PRODUÇÃO DE CONJUNTOS DE REATIVOS DE MACROAGREGADO DE SORO ALBUMINA HUMANO (MAA) PARA MARCAÇÃO COM ^{99m}Tc DESTINADO À CINTILOGRAFIA PULMONAR*

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RESUMO

Dentre os diversos processos descritos na literatura para preparação de Macroagregado de Soro Albumina Humano, desenvolvemos um método rápido e confiável para a produção rotineira de Conjuntos de Reativos de MAA liofilizados, destinados à marcação com ^{99m}Tc e utilização na Medicina Nuclear em estudos de perfusão pulmonar. Cada frasco da preparação contém uma formulação liofilizada com os seguintes componentes: 1,0mg de Macroagregado de Soro Albumina Humano, 0,12 mg de $SnCl_2.2H_2O$, 5,0mg de Soro Albumina Humano e 0,01mg de Tween-80 (agente emulsificante). O produto injetável recomposto com solução salina, apresenta um processo de etapas simples e uniformidade quanto ao tamanho das partículas entre 10 e 80 microns. O tamanho das partículas e o efeito do tempo de armazenamento na estabilidade dos kits liofilizados também foram avaliados. O MAA marcado com ^{99m}Tc apresentou boa pureza radioquímica em ensaios cromatográficos em papel Whatman nº 3 e metanol 85% (98% de pureza radioquímica). Estudos de biodistribuição em ratos demonstraram alta captação pulmonar (85% / D), baixa captação hepática (2% /D), e a relação pulmão fígado foi de 98%. As características apresentadas pelo produto marcado tornaram viável seu uso em cintilografias pulmonares e seu emprego em medicina nuclear.

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PRODUCTION OF LYOPHILIZED MACROAGGREGATED ALBUMIN (MAA) KITS TO BE LABELLED WITH ^{99m}Tc FOR LUNG SCANNING *

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ABSTRACT

Among the various macroaggregation procedures described in the literature, we have developed a rapid and reliable method for the routine production of lyophilized Sn-Macroaggregated Albumin (Sn-MAA) kits to be labelled with ^{99m}Tc for lung perfusion scanning. The reaction vial contains a lyophilized suspension having the following components: 1,0mg of Aggregated Human Serum Albumin (MAA), 0,12mg of hydrated stannous chloride ($SnCl_2.2H_2O$), 5,0mg of Normal Human Serum Albumin (HSA) and 1% of Tween 80 as emulsifying agent. In this method the Sn-MAA kits were performed in a single step process, and a consistent suitable range of particle size (10-80 mu) was obtained. The size of the particles and the effect of storage time on the stability of the lyophilized Kits were also investigated. The radiochemical purity, higher than 98% in ^{99m}Tc Macroaggregated was determined by using ascending paper chromatography (Whatman nº 3 and 85% methanol): Biodistribution studies in rats have shown the high uptake in the lungs 85%/D with low liver uptake 2%/D and lung-liver ratios 98 $\pm$ 1%. Excelent human lung images for clinical practice were obtained with these kits, which are prepared at IPEN-CNEN/SP for Brazilian Physicians.

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1. INTRODUCTION

The traditional compound for the scintigraphic evaluation of pulmonary diseases have been ^{99m}Tc-macroaggregated albumin (^{99m}Tc-MAA), widely applied for routine lung scanning and pulmonary embolism (5,8).

Radioiodine macroaggregates albumin were earlier prepared by Taplin et al. (13), in 1964.

Harper et al.(7) used macroaggregated labelled with ^{99m}Tc as an excellent lung perfusion scanning agent. Since then, various macroaggregation procedure had been described in the literature (2, 3, 10, 12). Other short-lived radionuclides such as ^{113m}In (1) and ^{68}Ga (6) have been used for the same medical purpose. Numerous investigations were developed to simplifly the methods of preparation of MAA, to get a high labelling efficiency with ^{99m}Tc, stability with time, good range of particle size and higher lung uptake (2). Lowes and Gydseen (9) have evaluated a lyophilized MAA kit.

