 10 mCi and was injected by Oldendorf's Technique.

Sequencial images (3/3s) showed the passage of the radiopharmaceutical through the heart and lungs. (Figure 9).

Static images could be taken 30 min. to 1 hr. after the injection (Figure 10).

MAA $^{99m}$Tc was used for lung scintigraphy.

Five patients were studied.

The dose used was 4 mCi for adults and the scanning was performed with a $\gamma$ camera.

The distribution of the radioactivity through normal tissues was homogeneous and the quality of the images was good (Figure 11).

## 4 – DISCUSSIONS

Stannous chloride has proved to be effective as a reducing agent for pertechnetate anion and this one, once reduced, is used in many radiopharmaceutical preparations.

To avoid the decomposition and the great instability of the reduced agent is was necessary to stabilize it, for the routine preparation of the kits , specially HSA and its aggregates MAA and MiAA. This scope was reached by lyophilization of the protein together with the reducing agent, in nitrogen atmosphere.

As the radiopharmaceutical is administered in human being, intravenous injection, sterile and pyrogen free equipment, techniques and solutions must be employed as described in "Farmacopéia Brasileira"[7]. The labelled product obtained must obey the international security laws[6].

Considering the HSA $^{99m}$Tc kit, it is necessary that the pH of the lyophilized product be within 6.0-6.5, in order to achieve a high labelling yield. At lower pH colloidal albumin is formed and it reaches the liver and spleen after administration. Hence, the scans of placenta and cardiac blood pool will be poor.

Above pH 6.5 the percentage of free technetium is higher and the product concentrates in the thyroid following intravenous injection.

To maintain the pH during the labelling process, the volume of sodium pertechnetate solution used must be less than two milliliters.

The absorption spectrum of pure albumin reaches a maximum at 300 nm while albumin/stannous chloride presents three maximum (310-340-360 nm). There seems to be a bond between stannous cation and albumin. The HSA/stannous chloride labelled with technetium 99m presents two maximum at 310 and 360 nm. This indicates that the reaction must occur between the stannous albumin and technetium.

