A MODIFIED PROCEDURE FOR THE PREPARATION OF MACROAGGREGATED ALBUMIN (MAA) KITS TO BE LABELLED WITH ^{99m}Tc FOR LUNG SCANNING.

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SUMMARY

A method is reported for the preparation of lyophilized MAA kits for ^{99m}Tc labelling in a single step process. High radiochemical yield was obtained. Lung and liver uptake in mice were determined to be 97% and 0.2% respectively.

Key Words : MAA kits, ^{99m}Tc, Lung scanning

INTRODUCTION

^{99m}Tc-MAA has been applied to lung scanning by Harper et al [1]. Since that time authors have proposed various methods for the preparation of MAA to be labelled with ^{99m}Tc, have increased the reproducibility of MAA with respect to the particle size and obtained a narrower size distribution and applied it in nuclear medicine [2,3,4] . We have labelled denatured human serum albumin (as microspheres and aggregates) with ^{99m}Tc for lung scanning [5,6] . In this report we describe a modified procedure for the preparation of ^{99m}Tc-MAA .

EXPERIMENTAL AND RESULTS

The procedure of Wolfangel for the preparation of denaturated macro-protien with divalent tin for tagging with ^{99m}Tc was essentially followed [7] . Human serum albumin (2.5 ml containing 500 mg) was added to 45 ml of 1% aqueous benzyl alcohol and the solution was heated for aggregation at 60°, 70°, 80° and 90°C for 10,15 and 20 minutes with different rates of stirring. Magnetic stirrer and regulator hotplate were used together with a water bath and a circulator. The vial with the MAA prepared at 82°C for 15 minutes with rapid stirring was cooled to 18-22°C using cold water. Two ml of 0.085 N HCl were added slowly with rapid stirring.

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The pH of the suspension was 4.9 . The suspension was reheated to 82 °C for 5 minutes with slow stirring . It was then centrifuged and the supernatant was replaced with 30 ml of acetate buffer solution (pH = 4.8) , 0.1 ml of stannous chloride solution in 1N HCl containing 15 mg of $SnCl_2.2H_2O$ was added and the suspension incubated at room temperature for 24 hrs. The MAA were washed three times by centrifugation with resuspension in 30 ml of fresh acetate buffer following each washing . The MAA were finally resuspended in 50 ml of the acetate buffer containg 1% by weight benzyl alcohol . One ml of the suspension was reacted with ^{99m}Tc in saline to get a final volume of 5 ml. 0.1 ml was used for organ distribution in mice.

Because of the high liver uptake another centrifugation step was performed after the final resuspension in 50 ml of the acetate buffer. The centrifugation was performed for 5 minutes at a rate of 1000 rpm . The suspension was diluted 1:5 acetate buffer containing 1% benzyl alcohol. 90% of the particles were with diameters between 10-60 μm . The organ distribution was determined

The stability of MAA particles was studied as a function of time and because the shelf-life of MAA particles was not long another procedure was followed .

0.3 ml of 20% HSA was mixed with one ml of tin solution containing 5 mg of $SnCl_2.2H_2O$ and 1.1 ml of 0.9% Nacl. The solution was sonified for 10 minutes using sonic dismembrator (Fisher , Model 300) The solution was transferred to a 100 ml capacity vial (and the pH was raised with phosphate buffer to 5-5.1 . One ml of 5% Tween 80 was added and the aggregation step was performed using a water circulator magnetic stirrer , regulator hotplate and a magnetic bar whose length was 2.8cm . The aggregation was performed with rapid stirring for 10 minutes at 70 °C . The suspension was cooled to 18-22 °C with cold water and it was then reheated for 5 minutes at 80 °C with slow stirring.

The particle size of the product (product no.1) was measured using a haemocytometer and a light microscope. In another experiment half of the supernatant was replaced with phosphate buffer (pH=5) and the particle size determined (product no.2) .

The third product was prepared by removing half of the supernatant and replacing it with phosphate buffer .

One ml portions were put in vials and the kits were lyophilized. After resupension with saline the particle size was measured (product no.3) . The results are shown in table I

The organ distribution of all these products was determined and the results are presented in TableII. The procedure which gave the optimal size distribution was applied to prepare MAA suspensions and the radiochemical yield was determined using suspensions kept at about 6°C , frozen and lyophilized. The analysis was performed by adding 4 ml of ^{99m}Tc eluate containg 500 µCi to one ml of MAA using paper chromatography (Whatman No.3 and 85% Methanol). The results are presented in table III.

Sterility and pyrogen tests were carried out using the MAA lyophilized kits and aseptic techniques were followed during the preparation.

The radiochemical purity of ^{99m}TC-MAA was also measured using thin layer chromatography (precoated TLC plates with silica gel 60). The results were in agreement with those obtained with paper chromatography.

Table I. Size distribution (%) of MAA particles

Product no.	<10 µm	10-60 µm	60-80 µm	> 80 µm
Product No.1	0.0	86.0	2.48	0.0
Product No.2	0.0	92.74	7.23	0.0
product No.3	4.02	95.33	0.62	0.0

TableII Organ distribution of ^{99m}Tc-MAA in mice 10 minutes post intravenous injection

Product	% of injected dose			
	Lungs	Liver	Spleen	Kidneys
Product No,1	92.3±0.41	1.4±0.07	0.07±0.01	0.4 ± 0.01
Product No.2	95.1±0.45	0.8±0.17	0.04±0.01	0.3 ± 0.01
Product No.3	96.9±0.45	0.2±0.03	0.04±0.01	0.1 ± 0.02

Table III: Effect of storage time on the stability of the MAA kits to be labelled with ^{99m}Tc for lung scanning .

Time preparation (days)	Radiochemical purity (%)		
	Kept at 6 °C	Frozen	lyophilized
1	99.89	98.57	99.85
39	98.25	98.20	99.60
60	98.07	98.60	99.64
75	99.88	99.52	99.80
115	34.40	-	99.68
180	-	-	99.14

DISCUSSION

The properties of MAA kits depend on the aggregation step which is largely related to the amount of human serum albumin, the concentration of stannous chloride , adjustment of pH and temperature and the rate of stirring . Emulsifying agents are also useful to prevent the aggregation of MAA particles .

The procedures published in the literature for the preparation of ^{99m}Tc-MAA for lung scanning involve the reaction of ^{99m}Tc with Sn-MAA suspensions and not with a lyophilized Sn-MAA kit which is the most suitable form for convenient despatch. In some papers the stannous chloride was added after the aggregation step giving rise to a short shelf life of the product.

Our method for the preparation of Sn-MAA kits for labelling with ^{99m}Tc involves the following advantages:

1- Suitable size distribution.
2- High and consistent radiochemical yield (99%) .
3- High uptake in the lungs (97%) with low liver uptake (0.2%). This is advantageous to minimize the interference during the diagnostic procedure.
4- Long shelf life
5- Ease of preparation in sterile and pyrogen-free form.

In recent years one of the aims of radiopharmacy is to facilitate and improve diagnosis and to streamline procedures and to minimize radiation exposure at the medical institution. The procedure applied in this report leads to a single step MAA kits for the preparation of ^{99m}Tc-MAA with high medical specification.

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