An Evaluation of Radiocolloid Sizing Techniques

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Techniques for sizing radiocolloids are reviewed. The small size range (1–100 nm) of many radiocolloids and their polydispersity limit the choice of the technique used. To compare several techniques directly, the particle size of technetium-99m sulfide colloid was studied using Nuclepore filtration, ultracentrifugation and electron microscopy. The last of these was adopted as the method of choice. Using this technique, the particle size and shape of colloids below 100 nm can be accurately determined. Technetium-99m antimony sulfide colloid and indium-113m hydroxide were then examined by electron microscopy, and the chemical nature of the particles was determined by x-ray fluorescence analysis. Results resolved the size discrepancies reported in the literature and demonstrated the importance of identifying the chemical nature of the particles under examination.

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Radiocolloids play an important role as diagnostic and therapeutic agents. Nevertheless, the properties of the radiocolloid dispersion, characterized by particle size, shape, charge, and stability, have not been systematically investigated. Consequently the biologic implications of variations in colloidal properties are not well understood. Reports show that particle size has a major influence on biologic behavior (*1–10*). The particle size of radiocolloids has been poorly defined, however, and discrepancies exist between the results of various sizing techniques. These discrepancies could be attributable to the limitations of sizing techniques in the colloid size range (1–500 nm).

This study was undertaken to evaluate techniques available for particle sizing and to establish a simple, reliable, and reproducible method for defining the size, shape, and stability of radiocolloids. Three techniques, Nuclepore filtration,* ultracentrifugation, and electron microscopy, were compared directly using technetium-99m sulfide colloid as a test material. Electron microscopy was ultimately selected as the method of choice, since it fulfilled the preceding requirements. Moreover, specimens prepared for this technique could be used directly for chemical analysis of individual particles. Technetium-99m antimony sulfide colloid and indium-113m colloid were then examined by electron microscopy and the chemical nature of the particles determined by x-ray fluorescence analysis.

REVIEW OF TECHNIQUES

With the possible exception of technetium-99m sulfide colloid, most radiocolloids are less than 100 nm in size. The application and usefulness of sizing techniques in this range is an important consideration in the selection of an appropriate method for the evaluation of the size and shape of particulate matter. Other parameters that can influence the selection of a sizing technique are:

1. Particle shape and polymorphism.
2. The number of components in the system (monodisperse, polydisperse).
3. The stability of the particles (aggregation, coagulation, flocculation).
4. The ease of sample preparation and analysis.
5. The reproducibility and accuracy of the technique.
6. The availability of facilities.

To illustrate the particle-sizing discrepancies en-

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countered, Table 1 summarizes the results of five different techniques used in the determination of the particle size of technetium-99m sulfide colloid. The same colloid is found to have a narrow size distribution by ultracentrifugation, a broad size distribution by Nuclepore filtration, and is found to be polydisperse by electron microscopy. Though all formulations were prepared by the same method, significant size differenees appear to be found, depending on the sizing techniques used. In this study we examined the particle-size distribution of a similar formulation of technetium-99m sulfide colloid.

In Table 2 the advantages and limitations of various sizing techniques are listed. Light microscopy and the Coulter counter cannot be used to measure accurately radiocolloids below 100 nm. In addition, though photometric techniques can be applied in this size range, the number of variables in the system, which must be analyzed to obtain reliable results, make the technique impractical. Electron microscopy, ultrafiltration, and ultracentrifugation appear to be the most favorable and potentially useful techniques for the sizing of colloids below 100 nm.

TABLE 1. TECHNIQUES USED IN SIZING Tc-99m SULFIDE COLLOID

Technique	Particle size range* (nm)	Ref.
Millipore filtration	500–1000	11
	450–1200	12
Nuclepore filtration	Less than 1000	13
	9–705, av. 110	14
Electron microscopy	20; aggregates 100	15
	800 ± 200	16
Ultracentrifugation	400–450	15
Light microscopy	800 ± 200	16
	500–2000	17
Coulter counter	290–790	18
	500–1000	11

* All formulations were prepared by the acid reduction of sodium thiosulphate in the presence of potassium perrhenate.

