The Preparation Parameters of Technetium-99m-Macroaggregated Albumin-Low Particle Number

Kenneth T. Cheng, Kenneth M. Spicer and Leonie Gordon

Medical University of South Carolina, Charleston, South Carolina

Objective: The preparation of technetium-99m-macroaggregated albumin-low particle number (^{99m}Tc-MAA-LPN) for lung scans of right-to-left shunt in neonates and young children involves the reconstitution of the reagent kit with 0.9% sodium chloride, USP, to obtain the desired low particle number before radiolabeling.

Methods: Volume effects of normal saline and ^{99m}Tc-pertechnetate were studied by using 2.5 to 10.0 ml of normal saline for reconstitution and 2.0 to 8.0 ml of ^{99m}Tc-pertechnetate for radiolabeling. The effect of time lapse after reconstitution was also studied by incubating the reconstituted kits for 5–60 min at room temperature before radiolabeling. Labeling efficiencies of the prepared ^{99m}Tc-MAA-LPN were evaluated using radiochromatography techniques at various time points up to 30 min after radiolabeling. The rate of ^{99m}Tc-MAA-LPN formation was calculated as the association rate constant (ka) from the fitted curves of labeling efficiencies versus time.

Results: The MAA labeling reaction with ^{99m}Tc proceeded at biphasic exponential rates. Both the reconstitution volume of normal saline and the ^{99m}Tc-pertechnetate labeling volume significantly decreased the initial reaction rate (ka_1). These results indicate that the labeling efficiencies of ^{99m}Tc-MAA-LPN preparations are highly dependent on the time of incubation after the addition of ^{99m}Tc-pertechnetate and this dependency increases with larger volumes of prepared ^{99m}Tc-MAA-LPN. In addition, the time between reconstitution and radiolabeling also has a significant impact on the reaction kinetics of radiolabeling.

Conclusions: This study suggests that the optimal labeling condition of ^{99m}Tc-MAA-LPN is achieved by minimizing both the volumes of reconstitution and ^{99m}Tc-pertechnetate radiolabeling immediately after reconstitution and a radiolabeling incubation time of 20–30 min.

Key Words: technetium-99m-macroaggregated albumin-low particle number; lung scan; neonate

J Nucl Med Technol 1994;22:173–177

For correspondence or reprints contact Kenneth T. Cheng, PhD, BCNP, Associate Professor/Director of Nuclear Pharmacy, Nuclear Pharmacy, H341G North Tower, Medical University of South Carolina, 171 Ashley Ave., Charleston, SC 29425.

Preparation of technetium-99m-macroaggregated albumin (^{99m}Tc-MAA) involves the addition of a suitable radioactivity concentration of ^{99m}Tc-pertechnetate in 0.9% sodium chloride injection, USP, to reconstitute the reagent kit which contains the pretinned MAA particles. The desired dose and number of particles per dose determine the exact volume and radioactivity concentration of ^{99m}Tc-pertechnetate added. Generally, the major limiting factor in the preparation procedure is the number of particles that can be given to the patients. Since the ^{99m}Tc-MAA particles localize by embolization in the lung capillaries, the number of particles given to patients with severe pulmonary hypertension, right-to-left cardiac shunts, neonates and children <3 yr old needs to be minimized (*1–3*).

When the ^{99m}Tc-MAA-low particle number (^{99m}Tc-MAA-LPN) preparation is required, a special preparation procedure is often needed to achieve the required particle concentration with the appropriate radioactivity (*4,5*). This is particularly important when commercial MAA kits with high particle numbers are used. The special preparation procedure commonly involves three steps: (1) the initial addition of 0.9% sodium chloride injection USP to reconstitute the MAA kit before radiolabeling; (2) taking out and discarding an appropriate volume of the reconstituted MAA solution to reduce the number of particles in the kit, or transferring the appropriate reconstituted volume to another vial for radiolabeling; and (3) adding suitable volume and radioactivity of ^{99m}Tc-pertechnetate to the reconstituted MAA solution and incubating for 5–10 min to radiolabel the MAA.

