IN-HOUSE PREPARATION AND CHARACTERIZATION OF READY-TO-USE TC^{99m}-Sn-MACROAGGREGATED ALBUMIN KIT FOR LUNG PERFUSION STUDIES

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SUMMARY: Macroaggregates of human serum albumin (HSA) are used as a basis for lung perfusion imaging. The present study involves in-house production of a macroaggregated HSA kit. A modified method for the preparation of Tin-macroaggregated albumin (Sn-MAA) kit, with high Tc^{99m} labeling efficiency (>98%) was developed for lung perfusion studies. The aggregative condition of HSA was stabilized by adding 5 mg/ml polyvinyl pyrolidone (PVP-30, 2.5% solution) and gave a uniform particle size distribution in the range of 10-60 μm. The kits were lyophilized and the stability of both wet as well as lyophilized kits were studied at room temperature 8°C and 0°C. The results revealed that lyophilized kits were stable for up to six months and retained their particle size uniformity and labeling efficiency. Biodistribution studies in the animals gave excellent lung images, with >90% of the injected radiation dose accumulating in the lungs.

Key Words: Macroaggregated Albumin, Tc^{99m}.

INTRODUCTION

Lung perfusion imaging is a non-invasive method to visualize pulmonary arterial bed. The underlying principle of perfusion imaging is pulmonary blood flow dependent distribution of particulate radiopharmaceuticals. Macroaggregates of HSA labeled with Tc^{99m} are used as one of the main clinical procedures in nuclear medicine for lung perfusion studies (19). A number of reports are available which evaluate the clinical usefulness of this radiopharmaceutical (8,20). Several investigations of the preparation, quality control, distribution, physiological response and metabolism of MAA have been published (1,6,9,12) and it has been proven that Tc^{99m}-Sn-MAA is more efficient for this purpose as compared to I^{131}-MAA due to better characteristics of its associated radionuclide. Although various tests have been used for the diagnosis of pulmonary embolism, ventilation perfusion scans using Tc^{99m}-MAA

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are considered the most useful and are employed regularly (4,14).

The aim of our present study is to establish a simple method for in-house development of ready-to-use Sn-MAA kit, thus making it available to the other nuclear medicine centers. The method of preparation has been modified and gives a particle size and radiochemical yield similar to that of commercially available kits, however, at a much lower cost.

MATERIALS AND METHODS

HSA 25% solution was obtained from Cutter Biologicals and Tc^{99m}-generator from Amersham (England). All other chemicals used were of AnalR grade.

Preparation of macroaggregates of HSA

HSA 25% solution equivalent to 20 mg was mixed with 1ml sodium acetate solution containing 300 mg sodium acetate in saline solution. After the addition of 0.4 ml stannous chloride solution (5 mg/ml), the mixture was diluted with normal saline solution to achieve a final HSA concentration of 2 mg/ml with constant gentle stirring. The solution was filtered through 0.22 μm millipore filter in an airtight container and stirred at 1000-1200 rpm for 10 minutes, followed by heating at 70-80°C for 15 minutes with continuous stirring. The solution was allowed to cool at room temperature for 5 minutes and re-heated to 70-80°C with stirring at 1000-1200 rpm. After 15 minutes, it was allowed to cool at room temperature and 2.5% PVP-30 solution was added to achieve PVP-30 concentration of 5 mg/ml in the final preparation. The solution was gently stirred for another 5 minutes and adjusted to pH 5.5 if necessary.

Table 1: Formulation of MAA cold kit.

HAS (2 mg/ml)	20 mg
Sodium acetate	300 mg
Stannous chloride	2 mg
PVP	50 mg
Physiological saline	Quantity sufficient
pH	5.5 - 6
Temperature	70 - 8°C

* For ten vials

Table 2: Labeling efficiency of cold MAA kits as a function of time stored at various temperatures.

