Filtered Technetium-99m-Sulfur Colloid Evaluated for Lymphoscintigraphy

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Several ^{99m}Tc-labeled radiopharmaceuticals have been developed for lymphoscintigraphy of the extremities. In the United States, however, these agents are not widely used clinically. This study evaluates the use of smaller particle sizes (< 0.1 μm) of ^{99m}Tc-sulfur colloid (^{99m}Tc-SC) for lymphoscintigraphy. **Methods:** The ^{99m}Tc-SC was prepared by kit, and the final preparation was filtered through a sterile 0.1-μm filter. The radiochemical purity (RCP) of the filtered ^{99m}Tc-SC was determined before administration. Nineteen patients with suspected lymphedema were injected with 18.5 MBq (500 μCi) filtered ^{99m}Tc-SC intradermally in each foot, and whole-body images were obtained immediately and 1, 3, 6 and 24 hr later. Local views over the inguinal or axillary lymph nodes were also obtained every 5 min for the first hour. **Results:** The average RCP value was 93.4% ± 4.2% (n = 19), and the RCP difference pre- and postfiltration of the ^{99m}Tc-SC preparation was −1.7% ± 1.4% (n = 40). Evaluation of the particle size with the polycarbonate filter showed that 89.9% ± 4.5% (n = 28) of particles were less than 50 nm, and the particle size was further determined by electron microscopy to be 38.0 ± 3.3 nm (n = 202). The mean particle sizes of two peaks measured by laser light scattering techniques were 7.5 and 53.9 nm (major peak). Clinical studies with filtered ^{99m}Tc-SC demonstrated similar lymphoscintigrams compared with those obtained with ^{99m}Tc antimony sulfide colloid (^{99m}Tc-ATC). Filtered ^{99m}Tc-SC showed a faster transport rate to the inguinal lymph nodes and lower radiation dosimetry for liver, spleen and whole body compared with ^{99m}Tc-ATC. **Conclusion:** Filtered ^{99m}Tc-SC can be easily prepared and is readily available for routine clinical use in lymphoscintigraphic studies.

Key Words: filtered technetium-99m-sulfur colloid; lymphoscintigraphy; membrane filtration; radiocolloid size determination

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Among a number of similar radiopharmaceuticals, ^{99m}Tc-antimony trisulfide colloid (^{99m}Tc-ATC) was previously the radiopharmaceutical most frequently used for lymphoscintigraphy in the diagnosis of the edematous extremity. This drug, which could be readily prepared from [^{99m}Tc]pertechnetate mixed with a cold kit, had an optimal particle size (3–30 nm) and yielded satisfactory scintigrams of the peripheral lymphatic channels and nodes after interdigital injection into the intradermal tissue. At present, commercial ATC kits are not available in the United States, and past users of ^{99m}Tc-ATC under Investigational New Drug sponsorship now need to look for a replacement.

A number of technetium-labeled agents have been investigated for use in lymphoscintigraphy in the past. The most significant ones are ^{99m}Tc-human serum albumin (^{99m}Tc-HSA), ^{99m}Tc stannous phytate and ^{99m}Tc-sulfur colloid (^{99m}Tc-SC). Although McNeill et al. (*1*) reported encouraging results from whole-body lymphangioscintigraphic studies using ^{99m}Tc-HSA in 10 patients we could not reproduce lymphoscintigrams of similar quality to ^{99m}Tc-ATC with ^{99m}Tc-HSA (unpublished data). In vivo, ^{99m}Tc-stannous phytate forms a colloid of about the same size as ^{99m}Tc-ATC (*2*) but has also been found to be inferior to ^{99m}Tc-ATC in clinical studies (*2,3*).

The clinical efficacy of the radioactive colloid preparation for lymphoscintigraphic studies is highly dependent on colloid particle size and stability because the radiopharmaceutical has to be absorbed by the peripheral lymph receptors to gain entrance into the lymphatic system. A uniform dispersion of small particles (< 100 nm) is necessary for the colloid to translocate from the interstitial injection site to the lymphatic channels and nodes. Large particles (500–2000 nm) remain trapped at the injection site and are unsatisfactory (*4*). Technetium-99m-ATC had a small particle size range, i.e., 3 to 30 nm, which was optimal for lymphoscintigraphy. Radioactive sulfur colloid produced by the hydrogen sulfide method (i.e., ^{99m}Tc sulfur minicolloid) has small particles (< 100 nm) and produced satisfactory lymph node scans (*5,6*); however, the method of preparation is technically cumbersome. Technetium-99m-SC produced by the thiosulfate kit method is an approved drug for liver scanning but is unsatisfactory for lymphoscintigraphy because of the relatively large particle size and range (100–1000 nm); therefore, particle migration from the injection site is poor (*7*). Although Sacks et al. (*8*) described a method of using either a 0.2-μm polycarbonate film filter or 0.22-μm cellulose membrane filter to remove larger

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^{99m}Tc-SC particles, this filtration method does not eliminate ^{99m}Tc-SC particle size between 100 and 220 nm. In addition, the yield of the ^{99m}Tc-SC filtered through those two filters is only approximately 10%.