From our experience, the preparation of ^{99m}Tc-MAA-LPN often produces inconsistent labeling efficiencies and the required radiolabeling incubation time varies from 2 to 30 min. This may be a result of the changes of ^{99m}Tc-labeling kinetics during the preparation procedure. Both the lower tin concentration after reconstitution with the 0.9% sodium chloride injection USP and the possible introduction of air into the reaction vial would significantly affect the ^{99m}Tc-labeling efficiency and reaction kinetics.

The goal of this study was to investigate various common preparation conditions of ^{99m}Tc-MAA-LPN that may influence the consistency of producing acceptable labeling

efficiency. In this study, we evaluated the following three preparation parameters: (1) reconstitution volume of 0.9% sodium chloride USP, (2) the time lapse between the reconstitution with 0.9% sodium chloride injection and radiolabeling and (3) the ^{99m}Tc-pertechnetate volume used for radiolabeling.

MATERIALS AND METHODS

In this study, the MAA kits were obtained from E.I. du Pont de Nemours and Co. (Pulmonite®, NEN Products, No. Billerica, MA) because they contain the lowest particle number than other commercial kits. Each Pulmonite® kit contains about 3.6–6.5 million particles. In this study, the lot of kits used contained 5 million particles (1 mg aggregated albumin, 10 mg human albumin, 0.13 mg total tin and 10 mg sodium chloride).

In the first study, normal saline volumes of 2.5, 5.0, 7.5 and 10.0 ml were added to reconstitute the MAA reagent kits (n = 5 for each variable). Appropriate volumes (2.0, 4.0, 6.0 and 8.0 ml) of the reconstituted MAA solution were withdrawn and discarded so that about 1 million particles (0.5, 1, 1.5 and 2.0 ml) remained in the kit. Immediately, appropriate (2.0, 4.0, 6.0 and 8.0 ml, respectively) volumes of 50 mCi of ^{99m}Tc-pertechnetate for radiolabeling were added to give final volumes of 2.5, 5.0, 7.5 and 10 ml of ^{99m}Tc-MAA-LPN, respectively. Instant thin-layer chromatographies (ITLC, 31ET Whatman paper) using acetone as the solvent were performed immediately on the radiolabeled kits at incubation times of 0.25, 0.5, 0.75, 1, 3, 5, 7, 10, 15 and 30 min after radiolabeling. The strips were dried and cut in half after development for counting in an automatic NaI(Tl) well counter or a dose calibrator.

In the effect of time lapse after reconstitution study, the MAA kits were reconstituted with 2.5 ml of normal saline. Two milliliters of the reconstituted solution were discarded and the remaining 0.5 ml was then radiolabeled with 50 mCi/2 ml ^{99m}Tc-pertechnetate to make a final volume of 2.5 ml of ^{99m}Tc-MAA-LPN. However, the reconstituted kits were allowed to incubate at room temperature for 5, 15, 30 and 60 min before radiolabeling with ^{99m}Tc-pertechnetate. Instant thin-layer chromatography tests were performed in a similar manner as described above for each kit at different times after radiolabeling.

A control study was done by preparing a ^{99m}Tc-MAA multidose kit in accordance with the manufacturer's recommendations (50 mCi/8 ml). The chromatography testing was performed in a similar fashion. The results from the control study were used for comparison purposes. Experimental data were calculated and expressed as labeling efficiencies (% ^{99m}Tc-bound MAA), and the data were statistically analyzed by the ANOVA (Newman Keul's range test at $p < 0.05$, SOLO BMDP Statistical Program, Los Angeles, CA). The data of labeling efficiencies (% bound) versus time (minutes) were also fitted with regression analysis (RSTRIP, MicroMath, Salt Lake City, UT), and the formation rate constants (ka) of the radiolabeled preparations were calculated from the half-life of the fitted curves.