Temperature	Time	Labelling (Percentage)
Room Temperature	0 hr	98.7
	3.5 hrs	98.8
	5.5 hrs	98.7
	24 hrs	89.2
	48 hrs	77.9
8°C	1 day	98.07
	3 days	98.16
	7 days	98.24
	14 days	97.5
	24 days	89.67
	48 days	84.7
Frozen	2 days	99.4
	4 days	99.5
	8 days	98.9
	24 days	98.7
	48 days	98.9
Lyophilized	6 months	>98

Effect of various parameters on kit development

a) Stabilizer: The kit was prepared using different stabilizers such as PVP-30, gelatin, PEG-1500, PEG-6000 and evaluated for particle size stability by ocular micrometry.

b) HSA solution concentration: Various initial concentrations of HSA solutions ranging from 0.5 mg/ml to 20 mg/ml were employed during the development of kit and its effect on particle size distribution was observed.

c) pH: The pH of the kit formulation was adjusted to different values from 3-8 and the kit was assessed for particle size stability by ocular micrometry and for radiolabeling efficiency by paper chromatography.

d) Stirring: The effect of stirring speed and length of stirring time on particle size distribution during the preparation and reconstitution was observed at different speeds of 800, 1000, 1200, 1400 and 2000 rpm for 5, 10, 15, 30 and 60 minutes.

Quality control by paper chromatography

Tc^{99m}-MAA complex formation was analyzed for any unbound sodium pertechnetate by paper chromatography. Strips of 2x15 cm size of Whatman No.1 filter paper were used. The prepared complex of Tc^{99m}-MAA was spotted over the lower portion of strip containing 10-20 μCi of the preparation. The strips were developed in acetone solvent system up to 15 cm and cut into 1 cm pieces after air drying. Activity at various portions of the strip was calculated by counting in a well type scintillation counter (Thorn EMI, UK).

Lyophilization of Sn-MAA kits

Lyophilization of the kits was carried out using freeze dryer (Consol-12, Virtis). One milliliter of Sn-MAA aliquots were added to each of the 2 ml vials (containing approximately 4 x 10^6 particles). The vials were shelf frozen to -40°C temperature, flushed with nitrogen and subjected to vacuum and sublimation followed by final sealing.

Particle size distribution

The size distribution of Sn-MAA particles was analyzed for 10 vials each of lyophilized and refrigerated kits. The contents of the kit were diluted with saline solution and mixed gently to avoid disintegration of HSA aggregates. A drop of this was placed on Neubaur's chamber and an average of 400 particles were measured per kit by means of a pre-calibrated micrometer using Olympus BH-2 microscope and analyzed for size distribution.

Figure 1: Effect of pH on the labeling efficiency of Tc^{99m}-MAA kit.

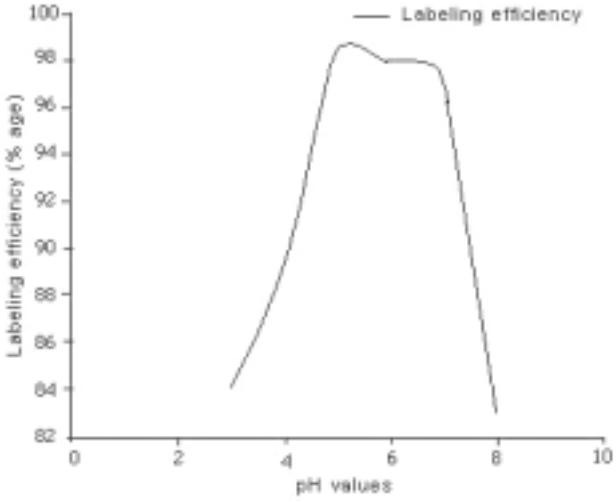
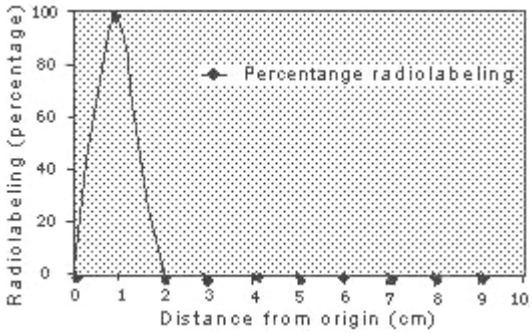


Figure 2: Distribution of radioactivity on a paper chromatogram of Tc^{99m}-MAA in methanol: water (85:15) solvent system.



