Particle Size Analysis of ^{99m}Tc-Labeled and Unlabeled Antimony Trisulfide and Rhenium Sulfide Colloids Intended for Lymphoscintigraphic Application

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Colloidal particle size is an important characteristic to consider when choosing a radiopharmaceutical for mapping sentinel nodes in lymphoscintigraphy. **Methods:** Photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM) were used to determine the particle size of antimony trisulfide and rhenium sulfide colloids, and membrane filtration (MF) was used to determine the radioactive particle size distribution of the corresponding ^{99m}Tc-labeled colloids. **Results:** Antimony trisulfide was found to have a diameter of 9.3 ± 3.6 nm by TEM and 18.7 ± 0.2 nm by PCS. Rhenium sulfide colloid was found to exist as an essentially trimodal sample with a $d_{v(max1)}$ of 40.3 nm, a $d_{v(max2)}$ of 438.6 nm, and a d_v of 650–2200 nm, where d_v is volume diameter. ^{99m}Tc-antimony trisulfide by MF showed that more than 96% of radioactive particles were smaller than 100 nm; however, ^{99m}Tc-rhenium sulfide showed that more than 21% were smaller than 100 nm. These radioactive colloids were used with seven different membrane compositions and found not to adsorb significantly to any of them. **Conclusion:** MF was validated as a simple and reliable technique to estimate the percentage radioactive particle size distribution.

Key Words: antimony trisulfide colloid; lymphoscintigraphy; sulfur colloid; particle size; radioactive particle size distribution

J Nucl Med 2001; 42:460–466

Since its description in 1965, ^{99m}Tc-antimony sulfide colloid has been used for bone marrow imaging (*1*), lymphedema assessment (*2*), and, more recently, scintigraphic mapping of lymphatic channels and sentinel nodes (*3,4*) in melanoma (*5*) and breast cancer (*6,7*). In lymphoscintigraphy, a radiopharmaceutical should move at a rate similar to that of the physiologic flow of lymph and should be sufficiently retained by intervening nodes. Colloids are highly desirable for such studies because their particulate nature allows them to be retained by the lymph nodes (*8,9*) by a phagocytic mechanism, yet the rate of colloid transport through the lymphatic channels is directly related to their particle size (*10*). Particles smaller than 4–5 nm have been reported to penetrate capillary membranes and therefore may be unavailable to migrate through the lymphatic channel. In contrast, larger particles (~500 nm) clear very slowly from the interstitial space and accumulate poorly in the lymph nodes (*11*) or are trapped in the first node of a lymphatic chain so that the extent of nodal drainage in contiguous areas cannot always be discerned (*5*). After injection, the radiocolloid travels to the sentinel node through afferent subcapsular lymphatics, where they are retained by the sinusoids and phagocytosed by the reticuloendothelial macrophages of the node (*2*). Particles smaller than 0.1 μm show the most rapid disappearance from the interstitial space into the lymphatic channels, with good retention in the lymph node (*12*). Most larger particles do not move easily beyond the first node site encountered. The permeability of colloids in lymph is maximal for particles smaller than 50 nm (*2*). An ideal radiopharmaceutical colloid should therefore comprise particles near, but not less than, 4–5 nm in diameter to avoid capillary penetration, reduce retention of activity at the injection site, and achieve high retention in the subsequent lymph nodes.

An early radiopharmaceutical used to image the lymphatic system was ^{198}Au-colloid, having a uniform particle size of 3–5 μm in diameter. However, this agent is not readily available today because of clinical limitations from its isotope being a β-emitter and emitting an unfavorable γ-ray for scintillation camera imaging (*13*) and because of the difficulties its manufacture represents (M. Jenson, written communication, July 1998). Other widely used technetium-bound radiopharmaceuticals that comprise particles approaching the size of the ^{198}Au-colloid include ^{99m}Tc-rhenium sulfide colloid and ^{99m}Tc-nanocolloid. ^{99m}Tc-sulfur colloid is a commonly used agent in the United States (*12*) for liver imaging, for oral gastric emptying studies (*14*), as a filtered (0.1 μm) preparation for lymphoscintigraphy (*15*), or as ^{99m}Tc-rhenium sulfide colloid (*16*). ^{99m}Tc-nanocolloid

Received May 30, 2000; revision accepted Nov. 14, 2000.