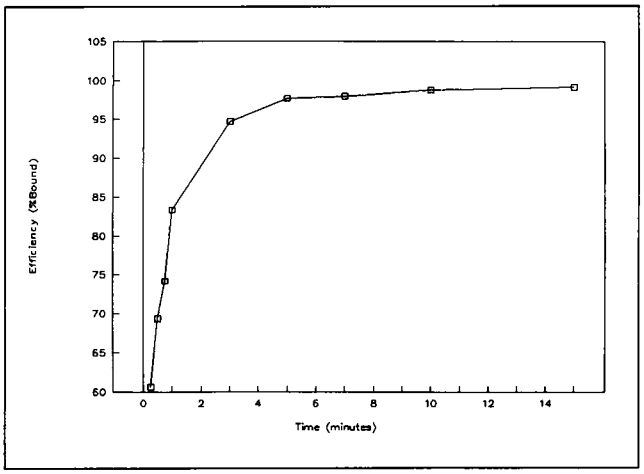

FIGURE 1. Labeling efficiencies versus incubation time of multidose ^{99m}Tc-MAA as the controls (8 mCi/5 ml) (mean ± s.d., n = 5)

RESULTS AND DISCUSSION

Changes in labeling efficiencies after radiolabeling from the control study (^{99m}Tc-MAA) are illustrated in Figure 1. For simplicity, only the labeling efficiencies at 1, 3, 15 and 30 min from the other experiments are shown. Labeling efficiencies from experiments using different reconstitution volumes (2.5, 5, 7.5 and 10 ml) and different volumes of ^{99m}Tc-pertechnetate (2.0, 4.0, 6.0 and 8.0 ml) are shown in Figures 2–5.

The results show that the MAA labeling reaction proceeded at biphasic exponential rates (Fig. 1). There was an initial phase of rapid labeling reaction within the first 3 min and then a much slower reaction phase rate from 3 to 30 min. Similar biphasic exponential reaction rates were observed in all experimental conditions. Based on these observations,

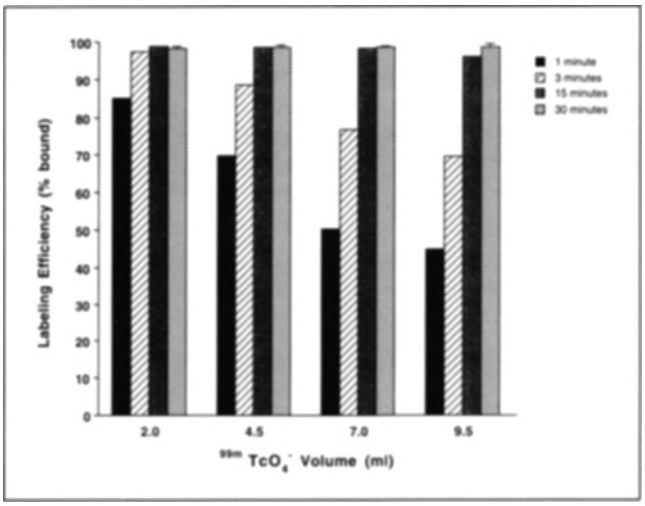

FIGURE 2. Labeling efficiencies of ^{99m}Tc-MAA-LPN using a 2.5-ml reconstitution volume and 0.5 ml (1 million particles) for radiolabeling with $^{99m}TcO_4^-$ (mean ± s.d., n = 5)

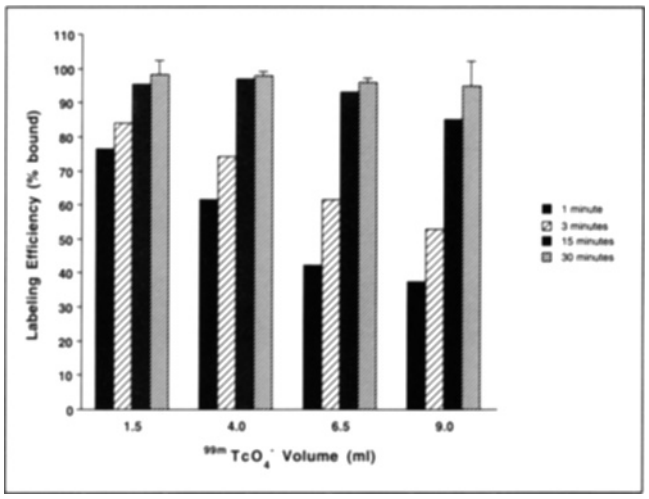

FIGURE 3. Labeling efficiencies of ^{99m}Tc-MAA-LPN using a 5.0-ml reconstitution volume and 1.0 ml (1 million particles) for radiolabeling with $^{99m}TcO_4^-$ (mean ± s.d., n = 5)