Figure 8 – Placenta Localization: – a. frontal view – b. lateral view

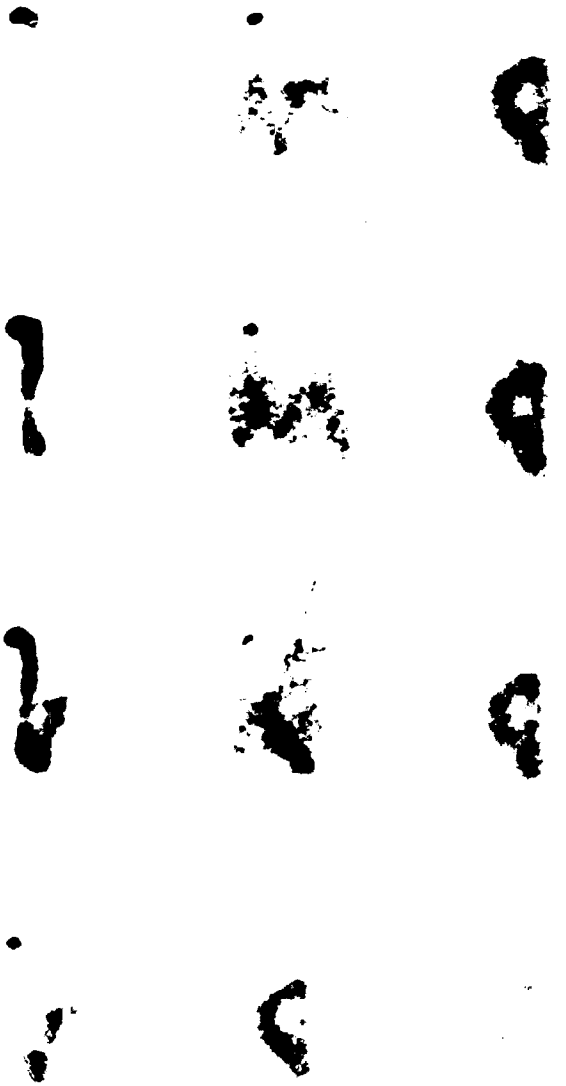

Figure 9 – Sequential Images Through the Heart and Lungs

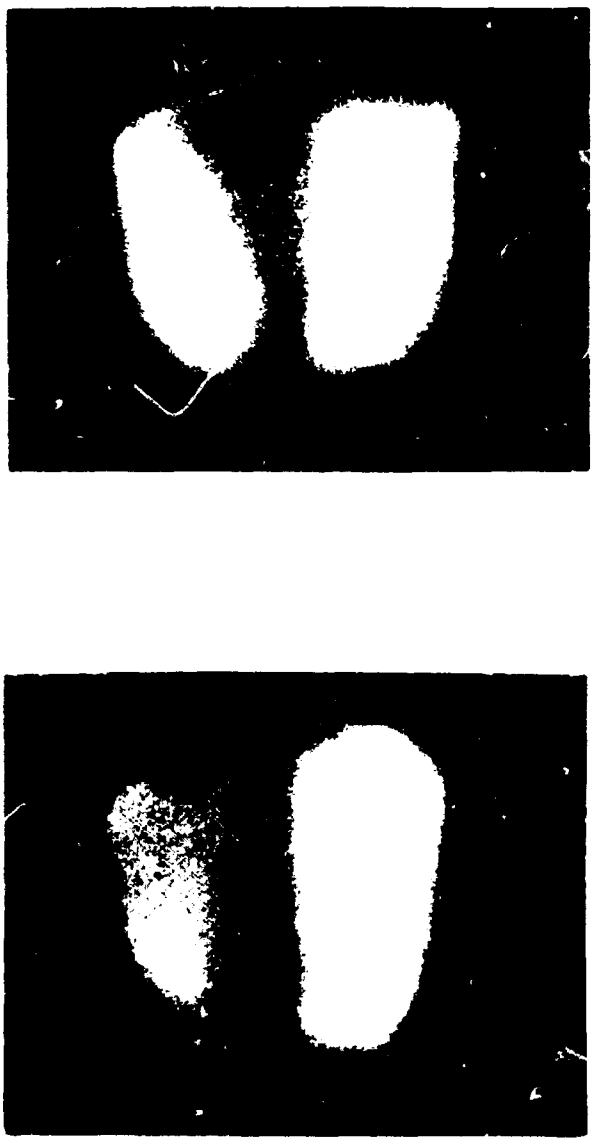

**Figure 10** – Pulmonary Scintigraphy. – a. Anterior – b. Posterior

Figure 11 -- Pulmonary Scintigraphy of Normal Fissure

