LOCAL PREPARATION, STANDARDIZATION AND QUALITY CONTROL OF TECHNETIUM LABELLED MACROAGGREGATED ALBUMIN FOR LUNG PERFUSION STUDIES

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ABSTRACT

Lung perfusion study is an important investigation in various pulmonary diseases. The radiopharmaceutical commonly used now-a- days is imported macroaggregated human albumin (in kit form), which is labelled with technetium (Tc99m-MAA). Due to its high cost the technique could not be fully exploited. We have tried to locally prepare freeze dried MAA particles. Various parameters like concentration of protein, pH value, temperature, quality and quantity of reducing agents were studied to find out the optimum conditions for radiolabelling and the desired particle size. More than 98% of the added radioactivity was found tagged to the MM particles in the final preparation (confirmed by paper chromatography). Labelled agent was found to be radiochemically stable for upto 6 hours. Initial animal and later human studies showed an ideal spectrum of particle size. (JPMA 41:167, 1991).

INTRODUCTION

Pulmonary artery occlusions were first visualized using Au^{198} adsorbed on Sop m carbon particles in 1951[1] Ag^{111} and Hg^{203} labelled ceramic microspheres were also employed for the same purpose in 1962-63[2,3]. These agents were unsuitable due to infinite retention in the lungs and associated tissue destruction due to radiation hazards. [^{131}] MAA (macroaggregated albumin) particles developed as an alternate lost popularity due to the high radiation dose to thyroid. The search for a more appropriate lung scanning agent continued and in 1969 Tc^{99m}-microspheres[4] and Tc^{99m}-Iron hydroxide macroaggregates[5] were introduced. The development of Tc^{99m} MAA in 197 1-72[6,7] brought a revolution in lung perfusion scanning. The agent is still universally employed. Lung perfusion scintigraphy is based on trapping of particles in the capillary bed of lungs. Approximately 106 particles (labelled with 2-4 mCi of Tc99m) of denatured albumin per injection block a negligible portion of the total number of blood capillaries in the lungs (approximately 280 billion) and do not pose any clinical hazard. The particle size should not exceed 100pm, as this may block the arterioles and cause pulmonary embolism. Antigenic reactions may also occur with the administration of albumin. Therefore, no more than 1 mg of the albumin should be used per injection[8]. In a series of experiments Tow and coworkers[9] demonstrated that the size of denatured albumin may be satisfactorywith an extraction efficiency of 80% (newer formulations give extraction efficiency in the range of 90-95%), and that particles are uniformly mixed with the blood before they arrive at the lungs. The particles are broken down into smaller size by mechanical movement of the lungs and enzymatic action (proteolysis) and then released into circulation to be removed by the reticuloendothelial system. The whole body radiation dose from Tc99m-MAA is 15 mRad/mCi and lungs receive about 280 mRad/mCi. Lung perfusion scintigraphy is routinely done at all Nuclear Medicine Centres of the Pakistan Atomic Energy Commission, using imported MAA kit, which is quite expensive, i.e., approximately 60 US $ per kit (each of 5 vials, each vial being sufficient for 5 patients). This laboratory is trying to replace high cost radiopharmaceuticals with locally produced ones to save on foreign exchange. Recently we developed a local technique to prepare Tc99m-MAA kit, which can be transported with good stability. The calculated cost for the local preparation is not more than 2 US $ per kit (total cost includes cost of HSA, chemicals, staffing and all related expenditures). The technique was evaluated in terms of particle size, concentration of albumin, radiochemical binding, radiochemical stability, safety and localization in target area.

MATERIALS AND METHODS

1. Preparation of the Aggregates

All procedures being carried out under aseptic conditions 0.2 ml (50 mg) of injectable 25% solution of HSA (Cutter Biological-USP) was diluted to 5 ml using sterile isotonic normal saline. The resultant solution contained 10 mg HSA per ml. The serial dilutions prepared from this mixture (using saline as diluent) were 10,5,3.,5,2.,5,1.,2, 0.6, 0.3, 0.16, 0.08, 0.04, and 0.02 mg HSA/ml respectively. pH of these mixtures was adjusted to 5.5 (checked with narrow range pH paper), which is the isoelectric point of albumin[8]. Each dilution was heated for 20 minutes at 100 °C with constant shaking. The heated mixtures were allowed to cool and checked for particle size using a light microscope and heamocytometer (Neubauer Chamber). It was observed that concentrations above 2.5 gave clots of size much bigger than the required range (10-100u m) and were rejected (Figure 1).