The purpose of this study was to determine whether satisfactory lymphoscintigraphy could be performed with a filter of smaller pore size to isolate the smaller particles (i.e., < 100 nm) in the ^{99m}Tc-SC radiopharmaceutical prepared by the thiosulfate kit method.

MATERIALS AND METHODS

Preparation of Technetium-99m-SC

We used the AN-SULFUR COLLOID® kit (CIS-US, Inc., Bedford, MA) in this study. The kit consists of one vial and two syringes. The 10-ml reaction vial contains, in lyophilized form, 2.0 mg anhydrous sodium thiosulfate, 2.3 mg edetate disodium and 18.1 mg gelatin. Syringe A contains 1.5 ml 0.148 *N* HCl solution and syringe B contains 1.5 ml of an aqueous solution of 38.8 mg anhydrous sodium biphosphate and 11.1 mg NaOH. Technetium-99m-SC was prepared according to the package insert (*9*) with 3 ml of 7400–9250 MBq (200–250 mCi) sodium [^{99m}Tc]pertechnetate injection (Ultra-TechneKow FM®, Mallinckrodt Medical, Inc., St. Louis, MO). The total volume in the ^{99m}Tc-SC vial was 6 ml. The sodium [^{99m}Tc]pertechnetate injection was initially tested and contained less than 10 μg aluminum per milliliter of ^{99m}Tc eluate.

Filtration of Technetium-99m-SC

A 1-ml aliquot of ^{99m}Tc-SC was withdrawn into a lead-shielded 3-ml syringe. For the filtration process, a sterile and nonpyrogenic 29-mm diameter membrane filter of 0.1-μm pore size (Millex®-VV, Millipore Corp., Bedford, MA) was used. The needle from the ^{99m}Tc-SC dosage syringe was carefully removed, and the syringe was aseptically attached to the female Luer-Lok™ (Millepore Corp., Bedford, MA) inlet end of a dry 0.1-μm filter (Fig. 1). A fresh and sterile 25-gauge needle was attached to the male Luer slip outlet end of the sterile filter (Fig. 1). The 25-gauge needle was aseptically placed into the rubber stopper of an empty 2-ml shielded serum vial, and ^{99m}Tc-SC injection was slowly pushed through the filter into the 2-ml vial. The filtered solution in the vial was visually examined for discoloration and particulate clumping.

Filter Retention of Technetium-99m-SC

The radioactivity of ^{99m}Tc-SC retained on the 0.1-μm filter was counted in a dose calibrator. The measured retained activity was divided by the total activity of the prefiltered ^{99m}Tc-SC preparation to calculate the percentage of the retained ^{99m}Tc-SC activity on the filter.

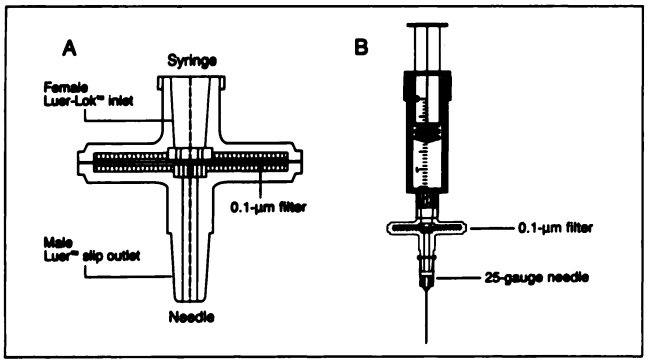

FIGURE 1. Setup for the filtration of ^{99m}Tc-SC. (A) Cut away diagram of the 0.1-μm filter. (B) The ^{99m}Tc-SC syringe and needle attached to 0.1-μm filter.