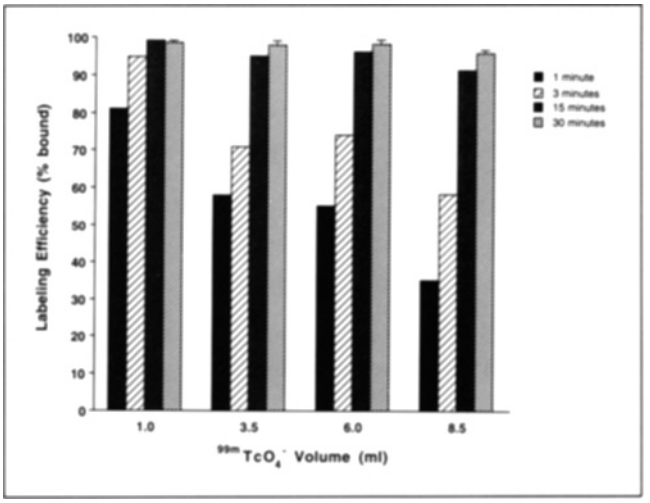

FIGURE 5. Labeling efficiencies of ^{99m}Tc-MAA-LPN using a 10-ml reconstitution volume and 2.0 ml (1 million particles) for radiolabeling with $^{99m}TcO_4^-$ (mean ± s.d., n = 5)

two reaction (association) rate constants (ka) were calculated. The first (ka_1) represents the initial fast reaction rate from 0 to 3 min and the second (ka_2) represents the slower reaction rate from 3 to 30 min (Tables 1–3).

There was a significantly greater volume effect on the initial reaction rate than on the second reaction rate. Both the labeling efficiencies within the first 3 min and the ka_1 changed significantly as the reconstitution volume and/or the ^{99m}Tc-pertechnetate volume changed (Figs. 2–5 and Table 2). There was a corresponding decrease in the initial reaction rate (ka_1) whenever the reconstitution volume and/or ^{99m}Tc-pertechnetate volume increased. However, the effect on the second reaction phase (ka_2, 3–30 min) was much smaller.

The ka_2 actually increased with an increase in the volume of reconstitution and/or ^{99m}Tc-pertechnetate. Most labeling reactions reached 95%–98% completion (labeling efficiencies) by 30 min with the exception of the final volume of ^{99m}Tc-MAA-LPN which reached 10 ml (84%–91%; Fig. 5, Table 2). Both the reconstitution volume and the ^{99m}Tc-pertechnetate labeling volume had similar negative impacts on the reaction rates.

In all these experimental conditions, only the 2.5-ml reconstitution volume (2.0 ml discarded) with 2.0 ml of ^{99m}Tc-pertechnetate (2.5 ml final ^{99m}Tc-MAA-LPN volume) had a similar ka_1 and ka_2 as the control multidose kit reaction (Tables 1 and 2). These results indicate that the labeling efficiencies of ^{99m}Tc-MAA-LPN preparations are highly dependent on the time of incubation after the addition of ^{99m}Tc-pertechnetate, and this dependency increases with larger volumes of prepared ^{99m}Tc-MAA-LPN. For example, the 5.0-ml volume of ^{99m}Tc-MAA-LPN (prepared from the different combinations of reconstitution volumes and ^{99m}Tc-pertechnetate volumes) required 15 min incubation × to achieve acceptable radiochemical purity (90%) while the 10-ml volume of ^{99m}Tc-MAA-LPN required at least 30 min.

In addition to the volume effects, the study results also show that the time between reconstitution and radiolabeling has a significant impact on the reaction kinetics of radiolabeling (Fig. 6 and Table 3). The ka_1 significantly decreased when the reconstitution time was longer than 5 min. The ka_2 was much less affected, but was also significantly changed. However, all labeling efficiencies reached 96% by 30 min of radiolabeling incubation time.