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is used extensively in Europe, and similar preparations are available (NANOCOLL and ALBU-Res; Nycomed-Amersham Sorin, Milan, Italy). These products result in good lymph uptake and images of flow (*17*), but poor retention limits their use in delayed images.

^{99m}Tc-antimony trisulfide colloid is currently commercially unavailable in the United States (*11*) but is an approved therapeutic drug in Australia and New Zealand for routine clinical use as a lymphoscintigraphic agent. (The Royal Adelaide Hospital Radiopharmacy, Adelaide, Australia, is the only current manufacturer of the lymphoscintigraphic agent Lymph-Flo [kit for the preparation of ^{99m}Tc-Colloidal Antimony Sulphide Injection], a product that has been commercially available in Australia and New Zealand for more than 10 y.) Antimony trisulfide colloid products have been previously commercially available (*5,18,19*). The ^{99m}Tc-antimony trisulfide colloidal particles have been reported as ranging from 3 to 30 nm, an optimum size for imaging lymphatic channels in lymphoscintigraphy (*20*). The most widely used measurement technique is transmission electron microscopy (TEM), which represents the projected area diameter (d_a) of a particle in stable orientation, but other techniques that measure and describe particle size, such as surface diameter and volume diameter (d_v), are also used. (*21*).

Particle size is a primary consideration when a radiocolloid is chosen for clinical mapping of lymphatic channels and nodes (Table 1). Based on this concept, three sizing techniques were investigated with two colloids, including photon correlation spectroscopy, TEM, and membrane filtration (MF) for the ^{99m}Tc-colloids. In particular, an aim of this study was to validate the simple MF technique for ^{99m}Tc-rhenium sulfide colloid versus ^{99m}Tc-antimony trisulfide colloid with different membrane compositions.

TABLE 1
Particle Size Diameter of Reported Colloids

Colloidal agent	Particle size (nm)	Percentage	Reference
Microaggregated albumin (nanocolloid)	<80	95	12
Rhenium sulfide	4–12	—	16
Sulfur colloid	100–300	—	16
	305–340	—	15
Lyocoll*	<200	74.4	
	200–1,000	22.4	29
TCK-1†	<200	58.2	
	200–1,000	24.0	
	1,000–3,000	7.8	
	>3,000	10.0	29
0.1-μm filtered	10	—	
	89–173	<0.1	15
	>200	56.8‡	
	30–200	7.3‡	
	<30	36.2‡	12
Antimony trisulfide	38.1 ± 3.0	—	37
	3–15 or 10–25	—	18
	3–18	—	24
	~9	—	38
	<30	—	25
	4–10	—	39

*Mallinckrodt Diagnostica, Petten, Holland.
†Oris-Industrie, Cedex, France.
‡48.3% were >0.2 μm, 5.4% were 0.2–0.03 μm, and 46.7% were <0.03 μm with old ^{99m}Tc-pertechnetate milked; 83.2% were >0.2 μm, 2.2% were 0.2–0.3 μm, and 14.7% were <0.03 μm with prolonged heating and freshly milked ^{99m}Tc-pertechnetate.

MATERIALS AND METHODS

Radiolabeling Procedures

The rhenium sulfur colloid kit and Lymph-Flo were manufactured in this radiopharmacy, where current batches were used for all experiments. ^{99m}Tc-pertechnetate was obtained from the daily milking of a wet-bed ^{99}Mo/^{99m}Tc generator (TC9M1; Australian Radioisotopes; Sydney; Australia). Normal saline (0.9%) for injection was used for dilutions and washes.

Preparation of ^{99m}Tc-Colloidal Antimony Sulphide Injection. Lymph-Flo consists of five separate nonradioactive components (antimony trisulfide, phosphate buffer, HCl, an empty sterile 10-mL vial, and a sterile 0.2-μm filter). The standard procedure for preparing this radiocolloid included adding ^{99m}Tc-pertechnetate (0.8–0.9 GBq/1.0 mL saline) and then HCl (1.0 mol/L; 0.1 mL) to the vial containing antimony trisulfide. This reaction vial was heated in a boiling water bath (100°C) for 30 min, allowed to cool to room temperature, and then adjusted to pH 5.5–6.5 with phosphate buffer (0.5 mL) before MF.