Radiochemical Purity Determination of Filtered Technetium-99m-SC

The radiochemical purity (RCP) of the pre- and postfiltered ^{99m}Tc-SC was performed with the use of Whatman® grade 31ET Chr paper (1 × 8.5 cm) (Whatman LabSales, Hillsboro, OR) as the stationary phase and 85% methanol as the mobile phase. The shielded 2-ml vial was shaken well before withdrawal of the filtered ^{99m}Tc-SC sample from the vial with a 1-ml syringe with 27-gauge needle. A 5-μl sample was applied at the origin (1 cm from the bottom of the chromatography strip), and the paper strip was then placed into a Venoject® blood collection tube (16 × 100 mm, 10 ml) (Terumo Medical Corporation, Elkton, MD). The glass tube was capped with a rubber stopper during the entire chromatography developing process to maintain an equilibrated solvent atmosphere in the tube. The relative front values for filtered ^{99m}Tc-SC and free ^{99m}Tc were 0.0 and 1.0, respectively. The acceptable RCP limit for the filtered ^{99m}Tc-SC was arbitrarily set at 90%.

Size Evaluation of Filtered Technetium-99m-SC

The radioactive particle size distribution of filtered ^{99m}Tc-SC was evaluated by membrane filtration with polycarbonate membranes (*10*), electron microscopy and laser light scattering analysis.

Membrane Filtration. Before the liquid filtration, the membrane of the 13-mm diameter filters of 15- and 50-nm pore size (Nuclepore® polycarbonate membrane, Costar Corp., Cambridge, MA) was rinsed with 0.5 ml sterile water for injection, USP, to make it easier for the filter to load the sample. Aliquots of 0.1 ml filtered ^{99m}Tc-SC preparation were passed through a prewetted Nuclepore® filter and then rinsed with 1.0 ml of sterile water for injection, USP. The percentage of radioactivity retained on the filter was determined by counting of the filter, and the activity was expressed as a percentage of the total activity (i.e., filter + filtrate + washing).

Electron Microscopy. The particle size and shape of filtered ^{99m}Tc-SC was determined from negatives obtained with a Philips CM12/STEM transmission electron microscope (Philips Electronics, Eindhoven, Holland). Filtered ^{99m}Tc-SC was absorbed onto plastic-coated copper grids (200–300 mesh) and allowed to air dry. The grid surface was then washed with distilled water to remove any water-soluble salts, such as free ^{99m}Tc, NaCl or phosphate buffer. Electron microscopic negatives of the particles were recorded at a magnification of 100,000×. An appropriate magnification calibration specimen was also recorded at 100,000× to be used for the subsequent digital image analysis.

Digital Image Analysis. Images were digitized and particles measured on a VIDAS image analysis system (Kontron Elektronik, Munich, Germany) with Kontron Elektronik software. Negatives from the electron microscope were placed on a light box and inputted into the system with a Hamamatsu Newvicon video camera (Hamamatsu Photonics, Hamamatsu City, Japan) equipped with a Zeiss 100-mm macro lens (Carl Zeiss, Inc., Oberkochen, Germany).

Laser Light Scattering Analysis. A Malvern laser light scattering system (System 4700c, Malvern Instruments Ltd., Malvern, England) for photon correlation spectroscopy (PCS) and total intensity measurements was used to size the particles of filtered ^{99m}Tc-SC. Instrumental details and specifications were as follows.

A Coherent Innova 60 argon laser (Coherent Laser Group, Santa Clara, CA) (tuned to the 514.5-nm band) was used in conjunction with the PCS components. The PCS Autosizer 4700 consists of the following: a temperature-controlled cell assembly with a range of 4 to 80°C and a precision of 0.1°C; a variable-angle goniometer (10–150° with a resolution of 0.01°), which supported a variable aperture and the photomultiplier tube detector; a 128-channel signal correlator with 8*N multibit data processing; sample times of 50–990 nsec; and input prescaling (1–256). The Autosizer 4700 was controlled with Malvern software.

Stability of Filtered Technetium-99m-SC

In Vitro Stability Test. Stability studies were performed on six preparations of filtered ^{99m}Tc-SC preparations by determining the RCP values at 0, 1, 3 and 6 hr postfiltration.

In Vivo/In Vitro Stability Test. Nine-milliliter samples of whole blood were collected from six individuals of a volunteer group with 1 ml of anticoagulant citrate dextrose solution, USP (solution A) (ACD) as the anticoagulant. Each 10-ml blood sample (9 ml whole blood plus 1 ml ACD solution) was then transferred to a 50-ml blood collection tube and mixed with 0.5 ml of filtered ^{99m}Tc-SC by vortexing. The mixture of the blood and filtered ^{99m}Tc-SC sample was divided into five aliquots and incubated in an oven at 37°C for 24 hr. During the incubation period, a 0.2-ml sample was transferred to a centrifuge tube that contained 2 ml of 0.9% NaCl at 0, 1, 3, 6 and 24 hr. This sample was then spun at 900 g for 5 min in a centrifuge. The amount of free ^{99m}Tc in the plasma layer was determined by the paper chromatography method described previously.