MATERIALS AND METHODS

Gelatin-stabilized technetium-99m sulfide colloid was prepared by the acid reduction of sodium thiosulphate in the presence of potassium perrhenate (*15*). Technetium-99m antimony sulfide colloid was prepared by a modification of the method of Garzon et al. (Warbick et al., in preparation), and indium-113m colloid by that of French (*27*).

NUCLEPORE FILTRATION

Particle size of technetium-99m sulfide colloid was evaluated using Nuclepore membranes with the following pore sizes: 0.1, 0.2, 0.4, 0.6, and 0.8 μm. One-tenth milliliter of the colloid was injected into a filter holder. Five milliliters of water were then passed through the filter, followed by approximately 5 ml of air to ensure complete filtration. The filter and filtrate were counted in a well counter.

ULTRACENTRIFUGATION

Technetium-99m sulfide colloid (0.2 ml) was layered on to a refrigerated 10–40% sucrose gradient. The gradient was then spun in an ultracentrifuge at 5°C. The spin time was 1.5 hr at 40,000 rpm (SW 40.1 rotor). The gradient was separated into fractions (7 drops per collection tube) and counted in a gamma sample counter.† The centrifuge tube was cut into 1-cm strips and the activity adhering to the sides and bottom of the tube was also counted.

Thin-layer chromatography (with Whatman No. 1 paper and 85% methanol) was used to confirm that the dissolution of technetium-99m sulfide colloid in sucrose did not affect its stability.

The polydispersity of the colloid on the basis of particle density using equilibrium centrifugation could not be demonstrated on sucrose gradients because the density of 60% sucrose (1.29) is less than the density of sulphur (2.07) and rhenium sulphide (4.866).

ELECTRON MICROSCOPY

Technetium-99m sulphide colloid was examined with a transmission electron microscope.‡ The samples were applied on plastic-coated (carbon-stabilized) copper or aluminum grids (200–300 mesh). The colloid was spotted or nebulized onto the grid surface and allowed to dry or partially dry. The grid surface was then washed with distilled water to remove any water-soluble salts such as free technetium-99m, sodium chloride, or phosphate buffer.

X-ray fluorescence analysis|| was used to identify the chemical composition of the particles. Samples for this procedure were prepared on aluminum grids because the energy peaks from copper tended to mask the rhenium peaks.

RESULTS AND DISCUSSION

The sucrose-gradient results (Fig. 1) show that the colloid is polydisperse with at least two major size components, one near the top of the gradient and the other pelleting and adhering to the bottom of the centrifuge tube. This observation was qualitatively confirmed by Nuclepore filtration (Table 3). For a sample sulfide colloid preparation, containing approximately 5% free pertechnetate at zero time, 22.3% of the particles were less than 100 nm, 39% were between 200 and 400 nm, with virtually all of