Preparation of ^{99m}Tc-Rhenium Sulfide Colloid. The rhenium sulfur colloid kit consists of four separate nonradioactive components (10% gelatin solution [10 × 1 mL], thiosulfate solution [19.2 mg potassium perrhenate and 80.0 mg sodium thiosulfate in 8.0 mL water for injection], 1.0 mol/L HCl [8.0 mL], and basic phosphate buffer [280.0 mg sodium dihydrogen phosphate dihydrate in 8.0 mL of 1.0 mol/L sodium hydroxide]). The standard procedure for preparing ^{99m}Tc-rhenium sulfide colloid included adding ^{99m}Tc-pertechnetate (0.5–1.0 GBq/0.5 mL saline), HCl (1.0 mol/L; 0.5 mL), and then thiosulfate solution (0.5 mL) to one vial containing 10% gelatin. This reaction vial was heated in a boiling water bath for 3–5 min, allowed to cool to room temperature, and then adjusted to pH 5.0–6.5 with basic phosphate buffer (0.5 mL) before MF.

Radiochemical Analyses

Radiochemical purity (% RCP) was determined (≤6 h of preparation for ^{99m}Tc-antimony trisulfide and <1 h for ^{99m}Tc-rhenium sulfide) by ascending instant thin-layer chromatography using silica gel–impregnated glass fiber sheets (Gelman Sciences, Ann Arbor, MI). Strips 1 cm wide × 20 cm long were marked from the origin every 1 cm for 10 cm and then were spotted with sample and developed in the solvent (0.9% saline). Radiocolloid remained at the origin, and free ^{99m}Tc-pertechnetate migrated with the solvent front. The strips were cut into 1-cm sections and counted in a γ counter (Packard Auto-Gamma 5650; Hewlett Packard, Palo Alto, CA) over a ^{99m}Tc window (70–210 keV). The % RCP limits for ^{99m}Tc-Colloidal Antimony Sulphide Injection and for ^{99m}Tc-rhenium sulfide colloid are greater than 95%. Radioactive colloids were used only in the MF and membrane adsorption analyses.

Particle Size Analyses

PCS. Each d_v value for antimony trisulfide and rhenium sulfide was determined as undiluted samples at 20°C according to a reported procedure (*22*) using a light-scattering photon correlation spectroscopy (PCS) instrument (Zetasizer 3000; Malvern Instruments, Malvern, United Kingdom). Rhenium sulfide was prepared in the same manner as ^{99m}Tc-rhenium sulfide colloid, except that saline was added instead of sodium ^{99m}Tc-pertechnetate. Measurements were made with reference to the viscosity of the solutions (20°C) that the colloids were dispersed in. These values were obtained by separately centrifuging (100,000*g*) antimony trisulfide and rhenium sulfide colloidal dispersions for 30 min at room temperature and then using the supernatants to determine their viscosities in reference to water through the flow rate method (*23*). The results for rhenium sulfide were obtained as unfiltered and filtered (0.1 and 0.45 μm; Millipore, Milford, MA) to accurately establish the volume mean values and corresponding size ranges of the smaller populations. The volume-distribution–averaged particle size results were used to determine the modality type of the colloidal sample and the mean diameter values per size distribution range. The harmonic-intensity–averaged size distribution results were used to determine the percentage area of the total particle population or the percentage of area greater than 20 nm. All experiments were performed in triplicate.

TEM. TEM was used to obtain images of antimony trisulfide colloid as previously reported (*24*) on an instrument (CM200 TEM/STEM; Philips Electron Optics, Eindhoven, The Netherlands). Random photographs (×52,000 magnification) were taken of the colloidal sample, and each particle ($n = 956$) was measured against a calibrated scale to determine d_a.