Labeling with Tc^{99m}

The vial containing Sn-MAA was placed in a lead container and 1 ml of Tc^{99m} eluate containing 5 mci radioactivity was introduced into the vial. The contents were shaken gently, taking care not to disintegrate the aggregates and incubated at room temperature for 10 minutes. The percentage labeling yield was quantified by paper chromatography on Whatman No.1 paper using acetone as solvent system. Percentage labeling yield was obtained by;

%age labeling = activity at the origin/total activity x 100

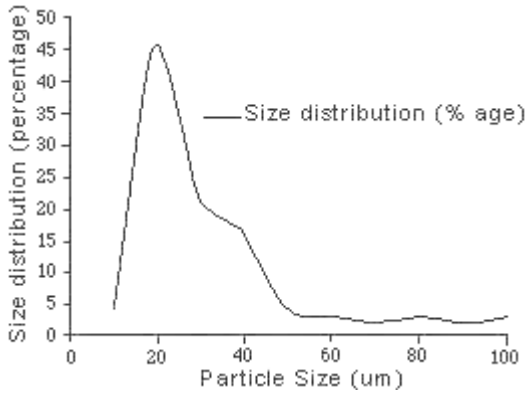
Stability studies

The stability of lyophilized and wet kits was studied as a function of time at room temperature, 8°C and 0°C, for six months. Ten kits of each were placed at specified temperature and analyzed for particle size distribution and labeling efficiency with Tc^{99m} by paper chromatography as described elsewhere at 0, 1, 2, 4 days; 1, 2 and 4 weeks. The stability of the radiolabeled MAA with Tc^{99m} was observed for 5 hours at 0, 1, 3, 1 and 5 hours at room temperature.

Biodistribution studies in rabbits

In vivo studies using Tc^{99m}-Sn-MAA were carried out in rabbits to determine the percentage of the injected dose concentrating in the lungs. Aliquots of 1 ml (2.5 mCi) Tc^{99m}-Sn-MAA was injected into the marginal ear vein of male rabbits (average weight 1.5 kg). The animals were imaged for 60 seconds for static images to study organ distribution pattern of the radiopharmaceutical. The animals were killed at 10, 60 and 300 minutes by injecting I/V thiopental and dissected to remove lungs, liver, spleen, bone-marrow and kidneys. These organs were weighed and counted for radioactivity concentration using Thorn EMI (UK) well counter.

Figure 3a: Particle size distribution in a freshly prepared coldMAA kit containing 5mg/ml PVP-30. The particle size was measured by ocular micrometry.



RESULTS

Macroaggregation of MAA was carried out by method proposed by Lyster *et. al.* (12) with slight modifications. The final formulation of the kit is shown in Table 1. Amongst the various factors studied for the optimization of conditions during the development of the kit formulation, the initial concentration of HSA solution was found to greatly influence the particle number as well as their size distribution. Larger aggregates were achieved at higher HSA concentration and smaller at lower concentrations. The optimum HSA concentration was 2 mg/ml. Similarly, the optimum stirring rate was 1000-1200 rpm in the presence of 5 mg/ml PVP-30 at pH 5.5 (Figure 1). The labeling efficiency and radiochemical purity of the kit by paper chromatography was >98% (Figure 2).

The range of particle size distribution was quite narrow with an average of >88% of the particles in the range of 10-60 μm (Figure 3a). Out of the total 4000 particles observed from lyophilized kits (Figure 3b), less than 12% were of size larger than 60 μm. Similar observations were made for refrigerated wet kits. Stability studies revealed that refrigeration or lyophilization did not effect the particle size distribution and the redispersibility of the particles. However, the wet kits stored at room temperature failed to retain their integrity with respect to particle size distribution.

The improvement of kit with regard to the particle size integrity may be attributed to the addition of 5 mg/ml PVP-30 which helped in stabilizing the particle size. The stability of the lyophilized kits was followed

Figure 3b: Photomicropgraph of HSA macroaggregates at an optimum concentration of 2mg/ml HSA, in a haemocytometer. The kit was lyophilized and stored in a refrigerator for one month.