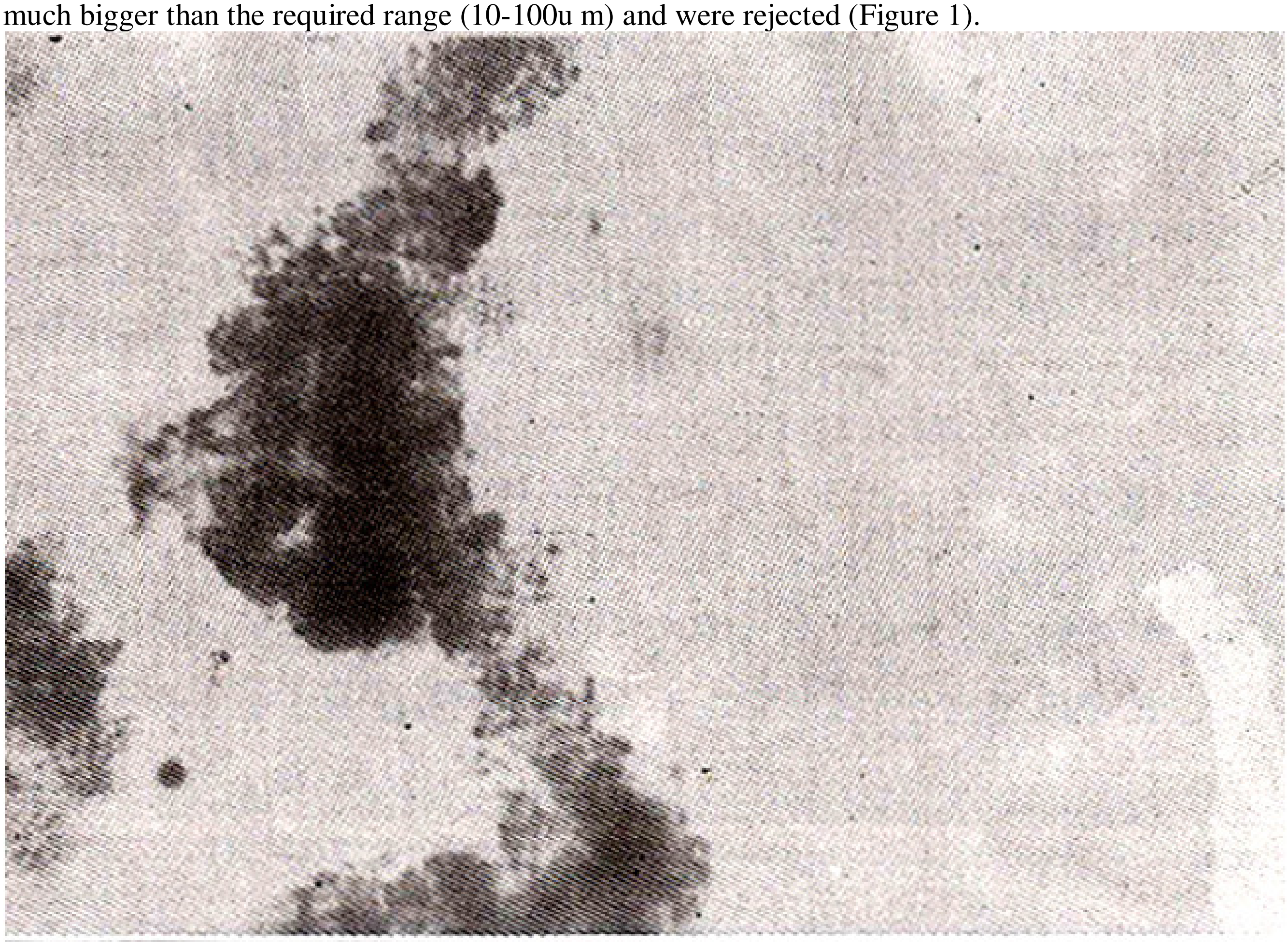

Figure 1.Microphotograph of clots obtained at Concentrations greater than 2.5 mg HSA/ml. (Note: Distance between two vertical lines represents 50 μm).

The concentrations from 2.5 to 0.6 mg/nil yielded particles of the required range (Figure 2).