Lymph Node Imaging with Filtered Technetium-99m-SC

Eighteen patients with suspected lymphedema were injected with 18.5 MBq (500 μCi) filtered ^{99m}Tc-SC intradermally into each foot (total dose 37 MBq, 1 mCi) between the second and third digits after povidone-iodine preparation. One patient was injected between the second and third digits of each hand and imaged for unilateral arm swelling. For the patients with lower extremity edema, the feet were strapped into a hydraulic, vacuum-powered exercise machine, and each patient peddled 1 min in every 5 min up to 60 min postinjection. For the arm swelling, the hands were exercised by gentle squeezing of a gauze roll for 1 min in 5 min increments up to 60 min postinjection. Local views over the inguinal or axillary lymph nodes were obtained every 5 min for the first hour, and those lymph nodes were also evaluated from the whole-body images performed at 1, 3, 6 and 24 hr postinjection. Lymphatic flow and lymph channels were evaluated for each extremity.

Radiation Dosimetry for Filtered Technetium-99m-SC

Lower extremity images were obtained immediately after intradermal injection of 18.5 MBq (500 μCi) filtered ^{99m}Tc-SC into each foot. Additional whole-body scans were performed at 1, 3, 6 and 24 hr postinjection. All whole-body scans were obtained on a Siemens (Des Plaines, IL) whole-body scanner. Simultaneous anterior and posterior views were obtained.

Regions of interest (ROIs) were drawn over the liver, spleen, lymph nodes and injection sites. The fraction of injected activity (FIA) at each site was determined with the relative geometric mean technique (*11*):

$$\text{FIA in ROI} = GM_r/GM_{inj},$$

TABLE 1
Quality Control of Pre- and Postfiltered Technetium-99m-Sulfur Colloid*

Time after preparation (hr)	Radiochemical purity (%)	
	Prefiltered ^{99m}Tc-SC	Postfiltered ^{99m}Tc-SC
0	95.0 ± 1.0	93.4 ± 1.7
1	94.5 ± 0.9	93.6 ± 1.7
3	94.2 ± 1.1	92.5 ± 1.5
6	93.8 ± 1.3	91.4 ± 1.8

*Values are presented in mean ± s.d. (n = 6).

where GM_r = geometric mean counts for the ROI and GM_{inj} = geometric mean counts for the two injection sites at time 0.

Whole-body remainder activity was calculated by subtraction of the organ activities from whole-body activity. The FIA, as a function of time postinjection for the four imaging sessions, was plotted and a biexponential least-squares fit of the data was performed with the SAAM (Simulation Analysis and Modeling) computer program distributed by the Resource Facility for Kinetic Analysis, University of Washington (*12*). The curves had the following form.

$$\text{FIA} = K_1 \times e^{-(P_1 \times t)} + K_2 \times e^{-(P_2 \times t)}.$$

The parameters K_1, P_1, K_2 and P_2 were determined during data fitting. The dose per unit administered activity was dose = $\tau \times S$, where S was the dose to the target organ from unit cumulated activity in the source organ. The value τ was source organ residence time defined as the time integral of FIA from 0 to infinity.

Source organ residence times were entered into the MIRDOSE2 program (*12*) to determine target organ dose.

The self-dose to the injection site was based on a spheric distribution of activity with diameter averaged from FWHM profiles taken through the injection site on both the anterior and posterior images at the four imaging times (*13*).

RESULTS

Filtered Technetium-99m-SC Preparation

The filtering process with ^{99m}Tc-SC was conducted in less than 1 min. The average radioactivity retained in the filter was 62.7% ± 10.1% (n = 22) of total activity. The RCP for filtered ^{99m}Tc-SC (n = 6) assessed by paper chromatography after filtration was 93.4% ± 1.7% (0 hr), 93.6% ± 1.7% (1 hr), 92.5% ± 1.5% (3 hr) and 91.4% ± 1.8% (6 hr) (Table 1). The RCP difference between the pre- and postfiltration of ^{99m}Tc-SC preparation was −1.7% ± 1.4% (n = 40).