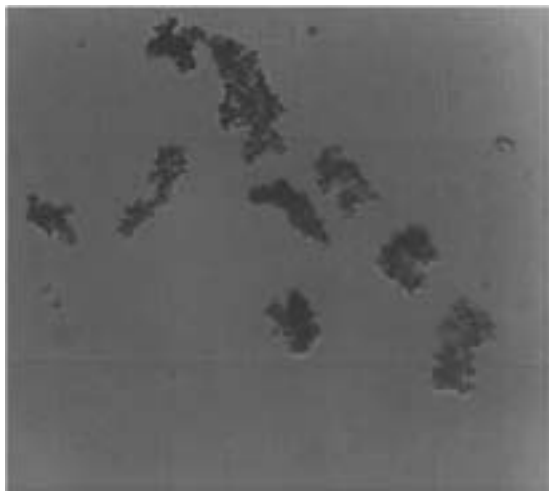
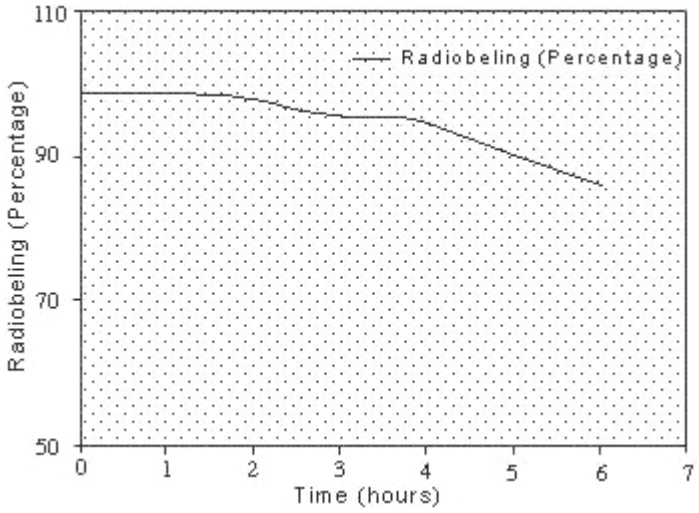


Figure 4: The effect of incubation at room temperature on the stability of radiolabeled Tc^{99m}-MAA kit.



for up to six months and they were found to retain >98% labeling (Table 2). However, wet kits stored at room temperature showed considerable decline in the radiolabeling efficiency (77.99%) after 48 hours. Radiolabeled Tc^{99m}-MAA when stored at room temperature remained stable for 4 hours post radiolabeling (Figure 4). Tissue distribution showed that lungs accumulated 90% of the activity with liver receiving 5.4%, spleen 0.35% and kidneys 5.2% radioactivity (Figure 5). Scintiscans taken during biodistribution studies revealed high and specific uptake of Tc^{99m}-MAA in the lungs (Figure 6).

DISCUSSION

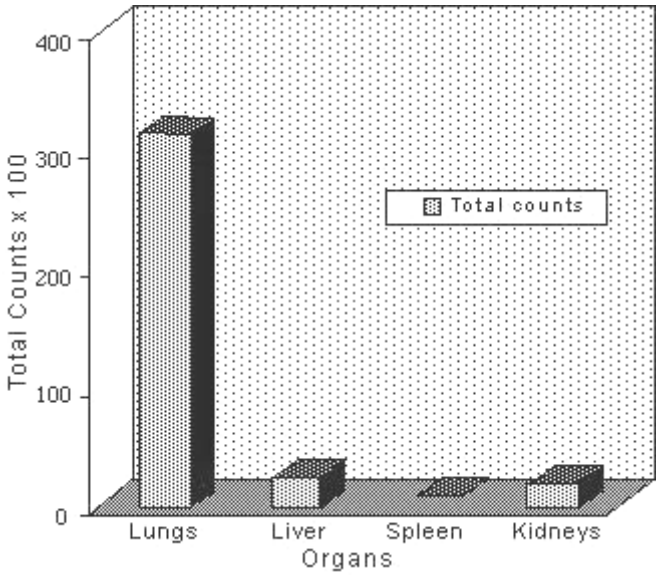
Thromboembolism in the lungs continues to be the major indication for radionuclide lung scanning (5, 7). When pulmonary embolism is detected, the significance of diagnostic imaging is to direct and validate the treatment (15). Various reports have been published wherein Tc^{99m}-Sn-MAA has shown excellent results in the differential diagnosis of pulmonary embolism from chronic obstructive lung disease, mediastinal and bronchial malignancies, detection of clots in the lower extremities by venography and of the capillary bed of any other organ (13,18).

Of all the agents, Tc^{99m}-labeled albumin based agents are extensively used in pulmonary imaging. The status of Tc^{99m}-MAA as the lung imaging radiopharmaceutical of choice is due to the particle size characteristics and biologically metabolizable nature. This is cleared rapidly from the lungs by enzyme metabolism and mechanical break down due to haemodynamic pressure (3). Moreover, the commercial availability of ready-to-use 'instant' MAA kit together with the availability of Tc^{99m} in the form of generator system, has enabled most nuclear medical centers to use Tc^{99m}-MAA for lung scanning. However, use of these commercially available kits is not cost-effective and hence, not feasible for routine use, especially in developing countries such as Pakistan. The in-house preparation of MAA ready-to-use kit will enable us to supply these kits at cheaper rates for routine clinical use.