The basic preparation of the macroaggregated particles are performed by denaturation of human albumin through heat and precipitation process with pH adjustment. With optimum aggregation conditions: concentration of human serum albumin and $SnCl_2.2H_2O$ and stirring rate, the particles of macroaggregated are formed with suitable range of particles size.

In the present work, we developed an improvement procedure for the preparation of lyophilized macroaggregated albumin kits to be labelled with ^{99m}Tc.

2. MATERIAL

Materials: The reagents utilized were:

- Human Serum Albumin (HSA) was obtained from the CUTTER BIOLOGICAL, at a concentration of 25 %; 0,2N hydrochloric acid; 0,2N sodium hydroxide; physiological saline solution (0,9%); $SnCl_2.2H_2O$; CH_3COOH.
- The technetium-99m-generator was produced at the IPEN-CNEN/SP.

Equipaments: The main items of equipament were:

- A precision water bath with a suitable attachment for continuous gentle agitation and a constant temperature control system.
- Scintillation counter from ANSER - ABOTT
- Microscope optical - ZEISS STANDARD
- Lyophilizator from INTERFRIGO S.A.

3. EXPERIMENTAL AND RESULTS

Aggregation procedure

Into a sterile 250 vial containing a magnetic stirring bar were added the following solution after going through a millipore filtration (0,22µm):

- 20,0ml of solution containing 100mg/ml of sodium acetate, 0,4 ml of 25% HSA (CUTTER) and 75ml of water.
- 3,0ml of solution containing 4,0mg/ml of stannous chloride in 1N HCl and 0,8 of 1% Tween-80.

The mixture of these solutions were carried out under nitrogen atmosphere and the volume was completed to 100ml with water. The pH from 5,4 - 5,5 was adjusted with 0,2N HCl or 0,2N NaOH.

The aggregation of the particles was performed using a heated water bath at 60 ± 5°C, with a continuous stirring for 20 min. It was reheated at 73 ± 2°C for 10 min.

The suspension was then cool down below room temperature by immersion in cold water.

The supernatant was decanted and the aggregates were rinsed three times, with a 0,9% of physiological saline solution. After then, it was forced through a 25 G gauge hypodermic needle twice.

Finally, the particles of MAA were resuspended in an equivalent volume of sterile 0,9% saline solution containing 10mg/ml of Human Serum Albumim (HSA).

Aliquots of 1,0ml from this preparation were dispensed in vials of 10,0ml capacity and lyophilized for 24 hours.

Aseptic procedures during the preparation of this radiopharmaceutical were employed.

Kit formulation

Each kit contains a lyophilized suspension, sterile, pyrogen-free of 1,0mg of Albumim Aggregated (MAA), 10,0mg of Human Serum Albumim (HSA), 0,12mg os stannous chloride dihydrate and 1% Tween-80 as emulsifying agent, and for the reconstitution of the kit a single step process was performed by adding a sterile ^{99m}Tc-sodium pertechnetate.

Particle size determination

For particle size determination the kits were reconstitued by addition of 1,0ml of sterile sodium saline solution. An aliquot of the MAA suspension was placed on a

hemacytometer grid and examined under a light microscope at 250x magnification, and the particle size distribution in each interval was given as a percentage of the total number of particles observed (4) (Figure 1).

At least 90% of the macroaggregated particles size were between 30-80 microns. No particle with size greater than 150 microns was found.