TABLE 2. A COMPARISON OF DIFFERENT SIZING TECHNIQUES

Method	Advantages	Limitations	Ref.
Electron microscopy	1. Resolution 0.2–0.3 nm. 2. Micrographs can be scanned with image-analysis computer to measure size distribution. 3. 3-dimensional images can be obtained by shadowing. 4. Particle identification by x-ray fluorescence analysis.	1. Larger particles may be subject to heating by the electron beam and may change or sublime off. 2. Difficulty in analyzing preparations containing stabilizer and contaminants. 3. Difficulty in identifying exact chemical nature of components in polydisperse system without energy-dispersive analysis of x-rays.	19–22
Light microscopy	1. Particle size greater than 200 nm. 2. Ease of sample preparation.	1. Visibility limit 120 nm. A halo effect occurs as the particle size approaches the wavelength of the incident light. 2. Inaccurate particle sizing below 2000 nm.	19, 20, 23
Coulter counter	1. Particle size greater than 400 nm. 2. Useful for sizing spherical particles. 3. Fast technique.	1. Difficult to differentiate particles below 400 nm from background electrical noise. 2. Measures particle volume, not shape. 3. Colloids that are good conductors may cause increases in current and present false results. 4. Requires preliminary calibration of equipment. 5. Requires dilute solutions so that particles pass through the orifice one at a time.	20
Ultrafiltration (a) Nuclepore filter	1. Practical particle size greater than 100 nm. 2. Membrane surfaces are not charged.	1. Does not measure particle shape. 2. Filtration through filters less than 100 nm is difficult.	13
(b) Millipore filter	1. Practical particle size greater than 80 nm.	1. Charged surfaces can cause particle retention. 2. Does not measure particle shape. 3. Filtration through filters less than 100 nm is difficult.	
Ultracentrifugation	1. Particle size limit less than 100 nm.	1. Preliminary calibration of apparatus required. 2. Factors to be considered in evaluating results: (a) limiting size for Stokes' Law; (b) centrifuge tube shape; (c) particle shape and weight; (d) colloid stability; (e) wall effects; (f) initial particle movement; (g) experimental errors caused by decantation, speed of rotation measurement, acceleration and imbalance. 3. With particle less than 100 nm, excessive time is required for determinations and inaccuracies occur due to Brownian motion. 4. Concentration must be kept low to avoid interference between particles. 5. Very time consuming.	21
Photometric determinations (Nephelometry, Turbidometry, Light scattering)	1. Ease of sample preparation. 2. Particle size limit less than 100 nm.	1. Most measurements are based on the assumption of sphericity. 2. Factors to be considered are: (a) number of components in dispersed phase and medium (polydispersity); (b) all components as to: (i) material differences; (ii) optical differences; (c) aggregation, coagulation, flocculation, micelle formation, polymerization and the reverse phenomena of subdivision. 3. Interaction of physical electro-magnetic radiation with the dispersion may be relatively complex and the complexity of the system will contribute to the number of such interactions.	24, 25, 26

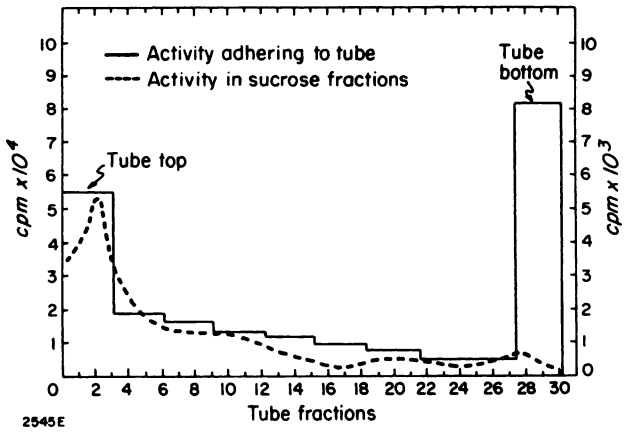

FIG. 1. Separation of technetium-99m sulfide colloid into two components by sedimentation in a 10–40% sucrose gradient. Spin time: 1.5 hr at 40,000 rpm, SW 40.1 rotor. Left-hand scale is activity adhering to walls of centrifuge tube; right-hand scale is activity in sucrose fractions. Note that most activity in technetium-99m sulfide colloid adheres to tube walls.

TABLE 3. NUCLEPORE FILTRATION PROPERTIES OF Tc-99m SULFIDE COLLOID

Pore size range (nm)	% activity 0 hr	4 hr
0–100	22.3	27.0
100–200	2.8	0.3
200–400	39.0	34.8
400–600	11.9	4.6
600–800	11.8	15.7

the remainder being larger. This size distribution did not change after 4 hr at room temperature. The possibility of two size populations has not yet been reported. Davis et al. (*13*) did not use a 200-nm Nuclepore filter when studying different sulfide colloids and consequently would have failed to observe the two size populations.