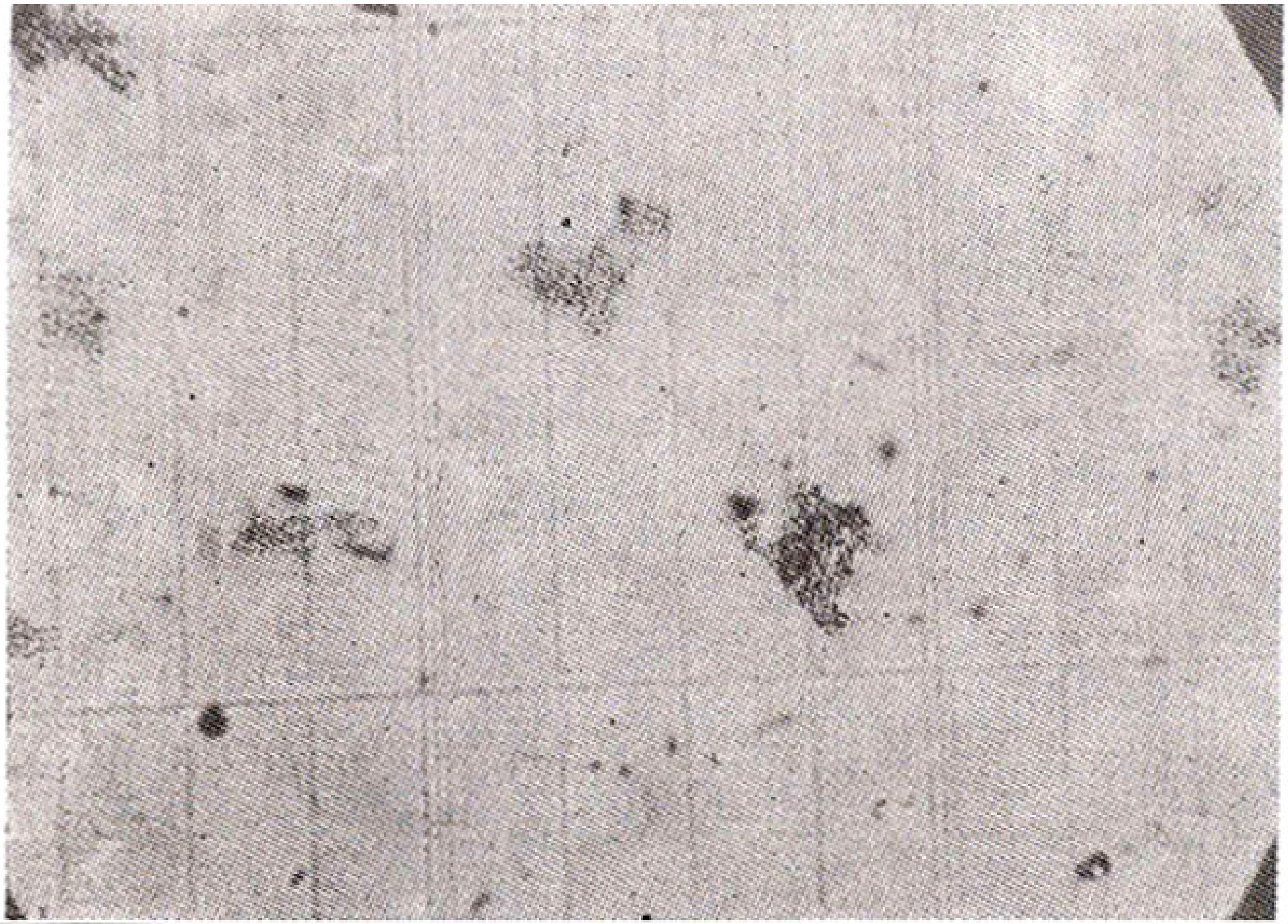

Figure 2. Microphotograph of particles obtained at Optimum Concentration: Concentration Range: 2.5-0.6 mg HSA/ml. Average: 1.43 mg HSA/ml. particle size range: 10-80μm.

Particle size was less than 10 u m in concentrations less than 0.6 mg HSA/ml (Figure 3)

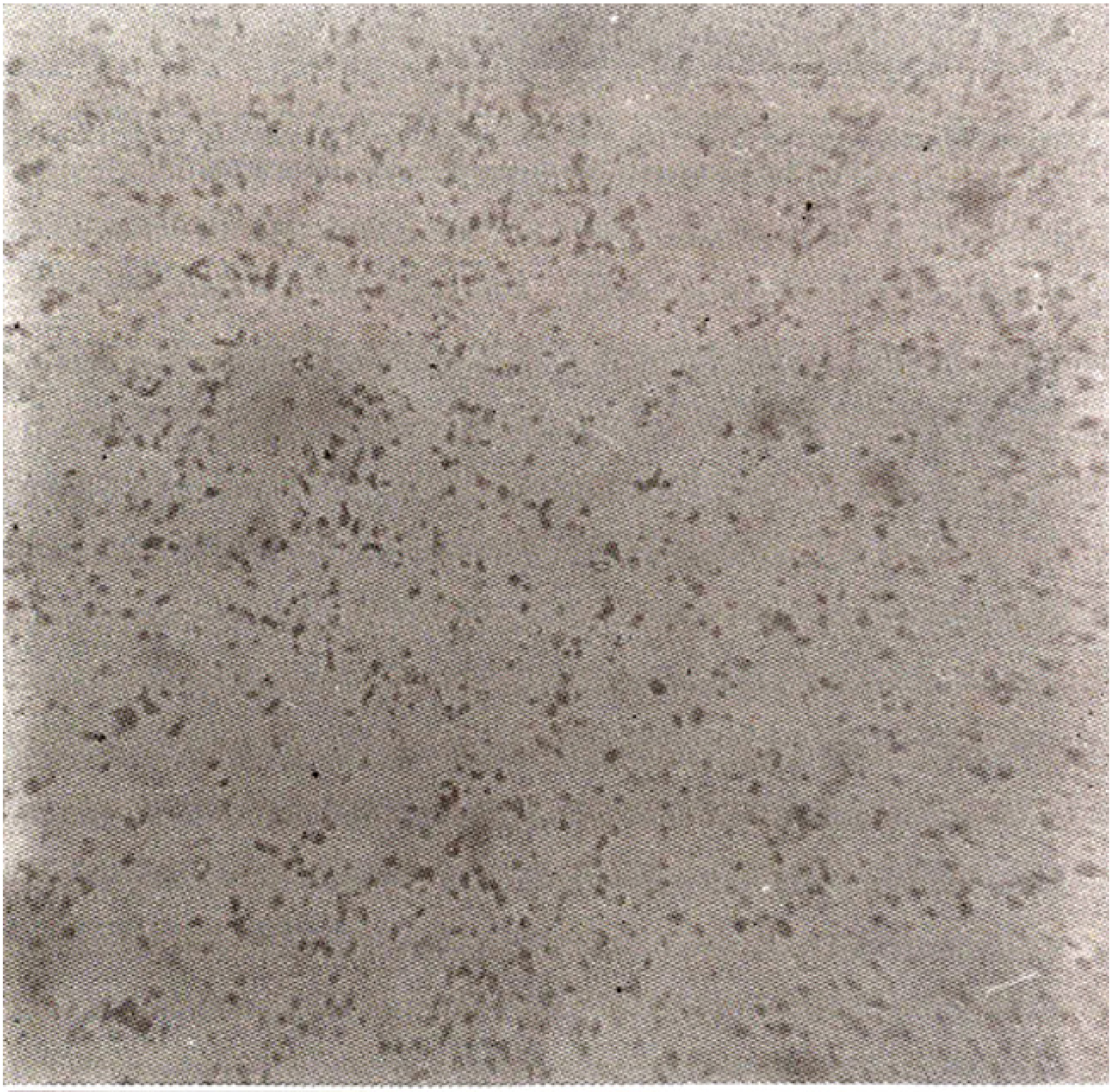

Figure 3. Microphotograph of percales at Concentrations less than 0.6 mg HSA/ml. (The particle size is less than 10 μm in these preparations).

andwere also rejected. An average concentration 1.43 mg HSA/ml was selected as the optimum concentration to get the desired particle range. Finally 10ml of 1.43 mg HSA/ml was prepared and processed for preparation of particles by the procedure described above and mixed with 2ml of a solution containing 6mg SnC12, 2H20 per milliliter in saline. The mixture thus obtained, contained approximately 1.2mg/nil HSA (aggregated) and 0.3mg/mi Sn+ +ion. This final preparation was divided into 2 mlaiiquotes in sterile vials, lyophilyzed and stored at -20°C until use. The agent for lung studies was prepared by adding 20-40 mCI of Tc99m-Tc04 in 2-4 ml saline into the vial. Paper chromatography was performed at 0,1,2,4 and 6 hours to check the radiochemical purity and stability.