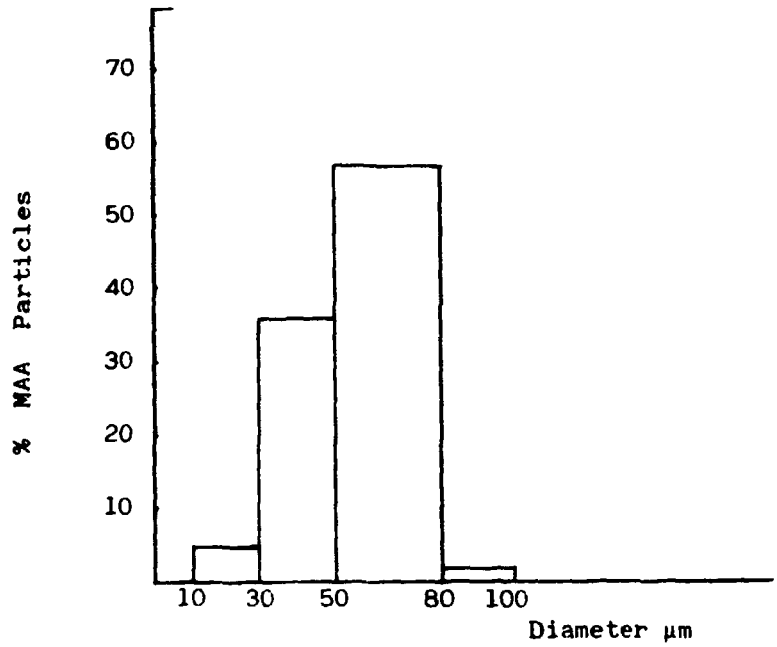

FIGURE 1 - Size distribution of MAA particles.

Radiochemical purity

The radiochemical purity of ^{99m}Tc - MAA was evaluated by paper chromatography using Whatman nº 3 in 85% methanol as solvent (11). In each vial of the lyophilized kit, the labelling was performed by adding 1-3ml of ^{99m}Tc - sodium pertechnetate, obtained from the generator IPEN-CNEN/SP.

Radiochemical analysis of ^{99m}Tc-MAA kit was carried out at interval of 15 to 360 min.. The results of the radiochemical yields are presented in Table 1.

The effect of storage time on the stability of the lyophilized kits was investigated during 10 months. The results are shown in Table 2.

Biodistribution Studies

The biodistribution studies of the ^{99m}Tc-MAA were made in male Wistar rats weighing 180-250g. After intravenous administration of the radiopharmaceutical , the animals were

sacrificed at intervals of 1 to 120 min., and the organs of interest were removed for counting in scintillation counter. The accumulated activity in each organ was calculated as a percentage of the total administrated dose (Table 3).

The clearance rate of the ^{99m}Tc-MAA preparation from the blood, after intravenous administration was also studied. One milliliter of blood sample, pos-injection was taken at different intervals and measured in a gamma counter. The radioactivity in the samples was evaluated as percent of the total administrated dose, assuming that the whole blood was 7% of the total body weight (Table 3 and Figure 2).

