MACROAGGREGATION OF AN ALBUMIN-STABILIZED TECHNETIUM-TIN(II) COLLOID

Max S. Lin and H. Saul Winchell

Donner Laboratory, University of California, Berkeley, California

A variety of macroaggregates labeled with ^{99m}Tc or ^{113m}In for lung scanning has been described (*1–10*). Reported methods for preparing these agents include macroaggregation of ^{99m}Tc-labeled human serum albumin (HSA) (*1,2*), ^{99m}Tc labeling of macroaggregated HSA (*3,4*), incorporation of ^{99m}Tc-sulfur colloids into HSA macroaggregates (*5,6*), and coprecipitation of ^{99m}Tc or ^{113m}In with Fe(III) (*7–10*). Control of particle size or particle fragility seems to be a problem with such techniques. With macroaggregates of ferric hydroxide, a prolonged pulmonary retention of the iron poses a potential problem (*10*).

A stable ^{99m}Tc-tin(II) colloid can be prepared simply by mixing an aqueous Sn(II) solution and ^{99m}Tc generator eluate followed by adding stabilizer HSA to the mixture (*11*). This report describes a two-staged method for converting this ^{99m}Tc-Sn(II) colloid into a ^{99m}Tc-labeled macroaggregate that is not fragile but metabolizable and has a controlled size limit.

MATERIALS AND METHODS

The ^{99m}Tc-Sn(II) colloid was prepared in 7-ml quantities by adding 5 ml of the ^{99m}Tc generator eluate and 1 ml of a stock HSA solution (5 mg/ml) to 1 ml of a stock Sn(II) solution (0.119 mg Sn/ml) in a vial as previously described (*11*). To prepare the macroaggregate, 1 ml of 0.2 *M* phosphate buffer at pH 5.6 was added to the 7-ml colloid, and the buffered colloid was converted into the macroaggregate by an aseptic technique in two stages.

In Stage 1, a suspension of oversize particles that were not fragile was prepared by heating the 8-ml buffered colloid in the vial in a boiling water bath. These particles ranged in size from a few hundred microns to about 1.5 mm. The suspending phase was watery clear. This appearance of the suspension was recognizable by a glance and served as an approximate endpoint of the heating. The required heating time varied from 2.5 to 4.0 min in preparing 21 batches of the macroaggregate using 11, 7, and 2 lots of stock solutions of Sn(II), HSA, and phosphate buffer, resepectively. Storage of the stock solution before its use in the preparation varied from 0 to 27 days for the Sn(II), 0 to 12 days for the HSA, and 0 to 6 months for the buffer solution. The heating was followed by 1-min cooling in run-

Received Nov. 15, 1971; revision accepted June 28, 1972.

For reprints contact: H. Saul Winchell, Donner Laboratory, Univ. of California, Berkeley, Calif. 94720.

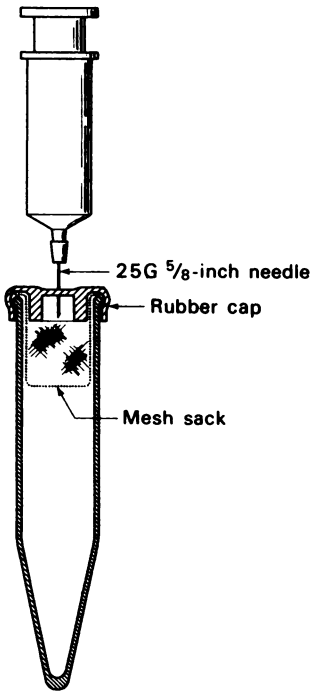

FIG. 1. Assemblage for disrupting oversize aggregates and sieving fragments of ruptured aggregate. Fragments exceeding 150 microns are retained by mesh. Syringe shield not shown.

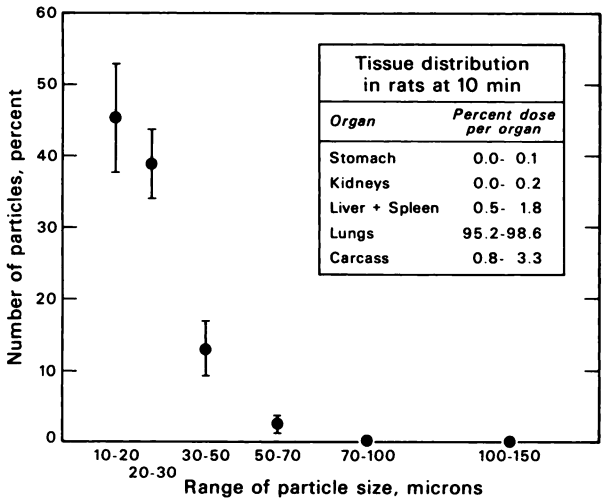

FIG. 2. Measured particle size distribution and results of bioassay in 10 batches of macroaggregate. Circles and vertical bars represent mean ±s.d. Only particles exceeding 10 microns were included in enumeration, 1,000 particles/batch. Inset shows tissue distribution of technetium 10 min after intravenous administration of 10 batches in rats (3 rats/batch).

ning water. While in the boiling bath and in the running water, the vial was moderately agitated at a rate of roughly 200 rpm.