2. Temperature effect on particle size

Effect of varying temperature on particle size was studied by heating 2 ml aliquotes of HSA solutions (concentration 1.2 mg/ml) at temperatures varying from 60°C to 100 °C. It was observed that the required particle size range can be obtained at temperatures from 85to 100 °C. 100°C was selected as the most suitable temperature, because the mixtures could simply be heated in boiling water bath without any temperature check.

3. pH and particle size

Effect of changing pH on particle size was studied by varying pH of the mixture of above composition with subsequent heating. It was observed that particle size varies with pH. At pH values greater than 7.5, cohesion among the particles is high and particles of much larger size were obtained (similar to those shown in Figure 1), whereas at lower pH values aggregates were of much smaller size (similar to those shown in Figure 3). The optimum particle size was observed at pH values between 4 to 7. pH 5.5 is the average.

4. pH and Radiolabelling

Effect of changing pH on radiolabelling was studied by adding 40 mCl's of Tc99m-Tc04 to 2 ml aliquotes of the final preparation with pre adjusted pH levels ranging from 3 to 10. Maximum binding was observed at pH levels 5-7 (Figure 4).

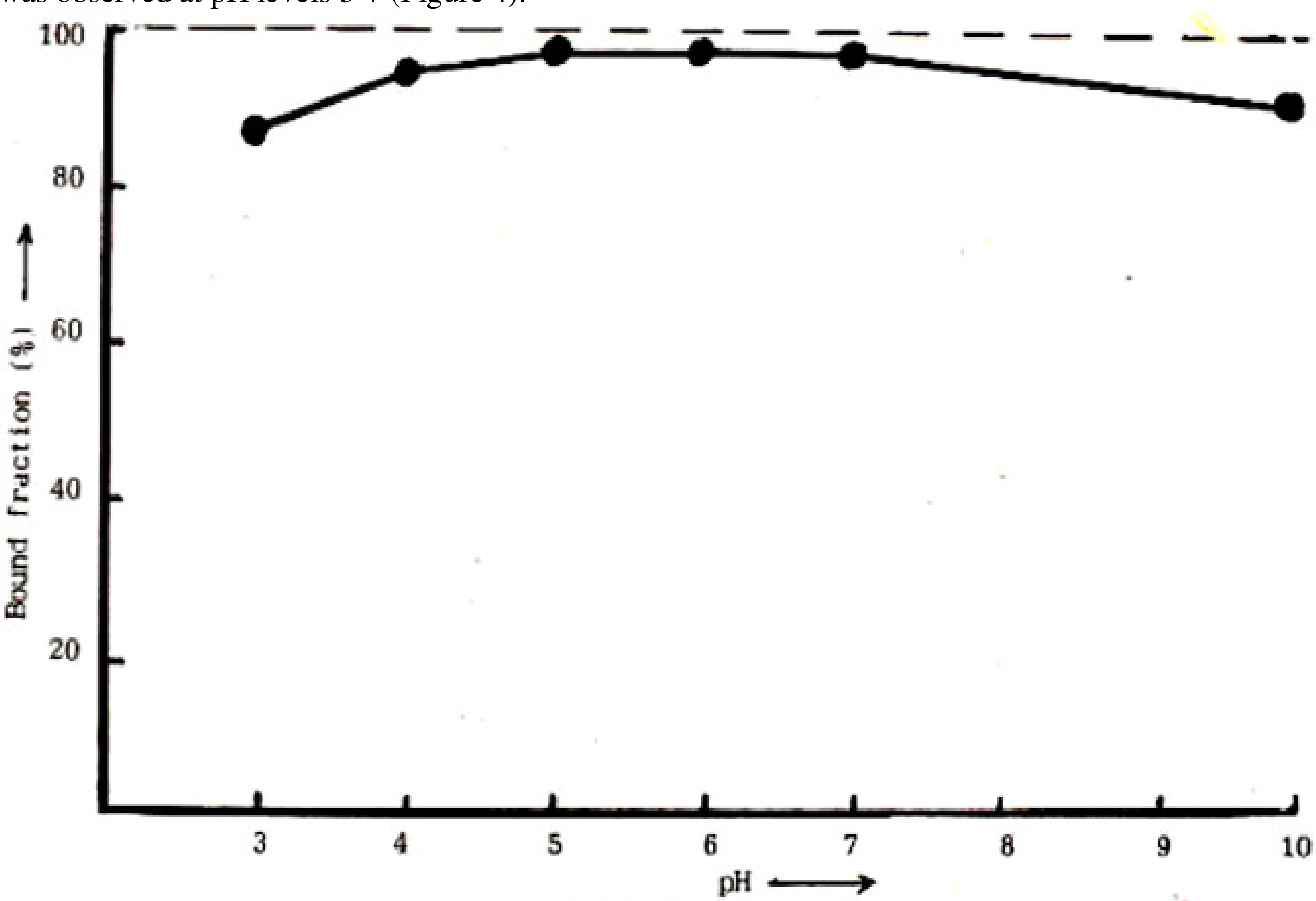

Figure 4. Effect of pH on binding of radioactivity to HSA.

RESULTS

The ranges of particle size atvarious concentrations are shown in Table.