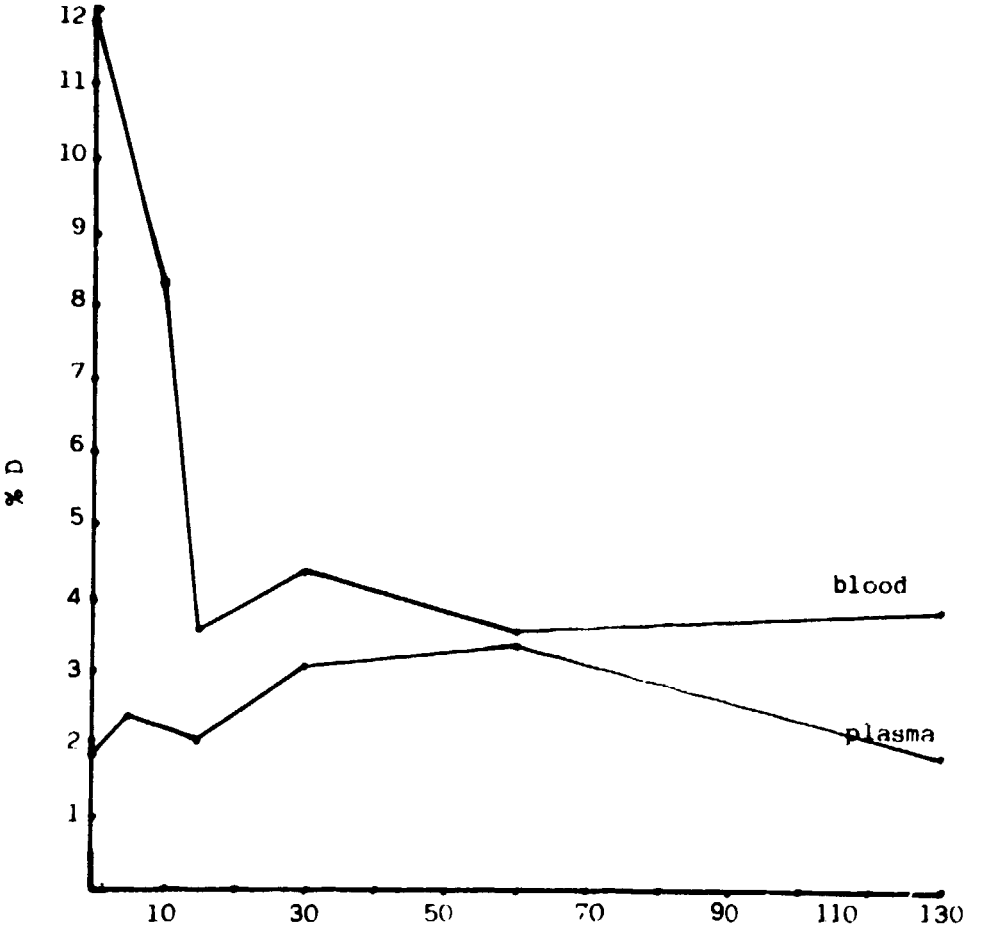

FIGURE 2 - Clearance of ^{99m}Tc-MAA from the blood and plasma of anesthetized rats. Each point is the mean $\pm$ SD from 5 different animals.

Time (min.) / Time (month)	15'	30'	60'	120'	180'	240'	300'	360'
0	99,63 ± 0,27	99,61 ± 0,15	99,67 ± 0,29	99,71 ± 0,18	99,49 ± 0,35	99,69 ± 0,09	99,47 ± 0,68	99,53 ± 0,38
1	99,62 ± 0,45	99,15 ± 0,82	99,65 ± 0,25	99,36 ± 0,68	99,34 ± 0,53	98,64 ± 1,62	99,48 ± 0,25	99,10 ± 1,02
2	99,75 ± 0,22	99,77 ± 0,13	99,58 ± 0,26	99,80 ± 0,07	99,52 ± 0,20	99,65 ± 0,40	99,65 ± 0,24	99,53 ± 0,30
3	99,68 ± 0,46	99,23 ± 0,61	99,59 ± 0,21	99,73 ± 0,41	99,83 ± 0,06	99,77 ± 0,25	99,32 ± 0,69	99,50 ± 0,82
4	99,85 ± 0,12	99,71 ± 0,26	99,36 ± 0,65	99,63 ± 0,53	99,92 ± 0,04	99,86 ± 0,12	99,83 ± 0,13	99,82 ± 0,18
5	99,50 ± 0,69	99,91 ± 0,04	99,17 ± 1,21	99,62 ± 0,46	99,54 ± 0,30	99,50 ± 0,17	99,63 ± 0,10	99,67 ± 0,27
6	98,35 ± 0,29	98,54 ± 1,33	99,23 ± 0,66	98,[illegible] ± 1,12	99,18 ± 0,68	98,63 ± 1,41	99,06 ± 1,35	99,09 ± 0,73
7	99,58 ± 0,13	99,16 ± 0,29	99,40 ± 0,53	99,22 ± 0,26	99,70 ± 0,17	99,48 ± 0,50	99,30 ± 0,36	99,44 ± 0,04
8	99,46 ± 0,24	99,64 ± 0,41	99,66 ± 0,23	99,56 ± 0,33	99,33 ± 0,23	99,70 ± 0,20	99,48 ± 0,26	99,55 ± 0,32
9	99,83 ± 0,11	99,68 ± 0,19	99,74 ± 0,12	99,45 ± 0,73	99,59 ± 0,20	99,30 ± 1,17	99,57 ± 0,36	99,85 ± 0,08
10	99,54 ± 0,53	99,33 ± 0,18	99,55 ± 0,33	99,49 ± 0,10	99,75 ± 0,21	99,66 ± 0,01	99,50 ± 0,26	99,72 ± 0,02

Table 1 - Radiochemical purity of ^{99m}Tc MAA during a period of time: minute after labelling and month after its preparation.