MF. All filters were preequilibrated with an initial wash of saline (1.0 mL) and finally rinsed with saline (1.0 mL) after filtration. Typically, a radiocolloid sample (1.0 mL) was withdrawn from the reaction vial and filtered through the 0.8-μm filter. The filter was rinsed, the filtrates were combined, and then filter and filtrate were counted separately in a validated counting unit (radioisotope calibrator CRC-12; Capintec, Pittsburgh, PA). A radiocolloid sample (1.0 mL) was withdrawn from the previous filtrate, filtered through the 0.45-μm filter, and rinsed. This filter and filtrate were counted. This process was repeated for the 0.2-μm, 0.1-μm, and 0.02-μm filters. All values were corrected for background. The radiocolloid sample volume was 0.5 mL when the 0.02-μm filter was used. ^{99m}Tc-rhenium sulfide was filtered above 0.2 μm ($n = 3$) and below 0.2 μm ($n = 6$). ^{99m}Tc-antimony trisulfide was filtered below 0.2 μm ($n = 5$) only.

TABLE 2
Mean Volume Diameter and Size Range Data by PCS

Colloid	d_v (nm)*	Size range (nm)*
Antimony trisulfide	18.7 ± 0.2	16.8–23.2
Rhenium sulfide	40.3 ± 9.6	21.7–65.7
	438.6 ± 14.5	255.0–562.4
	—	650–2,200

*$n = 3$.

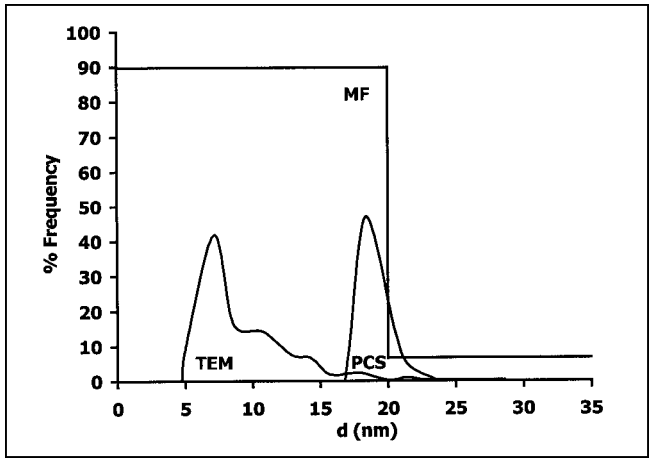

FIGURE 1. Comparison of three techniques to characterize size distribution of antimony trisulfide colloid (by TEM and PCS) and ^{99m}Tc-antimony trisulfide colloid (by MF). d = diameter.

Adsorption Studies

The sterile filters (of varying membrane composition) were 0.8 μm (Gelman Sciences), 0.45 μm (Gelman Sciences), 0.22 μm (Millipore), 0.2 μm (Nucleopore, Pleasanton, CA; Sartorius, Goettingen, Germany; Whatman, Chinatown Point, Singapore), and 0.1 μm or 0.02 μm (Whatman). Each filter was broken in half, and the intact membrane was cut away from the housing with care taken not to contaminate the membrane by touch. The intact membrane was cut into a small square piece (0.5–0.9 cm) and placed into an empty vial (10 mL). ^{99m}Tc-antimony trisulfide (26 MBq/mL; 1 mL) was added to the membrane piece and allowed to stand at room temperature (30–60 min) with occasional swirling (every 10 min). The radioactive liquid was removed from the vial by syringe, and the membrane was rinsed with saline (5 × 1.0 mL). All rinses were combined with the radioactive liquid. After the membrane was gently blotted dry (hand-towel paper; cellulose acetate composition), it was counted in the Capintec unit separately, as were the combined rinses plus blotting paper. ^{99m}Tc-rhenium sulfide (10 MBq/mL; 1 mL) was added to distinct membrane pieces as described above, and the individual rinse solutions plus membranes were then likewise counted to determine the percentage of adsorbed activity. All experiments were performed in triplicate.

RESULTS

PCS

The principal d_v values of antimony trisulfide and rhenium sulfide colloids by PCS are summarized in Table 2. The d_v is defined as the diameter of a sphere having the same volume as the particle. For antimony trisulfide particles, the mean d_v, $d_{v(total)}$, was found to be 18.7 ± 0.2 nm of the total population (Fig. 1). The diameter of the highest particle frequency in the total population $d_{v(max)}$ was 18.7 nm, and the range of the total particle population was 16.8–23.2 nm. Ninety percent of the more frequent particle population were in the range 17.3–21.7 nm. Of the total population of antimony sulfide particles, 96.2% were less than or equal to 20 nm.