Evaluation of the particle size of filtered ^{99m}Tc-SC with the Nuclepore® polycarbonate filter showed that 89.9% ± 4.5% (n = 28) of filtered ^{99m}Tc-SC was between 15 and 50 nm. The change in RCP values of filtered ^{99m}Tc-SC preparations after the particle size distribution analysis was −0.8% ± 1.6% (n = 28). Electron microscopic and digital image analyses (Figs. 2 and 3) revealed that the average particle was 38.0 ± 3.3 nm (n = 202). Based on the data from the laser light scattering analysis, filtered ^{99m}Tc-SC

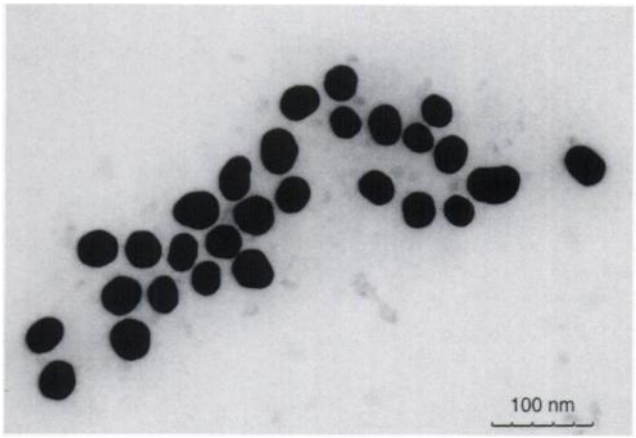

FIGURE 2. Electron micrograph of filtered ^{99m}Tc-SC.

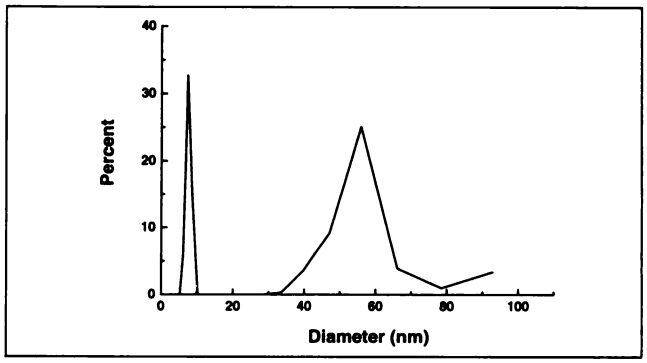

FIGURE 4. Particle-size distribution of filtered ^{99m}Tc-SC by the laser light scattering technique.

had two peaks of mean particle sizes of 7.5 nm (minor peak) and 53.9 nm (major peak) (Fig. 4).

The percentage of free ^{99m}Tc in the plasma after incubation of filtered ^{99m}Tc-SC in whole blood was 1.8% ± 1.0% (0 hr), 1.6% ± 0.7% (1 hr), 1.8% ± 1.0% (3 hr), 1.9% ± 1.1% (6 hr) and 2.0% ± 0.6% (24 hr).

Lymphatic Imaging and Radiation Dosimetry

Nineteen patients studied with filtered ^{99m}Tc-SC (RCP = 93.4% ± 4.2%) demonstrated similar lymphoscintigraphic patterns as would normally be seen with ^{99m}Tc-ATC (Figs. 5 and 6). Filtered ^{99m}Tc-SC showed a faster transport rate to the inguinal lymph nodes than that in our patients previously imaged and evaluated with ^{99m}Tc-ATC. All clinically normal extremities had radioactivity in the inguinal lymph nodes by 30 min postinjection compared with 60 min with ^{99m}Tc-ATC. There was somewhat less retention of activity seen in the extremities in patients with lymphatic obstruction and slightly more bladder activity on the filtered ^{99m}Tc-SC images compared with the ^{99m}Tc-ATC studies performed in the authors' institution.

The highest calculated radiation dose was to the injection site (each foot) with the FWHM used to calculate the volume of the activity (*13*) being 121 to 569 mGy (12.1–56.9 rad) per 18.5 MBq (500 μCi) dose and the second highest being each chain of inguinal lymph nodes with 0.2–16.7 mGy (0.02–1.66 rad) per 18.5 MBq (500 μCi) dose (Table 2). With filtered ^{99m}Tc-SC, the dosimetry to the liver (critical organ), inguinal lymph node, injection site and whole body (Table 2) was comparable to the previously published values for ^{99m}Tc-ATC (*14*).

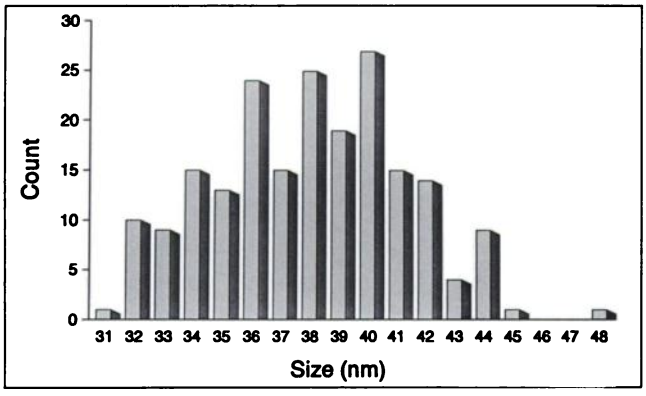

FIGURE 3. Particle-size distribution of filtered ^{99m}Tc-SC by electron microscopic and digital image analytical methods.