In Stage 2, the oversize particle was disrupted into fragments, and those fragments still exceeding 150 microns were eliminated with a sieve as illustrated in Fig. 1. The 8-ml suspension obtained in Stage 1 was taken with a 20-G needle into a shielded syringe and then forced out of a 25-G ⅝-in. needle over 12–16 sec into a meshed sac fitted inside a tube. The sac* had regular mesh openings of 175 microns when dry before autoclaving and of 150 microns when wet after autoclaving. The suspension in the tube was then recovered with a long needle bypassing the sieve into another shielded syringe and forced out of another 25-G ⅝-in. needle over 12–16 sec into a vial. The resultant suspension was the macroaggregate preparation.

Many aggregates adhered to the top portion of the vial during Stage 1 of the procedure. When no effort was made to dislodge and recover them for the Stage 2 operation, 60% ± 6 s.d. of the intial activity was recovered in the final preparation in eight batches. Under the circumstances, the vial retained 33% ± 6 s.d. of the initial activity, and the meshed sac 5% ± 2 s.d.

The macroaggregate was evaluated in rats by previously described techniques (*11*) except for the following modification. In tissue distribution studies, activities in the individual organs and the carcass were assayed with a scintillation camera and expressed as percent of the sum of these activities to approximate the percent body ^{99m}Tc in the organ or the carcass.

* Nylon mesh blood filter, Macbick Co., Billerica, Mass., Cat. No. 3525.

RESULTS

Figure 2 shows the particle size distribution and results of bioassay in ten consecutive batches of the macroaggregate. Particles of 10–50 microns comprised 95.4–99.4% (range) of all exceeding 10 microns. Those of 100–150 microns were rare, 0.0–0.2%. None exceeded 150 microns. The particle count was (0.88 ± 0.23) × 10^6/ml (mean ± s.d.). In 30 rats, 95–99% of the administered technetium localized in the lungs at 10 min. There was little free or colloidal activity in these preparations. Excellent visualization of lungs was obtained with a scintillation camera in rats and dogs.

As shown in Table 1, the macroaggregate was found to be virtually stable in vitro. Standing under air and moderate agitation in a shaker did not result in leaching of the technetium out of the particle or fragmentation of the particle that was detectable by the assay in rats.

In the rat, the rate of loss of the technetium from the body was found to be approximately 9% of the dose per day throughout the first 2 days. Measured distribution of the technetium in the body is shown in Table 2. The biological half-time of the technetium in the lungs was approximately 9 hr. At 24 hr, approximately ⅔ of the technetium remaining in the rat was found in the liver plus the spleen. The latter finding together with the finding that nearly all the administered technetium was initially localized in the lungs and the finding that only about 9% of the administered technetium was excreted by the rat in 24 hr allowed the conclusion that a major portion of the technetium initially localized in the lungs was transferred to reticuloendothelial organs in 24 hr. This transfer was considered to be an indirect evidence for a pulmonary degradation of the macroaggregate releasing its inorganic parts in colloidal forms.

DISCUSSION

During pilot studies in this work, attempts were made to heat the buffered colloid to produce the macroaggregate in a single stage. However, these attempts met at least two of the following three difficulties:

1. The suspension obtained had an opalescent suspending phase containing a substantial colloidal activity.
2. None of various combinations of temperature, duration, and agitation for the heating step resulted in a reasonably reproducible and ac-

TABLE 1. STABILITY OF THE MACROAGGREGATE IN STANDING AND AGAINST AGITATION*

Study	Percent dose/organ or carcass 10 min after i.v. injection				
	Stomach	Kidneys	Liver and spleen	Lungs	Carcass
Control	0.02 ± 0.00	0.07 ± 0.01	1.1 ± 0.1	97.8 ± 0.2	1.0 ± 0.2
10-hr standing	0.01 ± 0.00	0.06 ± 0.00	1.2 ± 0.1	97.8 ± 0.1	0.9 ± 0.1
Control	0.01 ± 0.00	0.10 ± 0.02	1.3 ± 0.2	97.3 ± 0.2	1.4 ± 0.1
200 rpm, 10 min	0.02 ± 0.00	0.08 ± 0.00	1.3 ± 0.1	97.2 ± 0.2	1.4 ± 0.1

* Upper half of table shows stability in standing under air at room temperature in two batches. Lower half shows stability against agitation in shaker in two other batches (7-ml preparation in 25-ml vial). All values shown represent mean ± s.e.m. of six rats.

ceptable distribution of the particle size. As a visual endpoint of the heating, particles of proper size for lung imaging were too small to be readily discernible by inspecting the suspension with an opalescent suspending phase.