Rhenium sulfide particles were found to exist as a polymodal distribution (Fig. 2) comprising a broad population of

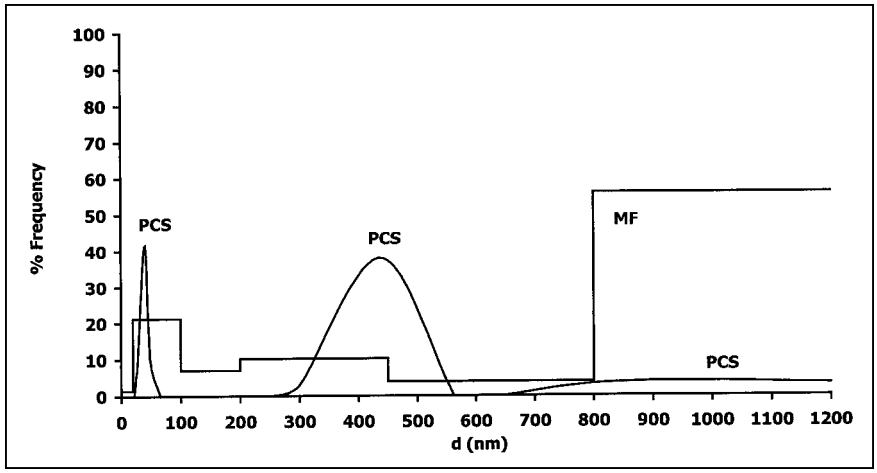

FIGURE 2. Comparison of two techniques to characterize size distribution of rhenium sulfide colloid (by PCS) and ^{99m}Tc-rhenium sulfide colloid (by MF). d = diameter.

31.1% between 650 and 2,200 nm (28.3% of particles were >800 nm), and 68.9% were less than 600 nm as a bimodal distribution in the unfiltered samples. The filtered samples enhanced the resolution of the two size ranges below 600 nm, in which 7.6% and 61.3% of the colloidal particles in the sample were characterized by a mean $d_{v(max1)}$ value of 40.3 nm and a mean $d_{v(max2)}$ value of 438.6 nm, respectively. The size was between 200 and 800 nm for 64.1% of the particles.

TEM

Antimony trisulfide particles were visualized as spheric by TEM, and size was thus described as d_a. The d_a is defined as the diameter of a circle having the same area as the particle viewed normally to a plane surface on which the particle is at rest in a stable position. The $d_{a(total)}$ of the total population of antimony trisulfide particles was found to be 9.3 ± 3.6 nm (n = 956). From Figure 1, the $d_{a(max)}$ of the highest particle frequency in the total population was 7.1 nm, and the range of the total particle population was 4.7–21.4 nm. Ninety percent of the more frequent particles were in the range 4.7–12.9 nm. Of the total population of antimony sulfide particles, 98.1% were less than or equal to 20 nm.

% RCP

The % RCP was 99.4% ± 0.2% for ^{99m}Tc-Colloidal Antimony Sulphide Injection (n = 8) and 99.1% ± 0.6% for ^{99m}Tc-rhenium sulfide colloid (n = 5).

MF

The results in Table 3 indicate that for ^{99m}Tc-antimony trisulfide, 89.7% of the total ^{99m}Tc-particles are smaller than 20 nm, accounting for more than 98% of the antimony trisulfide colloid peak by TEM ($d_{a(total)}$ = 9.3 nm) and more than 96% of the antimony trisulfide peak by PCS (d_v = 18.7 nm). In the case of ^{99m}Tc-rhenium sulfide, 21.3% of the total radioactive particles exist between 20 and 100 nm, accounting for 71.5% of the rhenium sulfide colloid peak characterized by PCS at a $d_{v(max1)}$ of 40.3 nm. Of ^{99m}Tc-rhenium sulfide particles, 14.2% exist between 200 and 800 nm, accounting for 31.8% of the rhenium sulfide peak (PCS) at a $d_{v(max2)}$ of 438.6 nm. Of radioactive rhenium sulfide particles, 59.9% exist at more than 450 nm, and all particles larger than 650 nm are ^{99m}Tc labeled.

Membrane Adsorption

The extent of membrane adsorption of ^{99m}Tc-rhenium sulfide and ^{99m}Tc-antimony sulfide colloid, each on different membrane compositions, is shown in Table 4. Neither of these colloids was significantly adsorbed to any of the seven membranes examined.