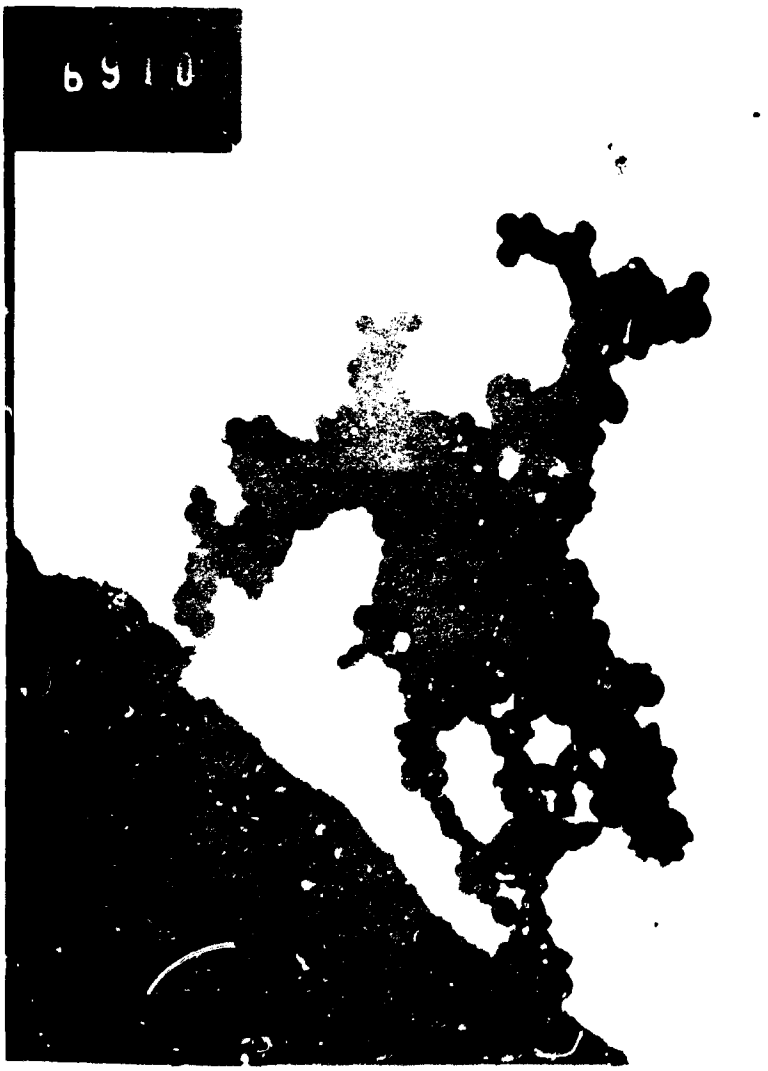


Photo 1 – Electron Micrograph of MAA–$^{99m}$Tc One Day After the Preparation (1800 x)

Photo 2 -- Electron Micrograph of MAA-$^{99m}$Tc 1 month After the Preparation (1800 x)

Photo 3 – Electron Micrograph of MAA–$^{99m}$Tc 6 months After the Preparation (28000 x)