3. Particles in the preparation obtained were fragile.

These problems led to the device of the two-staged method.

The formulation of "hard-boiled" oversize aggregates that required a forceful action for their disruption assured a mechanical strength of the product particle that was sufficient for withstanding ordinary handling of the preparation.

A sieve of 150-micron mesh was used in this study. Since more than 95% of the particle recovered in the preparation was 50 microns or smaller (Fig. 2), it would appear that the maximum particle size could be limited to 50 microns through using appropriate sieves without causing a substantial decrease in the activity yield in the preparation.

The two-staged method is essentially in a "kit" form and requires about 20 min in experienced hands. The only "radiochemical separation" step in the method is the mechanical sieving which provides for a desired upper limit in the particle size. The size control, strength, and degradability of the particle that are possible with this relatively simple approach make it a potentially useful one for producing a ready-for-use ^{99m}Tc-labeled macroaggregate by local radiopharmaceutical houses.

It is difficult to compare this macroaggregate with others (*1–10*). Data for the reproducibility and the stability of these other macroaggregates are hardly available for comparison (*1–10*). Several studies (*4,5,7,9,10*) provide data for lungs/liver uptake ratios which are generally several times lower than that found in this study. None of the methods in these other studies (*1–10*) employ a sieve. This study as well as others (*1*) indicates that the problem with particles exceeding 150 microns is a real one when the production method involves HSA macroaggregation without subsequent sieving.

Mechanical sieving is also employed in obtaining

TABLE 2. TISSUE DISTRIBUTION OF TECHNETIUM AS A FUNCTION OF TIME FOLLOWING ADMINISTRATION OF THE MACROAGGREGATE*

Time after i.v. injection	Batch of macro-aggregate	No. of rats	Percent body ^{99m}Tc/organ or carcass				
			Stomach	Kidneys	Liver and spleen	Lungs	Carcass
5 min	J	3	0.02 ± 0.01	0.05 ± 0.02	0.9 ± 0.3	97.7 ± 0.5	1.4 ± 0.3
10 min	A–J	30	0.02 ± 0.02	0.10 ± 0.05	1.0 ± 0.4	97.6 ± 0.8	1.3 ± 0.5
1 hr	I,K	6	0.06 ± 0.02	0.25 ± 0.07	2.9 ± 0.8	94.1 ± 1.2	2.6 ± 0.5
3 hr	F,I,J	9	0.20 ± 0.07	0.55 ± 0.30	6.9 ± 1.9	87.3 ± 3.3	5.0 ± 2.0
6 hr	F,H–J	12	0.27 ± 0.09	0.96 ± 0.48	16.9 ± 3.6	72.0 ± 5.3	9.9 ± 2.3
9 hr	G,H	6	0.15 ± 0.04	0.98 ± 0.14	31.7 ± 9.6	54.3 ± 11.3	12.9 ± 1.9
12 hr	G,H,K	9	0.13 ± 0.04	0.98 ± 0.17	43.5 ± 9.2	38.9 ± 11.0	16.4 ± 3.1
18 hr	F,K	6	0.11 ± 0.03	1.10 ± 0.43	61.6 ± 6.0	21.9 ± 7.4	15.3 ± 2.9
24 hr	F,K	6	0.08 ± 0.04	1.38 ± 0.58	66.9 ± 8.5	13.3 ± 5.7	18.3 ± 5.5

* Results from experiments involving 11 batches of macroaggregate prepared using 7, 6, and 2 lots of stock solutions of Sn(II), HSA, and phosphate buffer, respectively. Before preparation, Sn(II) solutions had been in storage for 0–27 days. Values shown are mean ±s.d.

a desired uniformity of particle size in a given batch of HSA microspheres (*12–14*). Recently, a simple kit became commercially available for preparing a ^{99m}Tc-labeled microsphere and the latter appears to be the current agent of choice for perfusion lung imaging. The ^{99m}Tc label tends to leach out of the microsphere (*14,15*). It is not clear at this stage of its development whether it is to become the exclusive agent for perfusion lung imaging.

SUMMARY

A rapid two-staged method was described for converting a technetium-tin(II) colloid stabilized with albumin into a technetium-labeled macroaggregate. In the first stage of the method, the colloid was heated to convert it into a suspension of oversize aggregates. In the second stage, the oversize aggregate was forcefully disrupted into fragments, and those fragments still exceeding 150 microns were removed with a sieve.

The macroaggregate prepared by this method has a controlled upper limit of the particle size, a low content of free or colloidal technetium, and a reasonable stability in vitro and degradability in vivo.

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