DISCUSSION

The advantages and limitations of particle size analysis techniques at the micron and submicron levels (*24*) have been reported for TEM, PCS (also known as laser or quasi-electron light scattering), MF, and gel filtration chromatography (*25*). Gel filtration chromatography is not practical because of its lengthy column-packing time and running time (*25*) and results in a radioactive particle size distribution rather than a particle size distribution. MF is the only other technique reported (*26*) to measure radioactive particle size distribution, but only with polycarbonate-based filters. PCS has the advantages of measuring particles in their traditional environment with little or no sample prep-

TABLE 3
Radioactive Particle Size Distribution

^{99m}Tc-colloid	% Activity in particle size range (d_v, μm)			
	>0.2	0.1–0.2	0.1–0.02	<0.02
Antimony trisulfide*	0.7 ± 0.1	2.9 ± 1.5	6.7 ± 5.0	89.7 ± 5.9
Rhenium sulfide†	72.1 ± 5.6	6.6 ± 1.7	20.0 ± 5.9	1.3 ± 0.3

*n = 5.

†n = 6. Percentage radioactive particle size distribution above 0.2 μm (n = 3): >0.8 μm, 56.0% ± 4.8%; 0.8–0.45 μm, 3.9% ± 1.8%; 0.45–0.2 μm, 10.3% ± 1.7%; 0.2–0.1 μm, 7.0% ± 2.4%; 0.1–0.02 μm, 21.3% ± 5.0%; <0.02 μm, 1.5% ± 0.3%.

TABLE 4
Membrane Adsorption of ^{99m}Tc-Colloids

Filter			Percentage nonadsorbed activity	
μm	Type	Membrane composition	^{99m}Tc-antimony trisulfide*	^{99m}Tc-rhenium sulfide*
0.8	Gelman Sciences	Acrylic copolymer on nylon (Versapor)	94.7 ± 1.1	98.3 ± 0.2
0.45	Gelman Sciences	Polysulfone (HT Tuffryn)	99.3 ± 0.2	98.4 ± 2.0
0.22	Millipore	Polyvinylidene difluoride (Duropore)	99.3 ± 0.3	99.7 ± 0.3
0.2	Whatman	Polysulfone	99.2 ± 0.4	98.9 ± 0.3
0.2	Sartorius	Cellulose acetate	99.4 ± 0.0	99.5 ± 0.2
0.2	Nucleopore	Polycarbonate	99.6 ± 0.4	99.0 ± 1.3
0.1, 0.02	Whatman	Aluminium oxide (Anopore)	96.3 ± 2.2	97.7 ± 0.3

*n = 3.

aration (*27*), over 3–30 min (*18*). PCS is limited in that its measurements are based on the assumption of sphericity, its use is more problematic when the size distribution of the sample is broad (*27*), and there may be an unknown effect of electromagnetic radiation with the dispersion (*24*). Also, instrument limitations make the results unreliable below a size threshold (*28*).

TEM has the excellent resolution (0.2–0.3 nm) necessary for particle analysis at the nanometer level (compare antimony and sulfur colloids) and can show the shapes of the studied samples. Microscopy is the only particle-sizing technique in which individual particles are observed and measured. A single particle can have an infinite number of linear dimensions (shapes); hence, these diameters are meaningful when sufficient particles have been measured to give average statistical diameters for each size range. TEM uses a vacuum for sample preparation such that the viewer observes dehydrated particles, a property that may not reflect the actual size (*24*) of particles in their original environment. In addition, particles studied by TEM are heated by an electron beam, possibly causing sublimation by elemental sulfur (*18*) and thus altering colloid size.

By TEM, antimony trisulfide particles were measured to be a $d_{a(total)}$ of 9.3 ± 3.6 nm. The magnitude of the SD implies that few smaller particles exist in the total population pool, such as sulfur particles possibly generated by interaction of the colloid with the instrument electron beam. This radiocolloid was concluded to be essentially monodispersed relative to ^{198}Au-colloid (*13*) and ^{99m}Tc-sulfur colloid (*15*), after consideration of the low degree of error involved with the electron microscopy technique used (*28*). A comparison of TEM (9.3 nm) and PCS (18.7 nm) indicates that these techniques found significantly different mean diametric sizes for antimony trisulfide colloid. Even when the viscosity of the solution was accounted for, a lower accuracy for the PCS instrument resulted in an overestimation of particle size at that size level. PCS was found to be more reliable for rhenium sulfide colloid particles because they are large enough to exceed the size-threshold limitation (*27*).