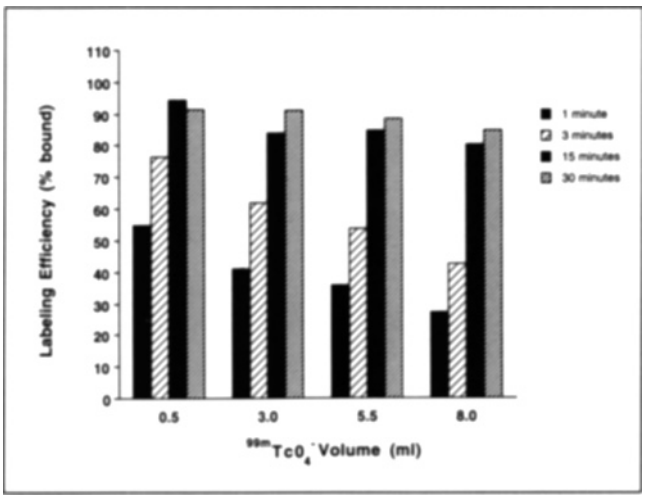

FIGURE 4. Labeling efficiencies of ^{99m}Tc-MAA-LPN using a 7.5-ml reconstitution volume and 1.5 ml (1 million particles) for radiolabeling with $^{99m}TcO_4^-$ (mean ± s.d., n = 5)

TABLE 1
Rate Constants for Controls (^{99m}Tc-MAA)*

ka_1 (0–3 min)	ka_2 (3–30 min)
137.5 (0.5)	0.15 (0.02)

*(hr^{-1}, mean ± s.d., n = 5)

TABLE 2
Reconstitution Volume Rate Constants*

$^{99m}TcO_4^-$ volume (ml)	ka_1	ka_2
	2.5-ml reconstitution volume	
2.0	137.1 (0.4)	0.10 (0.06)
4.5	74.9 (2.1)	0.15 (0.02)
7.0	51.5 (1.9)	0.40 (0.09)
9.5	40.1 (2.2)	0.61 (0.25)
	5-ml reconstitution volume	
1.0	84.4 (1.4)	0.17 (0.14)
4.0	71.3 (3.3)	0.47 (0.05)
6.5	39.7 (1.1)	0.75 (0.07)
9.0	38.4 (0.5)	0.95 (0.07)
	7.5-ml reconstitution volume	
0.5	86.6 (0.4)	0.18 (0.09)
3.5	43.1 (0.6)	0.56 (0.25)
6.0	42.4 (1.6)	0.72 (0.25)
8.5	37.7 (0.4)	0.93 (0.09)
	10-ml reconstruction volume	
0.5	41.7 (4.7)	0.24 (0.14)
3.0	39.2 (2.9)	0.75 (0.50)
5.5	37.7 (2.7)	0.93 (0.26)
8.0	34.7 (3.3)	1.27 (0.33)

*hr^{-1}, mean ± s.d., n = 5.

TABLE 3
Rate Constants for Incubation Time Study*

Incubation time (min)	ka_1	ka_2
0	137.1 (0.4)	0.10 (0.06)
5	65.0 (3.5)	0.25 (0.14)
15	49.1 (2.7)	0.44 (0.31)
30	35.6 (2.0)	0.45 (0.16)
60	26.8 (1.9)	0.57 (0.28)

*(hr^{-1}, mean ± s.d., n = 5)

CONCLUSION

From the study results, we conclude that both the initial reconstitution volume of normal saline and the ^{99m}Tc-pertechnetate volume can significantly affect the ^{99m}Tc-radiolabeling reaction kinetics and thus the labeling efficiency of ^{99m}Tc-MAA-LPN. This means the labeling efficiency of ^{99m}Tc-MAA-LPN is highly dependent on the incubation time of radiolabeling. Similar altered radiolabeling kinetics can occur when the reconstitution time is prolonged before radiolabeling. These effects are probably due to the lower tin concentration upon dilution and tin oxidation because of the introduction of air during reconstitution. The adverse impacts of lower tin concentration on ^{99m}Tc-labeling reactions are well documented in the ^{99m}Tc-labeled radiopharmaceutical literature (*6,7*). The radiolabeling efficiency also becomes more variable as the volumes of reconstitution and ^{99m}Tc-pertechnetate increase. This is evident by the increase in the standard deviations of labeling efficiencies with higher final volumes (7.5 ml, 10 ml) of ^{99m}Tc-MAA-LPN (Figs. 4 and 5).