	Time (months)								
	0	1	3	4	5	6	8	9	10
LUNGS	96,76 ± 0,61	98,67 ± 0,17	98,18 ± 1,09	98,53 ± 1,10	98,04 ± 0,56	98,83 ± 1,11	96,60 ± 0,57	96,71 ± 0,91	96,77 ± 0,60
LIVER	1,37 ± 0,09	1,33 ± 0,17	1,82 ± 1,09	1,47 ± 1,10	1,96 ± 0,56	1,17 ± 1,11	3,40 ± 0,57	3,29 ± 0,91	3,23 ± 0,60

Table 2 - Biological distribution of ^{99m}Tc MAA during 10 months after its preparation, expressed as % of injected dose per organ (lungs and liver)

Time (min.)	% injected dose (mean of six animals)					
	LUNGS	LIVER	KIDNEY	SPLEEN	BLOOD	PLASMA
1	85,42 ± 3,26	1,21 ± 0,06	0,33 ± 0,02	0,08 ± 0,03	0,50 ± 0,05	0,12 ± 0,01
5	85,30 ± 2,28	1,53 ± 0,07	0,50 ± 0,11	0,09 ± 0,01	0,27 ± 0,02	0,14 ± 0,01
15	82,19 ± 2,56	1,60 ± 0,08	1,72 ± 1,72	0,10 ± 0,02	0,21 ± 0,03	0,13 ± 0,01
30	76,57 ± 2,91	1,98 ± 0,10	1,58 ± 0,21	0,09 ± 0,01	0,26 ± 0,03	0,16 ± 0,01
60	71,69 ± 0,86	1,78 ± 0,10	1,96 ± 0,35	0,11 ± 0,01	0,21 ± 0,04	0,26 ± 0,04
120	68,23 ± 2,30	3,80 ± 0,66	6,64 ± 0,28	0,32 ± 0,03	0,23 ± 0,06	0,11 ± 0,03

Table 3 - Percent of injected radioactivity present in various organs in rats at time of sacrifice.

4. DISCUSSION AND CONCLUSION

In this paper we have developed a rapid and reliable method for the routine production of lyophilized ^{99m}Tc-MAA kits for lung perfusion scanning.

A total of 30 batches of ^{99m}Tc-MAA kits have been successfully prepared by this method.

The data obtained show that the present formulation gives a good radiopharmaceutical quality. The 90% of the particles ranged between 30-80 microns. The addition of small amount of Tween -80, as an emulsifying agent in the ^{99m}Tc-MAA kit preparation was usefull to prevent the aggregation of MAA particles. Removal of the supernadant and resuspension of MAA in saline solution containing Normal Human Serum Albumin (HSA), as a stabilizer for MAA particles, was necessary to obtain uniformity in the particles size. Reconstitution of the lyophilized kit and labelling with ^{99m}Tc was performed in a single step by adding pertechnetate eluated from the ^{99m}Tc generator.

Radiochemical quality control of the ^{99m}Tc-MAA kits has demonstrated high labelling efficiency greater than 98%. In all preparations the amount of impurity did not vary significantly and the tagged particles with ^{99m}Tc remained stable, "in vitro", for at least 6 hours.

Our MAA kit has also shown a prolonged stability for about 10 months when stored at 4-8 ^{o}C.

A biodistribution in experimental animals was realized to estimate the particle size of the MAA kits, in order to obtain and adequate quality in the lung image (Table 3). We could estimate that the lung uptake occurred very quickly, reaching a stable count rate after few minute pos-injection. It was not observed any significant radioactivity in the in the whole body, except in the lungs.