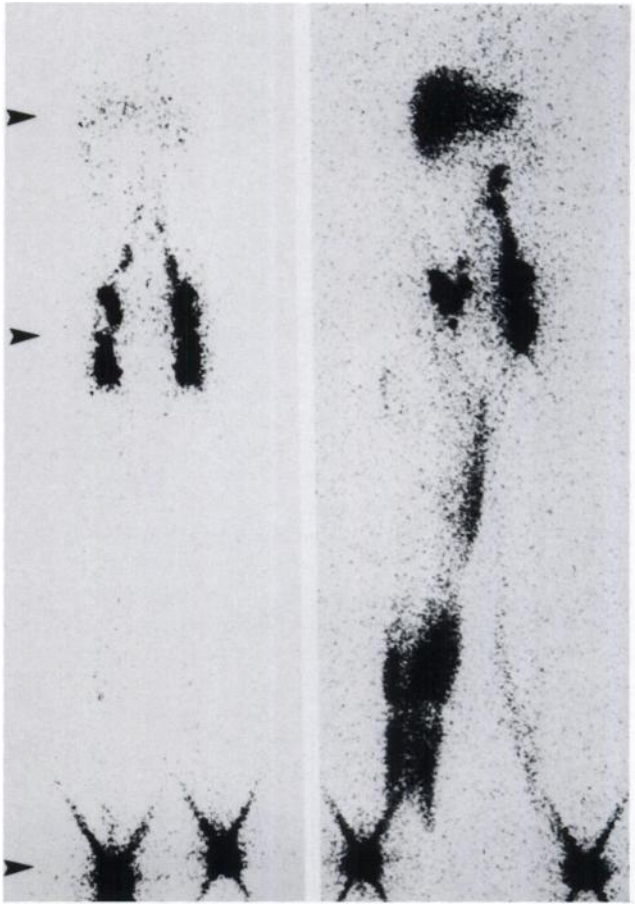

FIGURE 5. Abnormal lymphoscintigraphy study with filtered ^{99m}Tc-SC injection in a patient with right leg swelling. The study was interpreted to mean that the patient had localized lymphatic obstruction in the lower leg possibly because of previous infection. (Left) Anterior view 1 hr postinjection. (Right) Anterior view 6 hr postinjection.

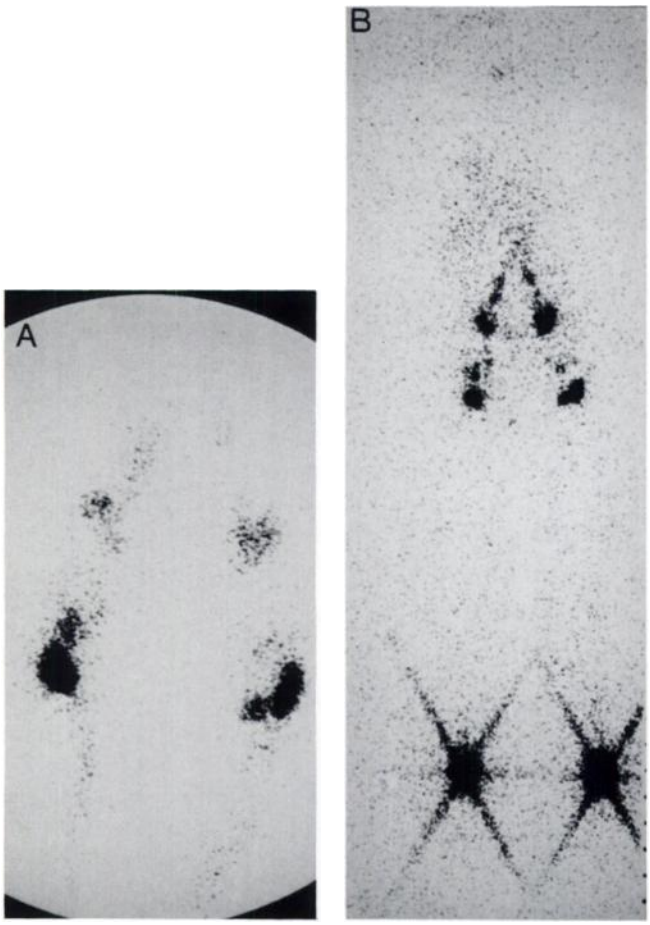

FIGURE 6. Normal lymphoscintigraphy study with filtered ^{99m}Tc-SC injection. (A) Dynamic view at 20 min postinjection. (B) Anterior view at 3 hr postinjection.