TABLE. Variation of particle size with concentration of HSA

S. No	Concentration mg HSA/ml	Particle Size (µm)
1.	10	Clots
2.	5	Clots
3.	3.5	Clots
4.	2.5	10--80
5.	2.0	10--80
6.	1.2	10--80
7.	0.6	10--80
8.	0.3	> 10
9.	0.08	> 10

The acceptable particle size range, 10-100 u m, was observed at concentrations from 2.5 to 0.6mg HSA/ml (see also Figure 2). Data on radiochemical purity and stability obtained by paper chromatography is shown in Figures 5 and 6 respectively.

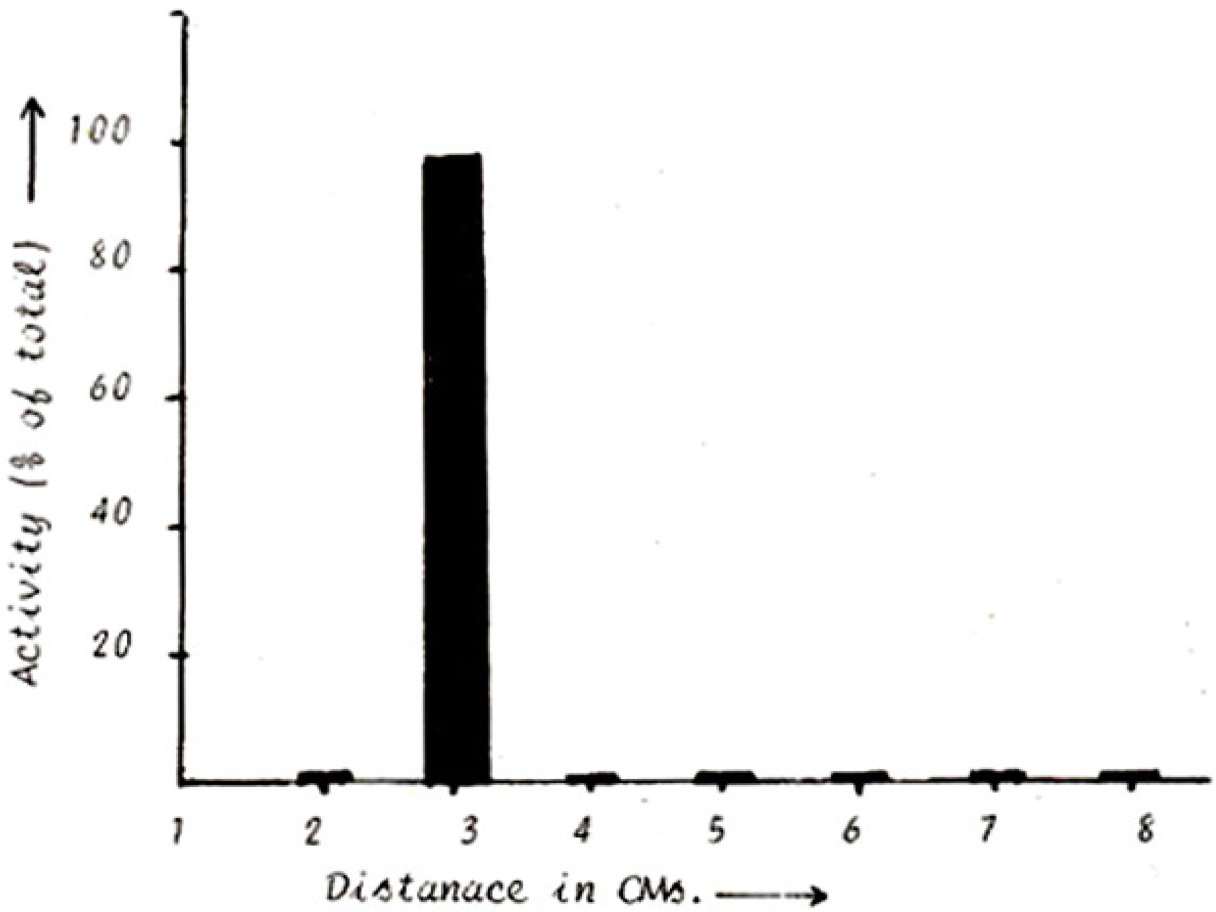

Figure 5. Radi chromatogram of Tc-99m MAA, showing more than 98% of added radioactivity tagged to Macroaggregates.