Firstly the ^{99m}Tc-MAA kit was prepared using a low purity albumin and consequently a degradation of the particules occurred in few days which is observed by the presence of "hot spots" in the lung image. But when high purity albumin was used for preparation of the kits, high efficiency in the lungs image was obtained and no significant uptake by the liver was observed. So, it is very important to know the purity of the albumin.

Various nuclear medical institutions in Brazil are applying these kits for routine clinical practice obtaining excellent human lung images.

The new formulation of the ^{99m}Tc-MAA exibits a good storage stability after lyophilization process and high efficiency of labelling with ^{99m}Tc, demonstrating that the method applied is suitable for the routine production of ^{99m}Tc-MAA.

5. REFERENCES

1. ALLEN, D.R.; HARTNETT, D.E.; NELP, W.B.; HAMILTON, G.W. A rapid reliable method of labelling Sn-MAA with $^{113m}InCl_3$. J.Nucl.Med. 15 (9): 821-2, 1974.

2. AL-JANABI, M.A.A.; YOUSIF, Z.M.; KADIN, H.M. A new technique for the preparation of ready to use Macroaggregated Albumin (MAA) kits to be labelled with ^{99m}Tc for lung scanning. Int.J.Appl.Radiat.Isot. 34 (10): 1473, 1983.

3. AL-UANABI, M.A. & MOUSSA, S.O. A modified procedure for the preparation of macroaggregated albumin (MAA) kits to be labelled with ^{99m}Tc for lung scanning. J.Lab.Comp.Radiopharm. 23 (5): 519, 1990.

4. CALLAHAN, R.J.; SWANSON, D.P.; PETRY, N.A.; BEIGHTOL, R.W.; VAILLANCOURT, J.; DRAGOTAKES, S. A multi-institutional in vitro evaluation of commercial ^{99m}Tc- Macroaggregated Albumin Kits. J.Nucl.Med.Technol. 14 (4): 206, 1986

5. COLOMBETTI, I.G.; MOERLIEN, S.; PINSKY, S. Rapid and reliable preparation of Macroaggregated Albumin suitable for lung scintilography. Int.J.Nucl.Med.Biol. 2: 184, 1975

6. GREGORY, A.E. & MARK, A. Gallium-68-labelled Macroaggregated Human Serum Albumin, ^{68}Ga-MAA. Nucl.Med.Biol. 16 (3): 319-21, 1989.

7. HARPER, P.V.; LATHROP, K.A.; JIMINEZ, F.; FINK, R.; GOTTSCHALK, A. Technetum-99m as a scanning agent. Radiology 85: 101, 1965.

8. JOVANOVIC, V.; CVORIC, J.; JOVANOVIC, O.; TEOFOLOVSKI, C. Production of lyophilized Albumin Macroaggregates and labelling with ^{99m}Tc. Nucl.Med. 17: 66, 1978

9. LOWES, M. & GYDEEN, F.R. Clinical evaluation of s new ^{99m}Tc-MAA kit for lung scanning. J.Nucl.Med. 14: 474-5, 1973

10. LYSTER, D.M.; SCOTT, J.R.; MINCOY, E.K.; MORRISON, R.T. Preparation of a ^{99m}Tc-Sn-MAA kits for use in Nuclear Medicine. J.Nucl.Med. 15: 198, 1974.

11. McLEAN, J.R.; WELSH, W.J.; ROCKWELL, L.J. Quality Control Procedures for ^{99m}Tc-MAA. Int.J.Nucl.Med.Biol. 6, 142-3, 1979.

12. ROBBINS, P.J.; FORTMAN, D.L.; LEWIS, J.T. A kit for the rapid preparation of ^{99m}Tc-Macroaggregated Albumin. J.Nucl.Med. 13, (6): 463, 1972.

13. TAPLIN, G.V.; JOHNSON, D.E.; DORE, E.K.; KAPLAN, H.S. Suspensions of radioalbumin aggregates for photoscanning the liver, spleen, lung and other organs. J.Nucl.Med. 5: 259, 1964