DISCUSSION

Because ^{99m}Tc-ATC has been removed from the market in the United States, there is a need for other radiopharmaceuticals to diagnose lymphatic obstruction. A satisfactory substitute was found in filtered ^{99m}Tc-SC. Because the overall particle size distribution of ^{99m}Tc-SC prepared by the thiosulfate kit method is unsatisfactory for lymphoscintigraphic study, the authors used a simple membrane filtration technique to isolate the smaller particles in the regular ^{99m}Tc-SC preparation.

TABLE 2
Radiation Dosimetry of Filtered Technetium-99m-Sulfur Colloid

Region of interest	Dosimetry	
	mGy/37 MBq (rad/1 mCi)	mGy/18.5 MBq (rad/500 μCi)
Liver	0.099–0.350 (0.0099–0.035)	
Spleen	0.150–0.550 (0.015–0.055)	
Lymph nodes (each chain)		0.2–16.7 (0.02–1.66)
Injection site (each foot)		121–569 (12.1–56.9)
Total body	0.130–0.170 (0.013–0.017)	

Earlier, we evaluated the feasibility of filtering the ^{99m}Tc-TSC kit (Medi-Physics) for use as a lymphoscintigraphy agent. Unfortunately, 99.8% ± 1.2% (n = 6) of the radioactivity of ^{99m}Tc-SC prepared by the ^{99m}Tc-TSC kit method was retained in the 0.1-μm filter (unpublished results); therefore, this kit was not used for the preparation of filtered ^{99m}Tc-SC. More recently, Medi-Physics, Inc. has discontinued the kit. Therefore, the only cold kit for the preparation of ^{99m}Tc-SC in the United States market is the AN-SULFUR COLLOID® kit from CIS-US, Inc. We used this kit to prepare filtered ^{99m}Tc-SC because of an unsatisfactory result with the Medi-Physics sulfur colloid kit, and because the average particle size of the CIS-US ^{99m}Tc-SC is 40 nm (written communication with Deneen M. Cipriani of CIS-US). According to our results from the filtration process with the 0.1-μm Nuclepore® membrane filter, 52.6%–72.8% of ^{99m}Tc-SC radioactivity was retained in the filter, which suggests that a significant portion of CIS-US ^{99m}Tc-SC particles were larger than 0.1 μm.

The Millex-VV 0.1-μm filter used in this study for the filtration of ^{99m}Tc-SC is a syringe-operated filter unit that can handle aqueous volumes from 1 to 100 ml. It is the smallest pore size filter that can be found on the market in which the filter unit is sterile, nonpyrogenic and nontoxic. Prewetting the membrane of the filter unit was not necessary for the filtration of ^{99m}Tc-SC because the radioactivity retained in the filter membrane was almost identical between the dry and prewetted filter membrane. The pressure generated in the 3-ml syringe during the filtration process was relatively low, which made the loading and filtering of ^{99m}Tc-SC easy and smooth.

To assure a proper intradermal injection of filtered ^{99m}Tc-SC for lymphoscintigraphic studies, one has to prepare the radiopharmaceutical in a small volume (i.e., usually about 0.1 ml). For a standard adult dose of 18.5 mGy (500 μCi) for the lymph node imaging study, this means that the specific concentration of the radiopharmaceutical preparation for lymphoscintigraphy must be 185 mGy/ml (5 mCi/ml). To inject the required concentration of filtered ^{99m}Tc-SC, it is necessary to prepare the regular ^{99m}Tc-SC kit with a highly concentrated solution of sodium [^{99m}Tc]pertechnetate. We have used 7400–9250 mGy (200–250 mCi) sodium [^{99m}Tc]pertechnetate injection to prepare the ^{99m}Tc-SC kit. With the final volume in the ^{99m}Tc-SC vial maintained at 6 ml, the specific concentration of the ^{99m}Tc-SC kit was 1233–1541 mGy/ml (33.3–41.7 mCi/ml). Given this high specific concentration, even if one prepares the filtered ^{99m}Tc-SC close to the expiration time of the regular ^{99m}Tc-SC preparation (i.e., 6-hr postkit reconstitution) (*9*), the filtrate would have more than enough concentration [e.g., 1233 mGy/ml × 50% (6-hr decay) × 63% (average retention) = 388.5 mGy/ml or 10.5 mCi/ml] for the lymphoscintigraphic study.