Over the years, various techniques for the preparation of Tc^{99m}-MAA have been reported in the literature (9-12,16). These attempts have met with many difficulties such as lack of reproducibility of the product with respect to particle size distribution and fragility of the aggregates.

Investigations have been continuing to simplify the method of preparation of MAA kit to overcome the

Figure 5: Tissue distribution of Tc^{99m}-MAA in rabbit after i/v injection.

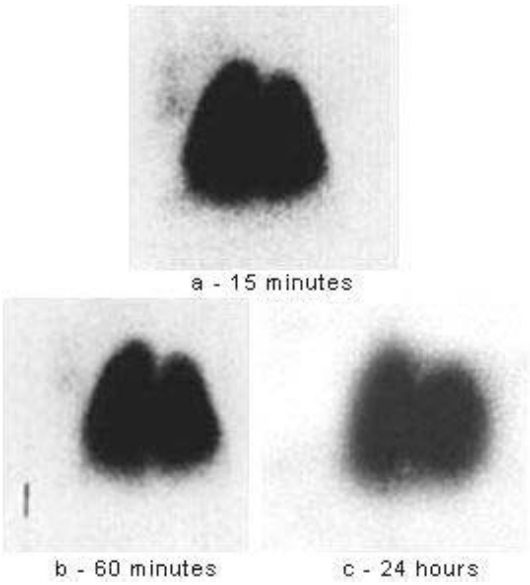


above-mentioned problems and enhance it's stability, labeling efficiency and lung uptake (1,2). During the present study, we have successfully attempted to simplify the methods of Lyster *et. al.* (12) and Al-Janabi *et. al.* (1) to produce an in-house, ready-to-use MAA kit. Our method of MAA preparation involves simple heating and does not include chemicals for this purpose. The aggregating solution is adjusted at pH 5.5 with sodium acetate which has been found to be critical with regard to the pH and particle size of the aggregates in the kit (Table 1). Furthermore, rate of stirring significantly influences the particle size distribution of the aggregates. From amongst the various stabilizers used during the development of the kit, PVP-30 at a concentration of 5mg/ml has been found to impart excellent stability and integrity to the aggregated HSA.

The initial HSA concentration influenced the size as well as number of particles obtained after macroaggregation. According to Robbins *et. al.* (17), 1mg/ml HSA provides 2.5 x 10^6 particles with 5-70 μm size. The number of MAA particles in the newly developed kit is kept at 4 x 10^6 particles/Vial and their size distribution is maintained between 10-90 μm (Figures 3a and 3b). Stability studies revealed that lyophilization did not affect the particle size distribution and redispersibility. The labeling efficiency with Tc^{99m} has been excellent. In terms of radiochemical purity of the radiolabeled MAA kit, paper chromatography results revealed >98% of the added radioactivity bound with MAA and negligible radioactivity present in free form (Figure 2). However, pH remained an important factor interfering with the labeling efficiency. Extensive experimentation showed that maximum labeling efficiency of MAA kit was achieved between pH 5.5-6 (Figure 1).

The stability study data shows that the newly developed kit is stable. It maintains particle size integrity as well as radiolabeling efficiency for more than 2 months when kept frozen below 0°C (Table 2). Thawing and reconstitution of the frozen kits during radiolabeling does not interfere with its labeling efficiency and particle size distribution. It is therefore believed, that this procedure could be followed to produce MAA kits in the nuclear medical centers devoid of freeze drying facilities. Lyophilization process has also not been found to interfere and change the kit characteristics. Rather, the shelf life of lyophilized kits have been found to be more than six months (Table 2). Lyophilized kits have the added advantage of simple

Figure 6: Lung scintiscans of rabbit using Tc99m-MAA at various time intervals post i/v injection.



storage at a cool place and easy transportation to other nuclear medical centers. However, storage at room temperature has been found to interfere with the labeling efficiency and the shelf life at room temperature is less than 48 hours, no matter the particle size distribution remains uneffected for up to 2 weeks. The MAA kit has been found to be stable for up to 4 hours after reconstitution and radiolabeling (Figure 4) at room temperature and does not show any change its biodistribution characteristics. The organ distribution studies and static images taken at various time intervals show high and specific lung accumulation of the radiopharmaceutical (Figure 6).

CONCLUSION

The kits of macroaggregated HSA have been successfully prepared. Its constitution in lyophilized form imparts stability as well as case of handling. It gives excellent imaging characteristics. The kit can be developed on larger scale to meet the needs of other nuclear medical centers in Pakistan, for which they have to spend precious foreign exchange.

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