Electron-microscopic analysis (Fig. 2) revealed the presence of two distinctly different particle sizes. One population was composed of large aggregates with subunits of approximately 140 nm. The other population was composed of smaller individual particles with a broad size distribution of 10–60 nm.

X-ray fluorescence analysis of the particles revealed that the smaller particles contained high rhenium, low sulphur, and low technetium levels. The larger particles composing the aggregates contained low levels of technetium and rhenium and negligible sulphur. Under the conditions of this analysis, sulphur can sublime (*28*), and the measured sulphur levels are therefore minimum levels. With this in mind, we believe the smaller particles to be rhenium sulphide and the aggregates to be colloidal sulphur. The low levels of technetium are consistent with the hypothesis that technetium-99m adheres as a positively charged ion to the double ionic layer (*20*) associated with colloidal sulphur and rhenium sulphide.

Comparison of the three different sizing techniques showed that Nuclepore filtration furnishes information on the distribution of colloidal particles within limits controlled by the sizes of Nuclepore membranes available. Though filters are available in pore sizes of 30, 50, and 80 nm, filtration through the 30- and 50-nm membranes requires high pressures,

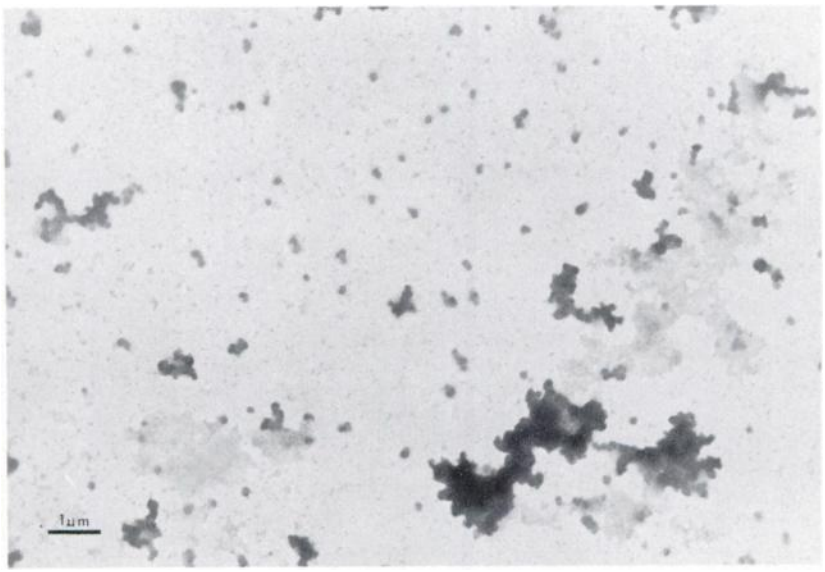

FIG. 2. Electron micrograph of technetium-99m sulfide colloid.

and is at best so difficult as to be impractical. Identification of the chemical components in a polydisperse system using this technique is not possible. Ultracentrifugation is a lengthy procedure requiring careful control and a large range of gradient and centrifugation conditions, since often a large variation both in density and size of the particles in the preparation must be analyzed. As rapid screening techniques for the sizing of radiocolloids, therefore, these two procedures are not suitable.

Since electron microscopy did not suffer from these drawbacks, it was adopted as the optimum method for determining colloid-particle size. Specimens for examination can be prepared easily, and accurate information on the shape and size of the particles below 100 nm can be obtained quickly. For particles that tend to rest on their largest surface (*21*), the third dimension of the particles can be investigated by shadowing techniques or with the scanning electron microscope. Furthermore, the chemical nature of the particles in question can be determined by x-ray fluorescence analysis.

As a demonstration of the usefulness of electron microscopy for radiocolloid particle sizing, technetium-99m antimony sulfide colloid and indium-113m colloid were examined. Specimens for electron microscopy were prepared as previously described. To rule out the possibility that variation in the preparative procedure could affect the particle size of antimony sulphide, three different preparations were studied. In addition, both indium-113m colloid and technetium-99m antimony sulfide colloid were studied by x-ray fluorescence analysis, and sized with the help of a computer.