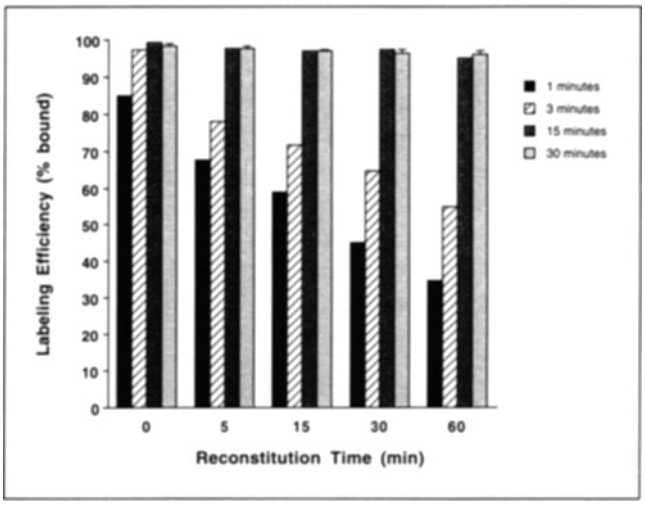

FIGURE 6. Labeling efficiencies of ^{99m}Tc-MAA-LPN using a 2.5-ml reconstitution volume at different reconstitution incubation times before radiolabeling 0.5 ml (1 million particles) with 2.0 ml of $^{99m}TcO_4^-$ (mean ± s.d., n = 5)

The biphasic exponential reaction phenomenon is interesting and may be explained by the fact that the aggregated albumin is a large molecule and may contain two or more different populations of functional groups (amino acid residues). These functional groups could react with ^{99m}Tc at different reaction rates.

Based on these data, we recommend the following guidelines for the preparation of ^{99m}Tc-MAA-LPN:

1. Use the lowest possible reconstitution volume, and avoid using volumes of normal saline that are greater than 5 ml.
2. As suggested by Bolstad et al. (*5*), use the lowest possible volume of ^{99m}Tc-pertechnetate for radiolabeling, and avoid using volumes of ^{99m}Tc-pertechnetate that are greater than 5 ml.
3. Allow an incubation time of about 20–30 min after radiolabeling for the completion of the radiolabeling reaction.
4. Perform radiochemical purity testing (ITLC) before patient administration in all possible circumstances.

ACKNOWLEDGMENTS

The authors thank Mrs. Lisa Marcou and Mr. Rajenda V. Deshpande for their technical support, and Mrs. Tracy Hackett for her invaluable help in preparing this manuscript.

REFERENCES

1. Kowalsky RJ, Perry JR. *Radiopharmaceuticals in nuclear medicine practice*. Norwalk, CT: Appleton and Lange: 1987:242–247.

2. Kowalsky RJ. Safety and effectiveness considerations with particulate lung scanning agents. *J Nucl Med Technol* 1982;10:223–227.
3. Heyman S. Toxicity and safety factors associated with lung perfusion studies with radiolabeled particles. *J Nucl Med* 1979;20:1098–1099.
4. Levine EK, Perritt JS, Gordon L. Particle reduction of a macroaggregated albumin kit: simplified calculations. *J Nucl Med Technol* 1989;17:143–144.
5. Bolstad DM, Valley TB, Wilson ME, Hung JC. An improved technique for reducing the number of particles in a technetium-99m macroaggregated albumin injection. *J Nucl Med Technol* 1992;20:220–223.
6. Owunwanne A, Church LB, Blau M. Effect of oxygen on the reduction of pertechnetate by stannous ion. *J Nucl Med* 1977;18:521–523.
7. Kowalsky RJ, Perry JR. *Radiopharmaceuticals in nuclear medicine practice*. Norwalk, CT: Appleton and Lange; 1987:82–86.