With polymodal-sized colloidal sols, more radioactive atoms would be expected to bind to larger particles as a result of a larger surface area of the exposed reactive atoms. This effect can be a major disadvantage when the smaller particles in the range are needed, where filtering off the larger particles leaves a low concentration of smaller radioactive particles. However, ^{99m}Tc-sulfur colloid is thought to form by a nucleation of ^{99m}Tc atoms followed by sulfur atoms; therefore, the smaller particles generally contain high levels of technetium (*29*). This is in contrast to ^{99m}Tc-antimony trisulfide, in which the radioactive metal is more evenly dispersed among the colloidal particles. In effect, covalent binding of ^{99m}Tc to the surface of preformed antimony trisulfide particles during the radiolabeling reaction would not cause a significant alteration in size. The reasons relate to the number, size, and chemical reactivity of these particles compared with ^{99m}Tc atoms.

The ionic diameter of Tc(VII) is 0.196 nm (*30*), and that of Tc(V) is 0.148 nm (*31*). The oxidation state of technetium bound to the antimony colloid surface is not known, but if the largest-diameter value is chosen, then ideally, a monolayer of technetium atoms covering the surface of one particle (d_v or d_a = 10 nm) would result in a minor increase of 8% in the projected area of a circular particle or 12% in the volume of a spheric particle (*21*). Consequently, the radioactive particle is only slightly larger than the nonradioactive colloidal particle with a monolayer of technetium atoms. The % RCP of ^{99m}Tc-antimony sulfide was found to be 99.4%, indicating a quantitative conversion of pertechnetate moles to ^{99m}Tc product. In practice, a generator (*32*,*33*) eluate milked daily would have fewer moles in 1,000 MBq $^{99m}TcO_4^-$ (activity used for kit preparation) than the 3.1 μmol in an antimony trisulfide vial. This indicates that an excess of cold antimony trisulfide particles exists in the radiolabeling solution and that few technetium atoms are bound to the surface of one colloid particle. Furthermore, the low ^{99m}Tc concentration also suggests that any bound ^{99m}Tc atoms will not significantly affect the rate of reaction of colloid with other atoms. The colloidal particles are certainly not spheric at the molecular level, yet for each

uniformly sized, chemically homogeneous particle, the kinetics of the labeling reaction should be the same. Therefore, we can study the size of preformed antimony sulfide colloidal particles and approximate the results to ^{99m}Tc-antimony trisulfide colloidal particles with confidence.

The most rapid and simplest technique for measuring the radioactive particle size distribution is MF. The principle of size measurement for this technique is based on d_v. These experiments showed that, of the total radioactivity associated with colloidal particles of antimony trisulfide, almost 90% was associated with particles smaller than 20 nm. With TEM, the relative percentage frequency by number (Figs. 1 and 2) indicates that more than 98% of antimony trisulfide particles are 20 nm or smaller, a value that is higher than that determined for the radioactive particles by MF. Perhaps the small error ($\pm$5.9%) with MF, in conjunction with a slight adsorption to the filter, explains the underestimation of the percentage of ^{99m}Tc-antimony trisulfide particles smaller than 20 nm. The PCS results better correlate with the MF data, indicating that the colloidal particles between 20 and 23.2 nm (3.8%) could account for the radioactivity ($\sim$7%) associated with particles in the 20- to 100-nm filtrate fractions. However, more than 70% of activity was associated with rhenium sulfide colloidal particles greater than 200 nm, and more than half of radioactive particles were larger than 800 nm. Approximately 30% of particles found in the $>$800-nm range by PCS were labeled and associated with this bulk ^{99m}Tc activity. The small population of rhenium sulfide colloidal particles in the 22- to 66-nm range accounted for more than 20% of total radioactivity, with approximately three fourths of these particles being ^{99m}Tc-labeled. The largest population of particles between 200 and 800 nm accounted for more than 14% of total activity, with approximately one third of these being ^{99m}Tc-labeled. Thus, unlike the case for antimony trisulfide colloid, the ^{99m}Tc-particle size distribution is clearly not proportional to the particle size distribution for unlabeled rhenium sulfide colloid.