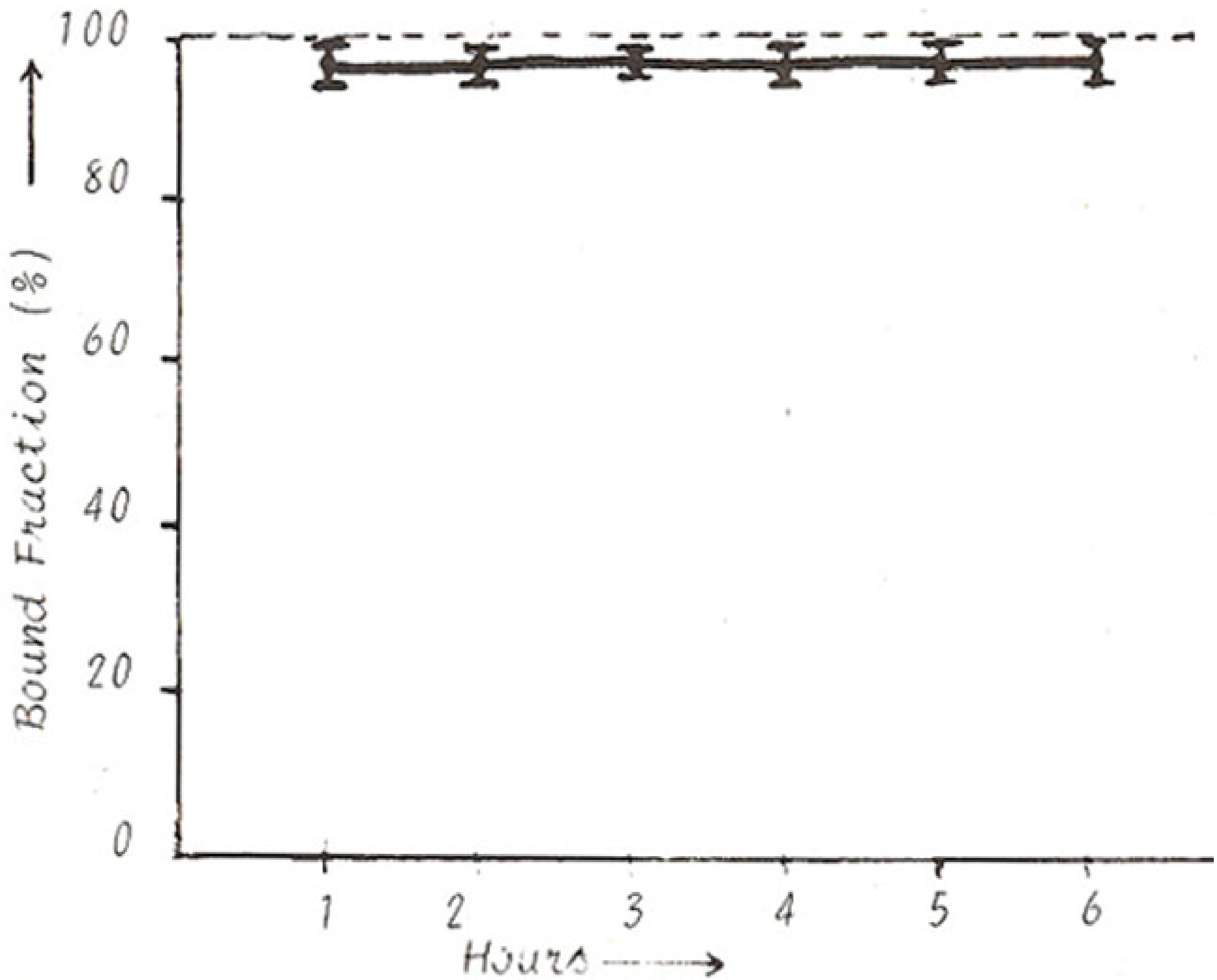

Figure 6. Stability of Tc-99m MAA. Bound Fraction Versus Time.
(Averaged over 5 preparations).

The radiochemical yield measured immediately after labelling is greater than 98% and is stable over 6 hours. The lung scanning performed in rabbit (Figure 7)

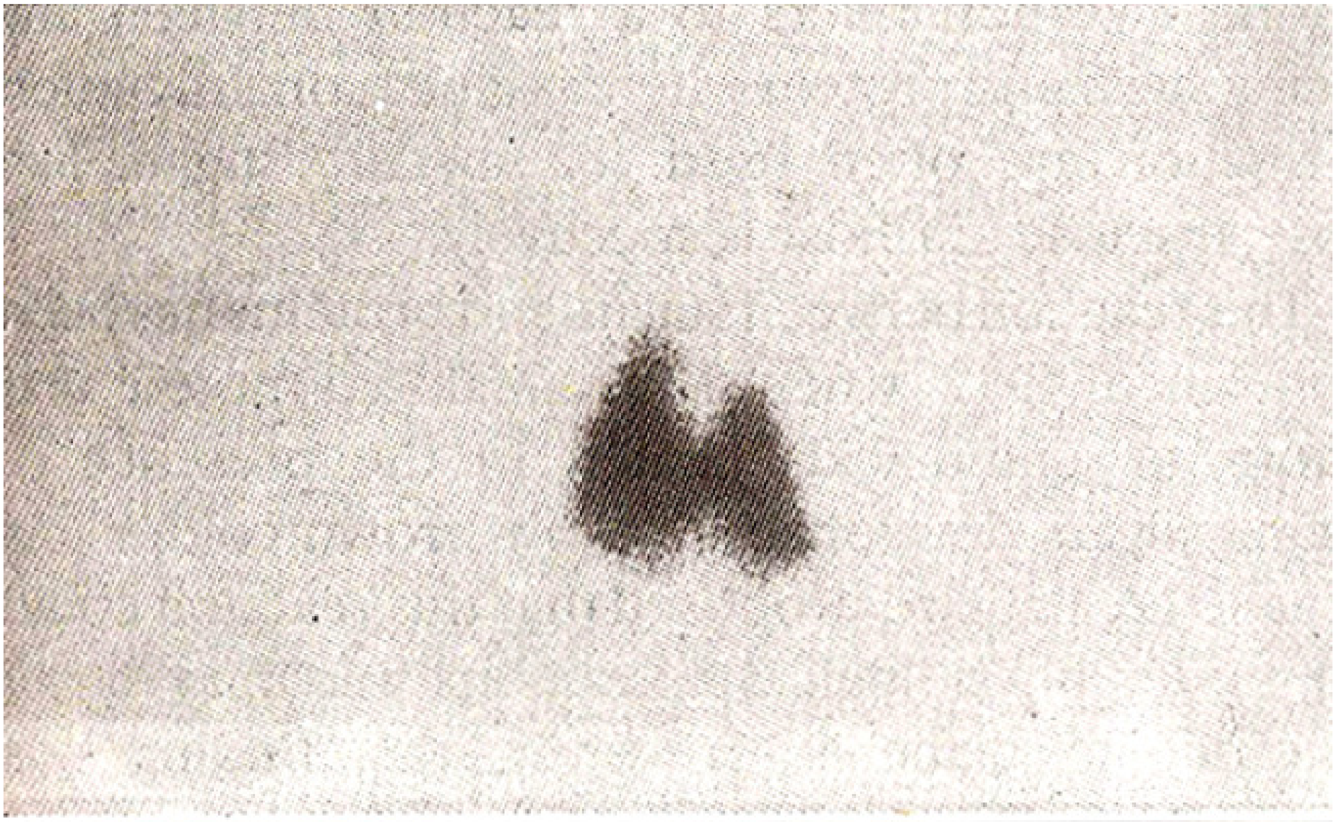

Figure 7. Lung scan of rabbit, using locally prepared ^{99m}Tc-MMA showing satisfactory perfusion of agent into lung. Anterior view.

shows satisfactory perfusion of agent in lungs. No pyrogenic reaction was observed in rabbit when injected with 10mg albumin preparation. Normal lung scan of one volunteer alongwith scans of patients with obstructive lung disease is shown in Figure 8.