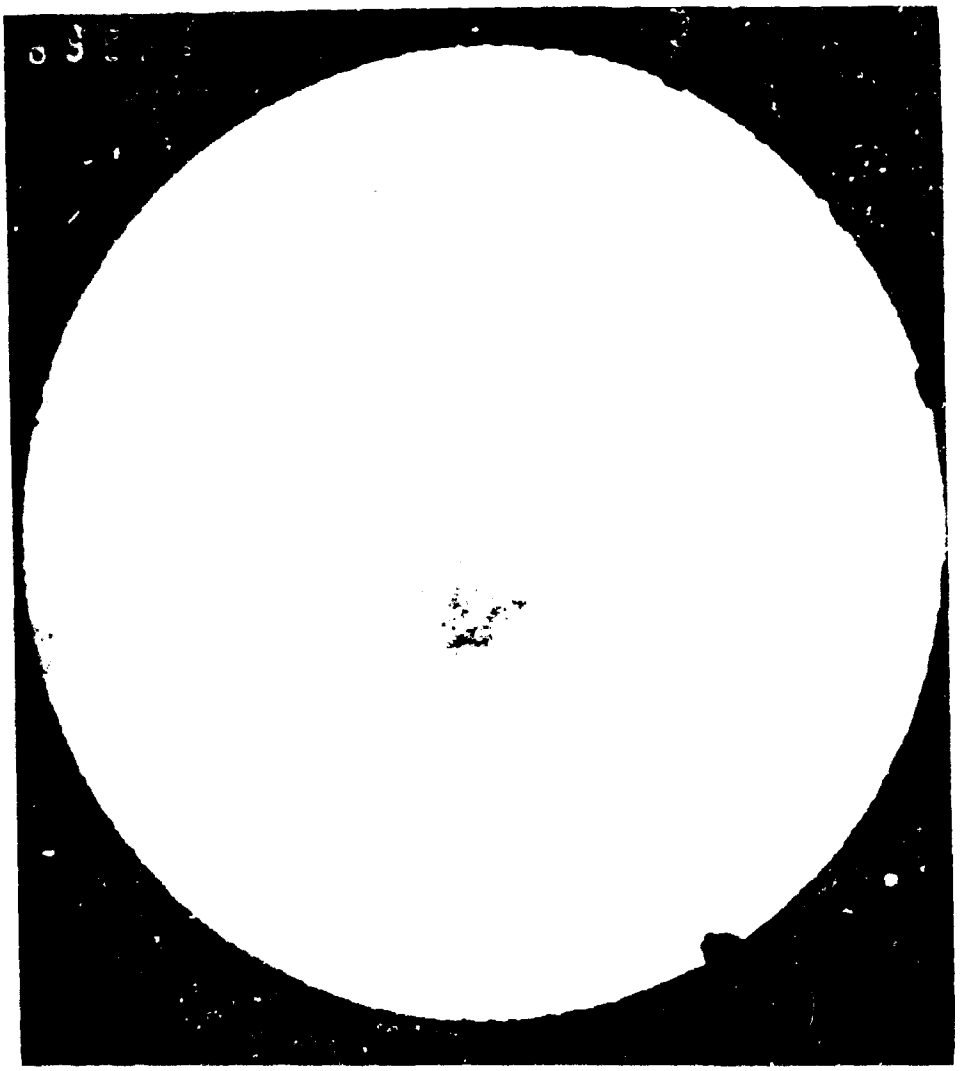

Photo 4 – Electron Micrograph of MIAA-$^{99m}$Tc, 1 month After the Preparation (28000 x)

Photo 6 – Electron Micrograph of MIAA-$^{99m}$Tc, 6 months After the Preparation (28000 x)

The analysis of the spectrum of labelled albumin shows that the typical peaks of the pertechnetate anion (240-290 nm) disappear and this indicates a total reduction.

The same proportion of reagent and reducing agent (250:1) was used in the preparation of HSA, MAA and MiAA Kits .

Considering HSA, it seems to occur an initial reduction of the pertechnetate anion (Figure 2) which labels the protein after a certain percentage is reached.

The protein, once labeled, remains stable for a long time, about 22 hours, which indicates a stable bond.

For derivatives, MAA and MiAA, the time required for the reaction is extremely short, of about minutes.

It seems that the thermic treatment during the aggregation destroys the sites of complexation between the albumin and tin.

## 5 — CONCLUSIONS

The lyophilized kits reached a high labelling yield technetium 99m.

The HSA $^{99m}Tc$ kit has a stability under storage over 45 days.

MAA $^{99m}Tc$ kit and MiAA $^{99m}Tc$ kits must be used within a month after their preparation.

The particle size of the MAA permits the lung scanning and the MiAA the liver scanning.

For the preparation of the kit an adequate technique to obtain a high degree of radiochemical labelling (nitrogen atmosphere) and all the cares necessary for injected products are employed.

Representative samples of each batch are submited to radiochemical control and sterily tests in order to obtain products with the best quality.

## ACKNOWLEDGEMENT

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## RESUMO

Estuda-se neste trabalho os resultados experimentais da preparação e conservação do soro albumina humana e de seus derivados macro (MAA) e micro (MiAA) agregados. Os compostos liofilizados são marcados com tecnécio 99m.

Analisam-se os compostos marcados pelas técnicas de cromatografia em papel e espectroscopia de absorção.

Examinam-se os dados obtidos na microscopia óptica e eletrônica de MAA e MiAA, a distribuição biológica e os ensaios de toxidade.

Mostram-se os mapas obtidos na placentografia, a cintilografia dos pulmões e fígado e os estudos de "pool" cardíaco sangüíneo.

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