Of the ^{99m}Tc-pertechnetate activity added to make ^{99m}Tc-rhenium sulfide colloid, only 21% was used to produce radioactive particles (compare 0.1-μm filtered preparation) useful for lymphoscintigraphic studies. For antimony trisulfide, in contrast, 96% of the initial ^{99m}Tc-pertechnetate added is converted to ^{99m}Tc-antimony trisulfide particles ($<$100 nm), showing a far more efficient use of activity. For ^{99m}Tc-rhenium sulfide colloid synthesis, the conditions used in this laboratory resulted in radioactivity dominating the larger particles, suggesting that the ^{99m}Tc atoms continue to bind with sulfur in the mechanism (*34*) of colloid formation long after nucleation.

Studies from the literature have shown a preference for using Nucleopore filters for measurement of radioactive particle size distribution. Nucleopore filters have membranes composed of polycarbonate films with cylindric pores distributed evenly over the filter (*26*). Anopore filters (0.02 and 0.1 μm, Whatman) also have inorganic membranes with vertical (hexagonal) pores (*35*); however, other filters contain organic membranes, such as acrylic copolymer on nylon (0.8 μm, Gelman Sciences), polysulfone (0.45 μm, Gelman Sciences, and 0.2 μm, Whatman), polyvinylidene difluoride (0.22 μm, Millipore), and cellulose acetate (0.2 μm, Sartorius), that comprise a tortuous network throughout their thickness (*36*). Nucleopore filters are not readily available in Australia, prompting examination of other commercial filters for application in measurements of radioactive particle size distribution.

The typical colloidal sol is sensitive to the concentrations and charges of its environment (*23*), and it was unknown whether the surface-charge density of filter membranes could influence the sol to induce particle adsorption. An earlier report (*26*) showed erroneous size distribution data when ^{198}Au-colloid was significantly retained after filtration through Millipore filters with cellulose fiber membranes. Hence, the adsorption properties of the labeled antimony trisulfide and rhenium sulfide colloids with different filter membrane compositions were investigated. ^{99m}Tc-antimony sulfide colloid was found to adsorb slightly to Versapor (Gelman Sciences) (5.3%) and Anopore (3.6%) membranes, yet insignificantly ($<$1.0%) to the others. Likewise, ^{99m}Tc-rhenium sulfide colloid was found to adsorb slightly to Anopore (2.3%) and insignificantly to the others. These results provide evidence that adsorption of ^{99m}Tc-antimony trisulfide and ^{99m}Tc-rhenium sulfide colloids to these membranes does not influence the percentage radioactive particle size distribution determined by the MF method.

CONCLUSION

Antimony trisulfide colloidal particles were determined by TEM to have a diameter of 9.3 nm. TEM is a desirable size-measuring technique because it is more reliable than methods such as PCS, which cannot adequately cope with sizing particles at approximately the 10-nm level without accruing significant error. PCS is better suited for larger particles such as rhenium sulfide colloid, which, when prepared in this laboratory, gave a polymodal distribution at a $d_{v(max1)}$ of 40.3 nm, a $d_{v(max2)}$ of 438.6 nm, and a d_v of 650–2200 nm. When radioactive particle size distribution was compared with particle size distribution, the results were found to be directly proportional for preformed antimony trisulfide colloid but not for ^{99m}Tc-rhenium sulfide, because this radiocolloid is formed by a nucleation reaction in situ. However, the radioactive particle size distribution of ^{99m}Tc-antimony trisulfide or ^{99m}Tc-rhenium sulfur colloids can be successfully determined using MF, a rapid and reliable technique.

ACKNOWLEDGMENTS

The author thanks Phil Harrip (ATA Scientific Pty Ltd., Sydney, New South Wales, Australia) for invaluable assistance with the PCS measurements. The author also thanks John Terlet and Dr. Marilyn Henderson (The Center for

Electron Microscopy and Microstructure Analysis, Adelaide, South Australia, Australia) for invaluable assistance and use of their facilities for the TEM measurements.

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