Figure 8. Lung scans of human subjects, using locally prepared ^{99m}Tc-MAA. Anterior views. (a). Scan shows perfusion in both lungs and visualized functioning normally.

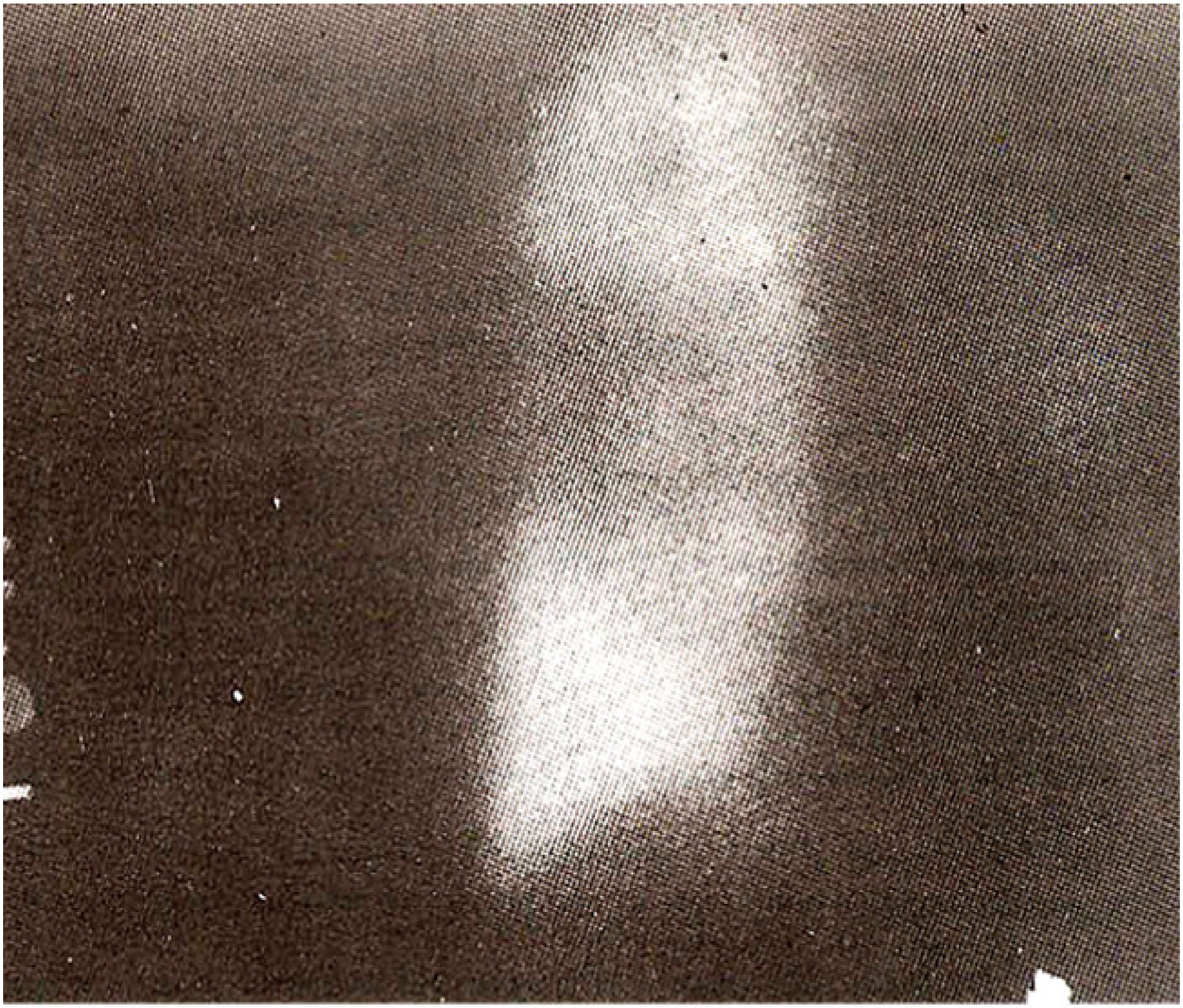

Figure 8 (b). No perfusion in left lung (non functioning), while perfusion defects in left lung suggest pulmonary emboli.