According to the United States Pharmacopeia, 23rd edition, and the National Formulary, 18th edition, the RCP

limit for ^{99m}Tc-SC is 92% (*15*). The minimum RCP limit for filtered ^{99m}Tc-SC has been arbitrarily set at 90% because the RCP difference between pre- and postfiltration of the filtered ^{99m}Tc-SC preparation was $-1.7\% \pm 1.4\%$ (n = 40). The data presented in Table 1 also indicate that the RCP value for filtered ^{99m}Tc-SC decreased to $92.5\% \pm 1.5\%$ and $91.4\% \pm 1.8\%$ at 3 and 6 hr postfiltration, respectively. Therefore, a lower RCP limit had to be used for filtered ^{99m}Tc-SC (i.e., 90% for filtered ^{99m}Tc-SC compared with 92% for ^{99m}Tc-SC). This RCP limit, however, was comparable with the minimum RCP acceptance level for most other ^{99m}Tc-labeled radiopharmaceuticals listed (*15*).

Davis et al. (*10*) suggested the use of Nuclepore® membrane filtration for the determination of the radioactive particle size distribution of radioactive colloids. We used this method with the prewetted 13-mm diameter filters of 12.5- and 50-nm pore size and showed that more than 89.9% of the particles of filtered ^{99m}Tc-SC were between 12.5 and 50 nm at the time of preparation. Therefore, the ultrafiltration method with the Nuclepore® membrane provides only particle size range and not the actual size of the individual filtered ^{99m}Tc-SC particle. In addition, the membrane filtration technique does not measure particle shape (*16*).

To measure the shape and size of the filtered ^{99m}Tc-SC particle accurately, electron microscopy and digital image analysis techniques were utilized in this study. Davis et al. (*10*) suggest that the relatively complex mixture of chemicals in a ^{99m}Tc-SC sample may make it difficult to measure the size of radioactive colloids under the electron microscope. We did not encounter such technical difficulties in the sizing of filtered ^{99m}Tc-SC with electron microscopy, and we believe that the use of the 0.1-μm membrane filter to prefilter the ^{99m}Tc-SC preparation might help to remove that complex mixture of chemicals in the ^{99m}Tc-SC preparation. The shape of the filtered ^{99m}Tc-SC particles, however, seemed to be not as spheric as the regular particle shape for ^{99m}Tc-SC (*16*) and ^{99m}Tc-ATC particles (*17*). This slight change in the particle shape might be caused by the filtration process or by the heat generated by the electron beam in the electron microscope.

It is also interesting to note that almost none of the filtered ^{99m}Tc-SC particles were between 45 and 100 nm measured by the electron microscope (Fig. 3). One possible explanation for this is that the 0.1-μm membrane filter not only retains particles larger than its pore size, but it also traps some portion of smaller particles, especially those nearest to the pore size (i.e., 0.045–0.1 μm) within the membrane matrix. It seems that larger ^{99m}Tc-SC particles tend to form a coarse mat on the membrane filter surface, which acts like a depth filter to retain particles smaller than the rated pore size. When electron microscopy and digital image analysis was used to evaluate the particle size of the filtered ^{99m}Tc-SC, the samples and the fields under the electron microscope were randomly selected, and no particle sizes of the filtered ^{99m}Tc-SC sample were found that were larger than 50 nm.

The electron microscopic technique may create artifacts such as sublimation of smaller particle size and/or distortion of the particle shape of filtered ^{99m}Tc-SC particle caused by the drying process on the grid and the heat from the electron beam. The laser light scattering analysis offers a nonobstructive way to study the particle size of radiocolloids while the particles are still in the original solution and there is no need for special sample preparations. Therefore, the laser light scattering technique minimizes possible artifacts associated with the electron microscopic method. The laser light scattering technique, however, does not measure the particle shape, and therefore the method cannot distinguish contaminating particles such as dust from the sample particles. Nevertheless, the laser light scattering analysis revealed that the particle sizes of filtered ^{99m}Tc-SC were in two peaks, at 7.5 and 53.9 nm (Fig. 4). The lowest peak may be fragments of ^{99m}Tc-SC or contaminating particles such as dust.

CONCLUSION

Filtered ^{99m}Tc-SC has similar imaging characteristics and radiation exposure comparable to ^{99m}Tc-ATC, and we believe that filtered ^{99m}Tc-SC can be considered an acceptable and safe radiopharmaceutical to replace ^{99m}Tc-ATC for lymphoscintigraphy. Filtered ^{99m}Tc-SC can be easily prepared and is readily available for use in lymphoscintigraphic studies.

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