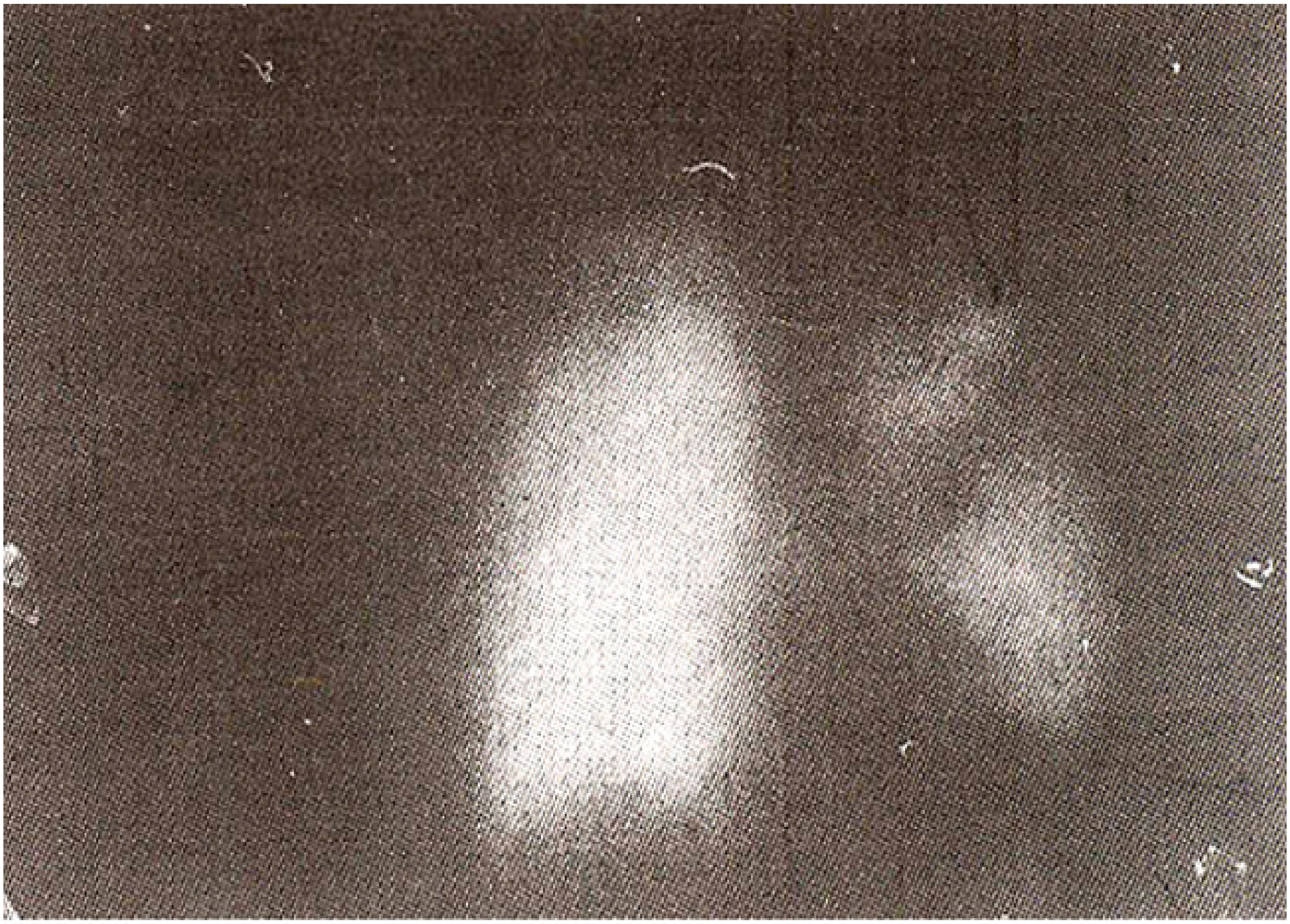

Figure 8 (c). Lung scan showing perfusion defect in the left lung, in the upper part of upper lobe, which could be bronchogenic carcinoma.

DISCUSSION

Radiolabelled MAPS has been popular for the last two and half decades I^{131}MAA a biodegradable and metabolizable material[10-12] was unchallenged from 1965 to 1970[13]. One major drawback with this agent I^{131} which after liberation from protein was taken up by the thyroid gland. The search for suitable replacement of I^{131}-MAA therefore, began.

In^{113m}-Fe (OH)3 macroaggregates were introduced by Stern et.al[14], which later on lost popularity due to unsuitable isotope (internal conversion), less degradation and clearance from lungs (effective tl/2 =18 hours) and less suitability of gamma camera for 393 KeY photons from In^{113m} Development of Tc99m-Iron hydroxide macroaggregates and the Tc^{99m} HSA microspheres brought an end to I^{131}MAA[4,5,15,16,18]. The Tc^{99m}-HSA microspheres offered advantages over macroaggregates in terms of particle size, quality control and advantages of Tc^{99m} over I^{131}. The problems with this agent were, however, time consuming labelling procedure, poor efficiency of labelling and tendency of microspheres to aggregate[13] . A new agent named Technetium labelled Macroaggregated Albumin (Tc99mMAPS) was therefore introduced[6,19-21]. . This is now the agent of choice due to following reasons: (i) it employs Tc^{99m} a short lived easily available radioisotope with optimum physical characteristics; (ii) it is used in quantities which are non-toxic and non-antigenic (maximum toxic dose is 125-150 times the average lung scanning dose?, (iii) is biodegradable and metabolized within proper time (effective tl/2 = 1.5 hours); (iv) has high extraction coefficient., (v) particle size range in these preparations is 10-2Opm, 66% 20-40uM, 26% 40-60 u m,6% and 60-80 um,2%[13]. Differential diagnosis of pulmonary embolism from chronic obstructive lung disease requires perfusion and ventilation studies[22] For perfusion studies and mediastinal and bronchial malignancies Tc99m-MAA is selectively used; In addition it is also used for detecting clots in lower extremities, a procedure called venography[22] and for the study of capillary bed of any other organ. The use of commercial MAA-kits, needs to be replaced to reduce cost of these studies. We have established a procedure for local preparation of Tc-99m-MM kit. The lung images (in animals and humans) obtained with local agent show satisfactory accumulation of bolus in target area, with high target to non-target ratio and complete safety of the agent. The efficiency of the agent to detect perfusion defects is same as that of commercial kit. The procedures described to standardize the agent are very simple and do not require complex chemical manipulations and extensive instrumentation. We feel that such simple techniques can be adapted by other radio pharmaceutical laboratories of our country as well.

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