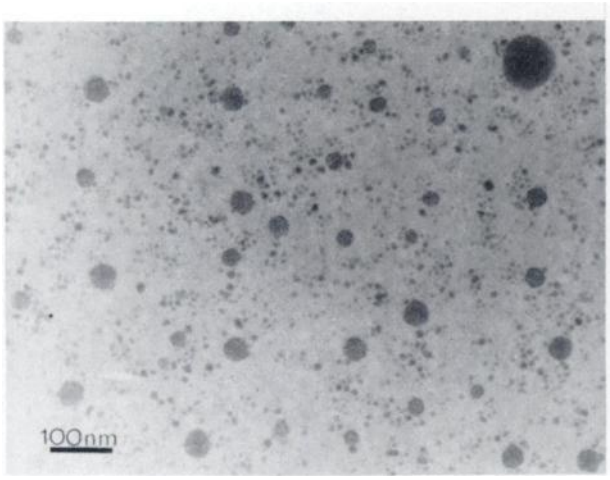

FIG. 3. Electron micrograph of technetium-99m antimony sulfide colloid, in-house kit prepared at The Princess Margaret Hospital.

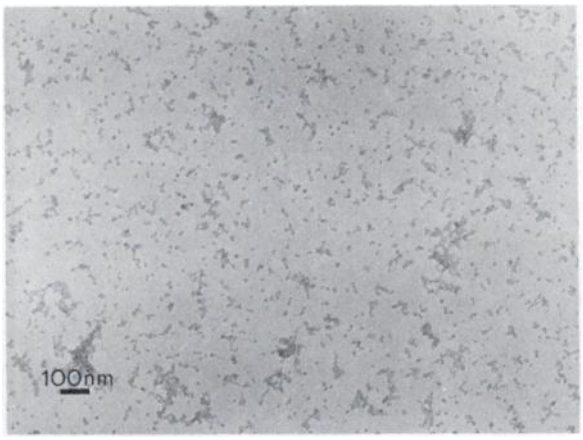

FIG. 4. Electron micrograph of technetium-99m antimony sulfide colloid, prepared without stabilizer.

TECHNETIUM-99m ANTIMONY SULFIDE COLLOID

The electron micrograph of technetium-99m antimony sulfide colloid (in-house kit), shown in Fig. 3, shows both large and small particles. X-ray fluorescence analysis revealed that the smaller particles (3–15 nm) were antimony sulfide (Sb_2S_3). The larger particles (60–200 nm) were polyvinylpyrrolidone (PVP), the stabilizer in the preparation. Absence of PVP resulted in the aggregation and settling of the colloidal preparation (Fig. 4). Table 4 gives the size distribution of three antimony sulfide preparations. The distribution is log normal and in all cases the size range is narrow. The three distribu-

TABLE 4. PARTICLE SIZE DISTRIBUTION DATA FOR SEVERAL PREPARATIONS OF Tc-99m ANTIMONY SULFIDE COLLOID, AS DETERMINED BY ELECTRON MICROSCOPY

Diameter class interval (nm)	Mean of interval (nm)	Frequency per 1000		
		Lot 6890	Lot 6445	Lot 3230
2–3	2.5	2.3	0.0	1.9
3–4	3.5	4.5	2.9	0.0
4–5	4.5	20.3	11.6	21.0
5–6	5.5	51.8	47.6	38.1
6–7	6.5	110.4	108.9	104.8
7–8	7.5	227.5	190.1	181.0
8–9	8.5	229.7	187.2	190.5
9–10	9.5	153.2	194.5	190.5
10–11	10.5	110.4	136.4	110.5
11–12	11.5	58.6	63.9	95.2
12–13	12.5	13.5	34.8	30.5
13–14	13.5	9.0	8.7	9.5
14–15	14.5	9.0	4.4	7.6
15–16	15.5	0.0	2.9	7.6
16–17	16.5	0.0	5.8	5.7
17–18	17.5	0.0	0.0	3.9

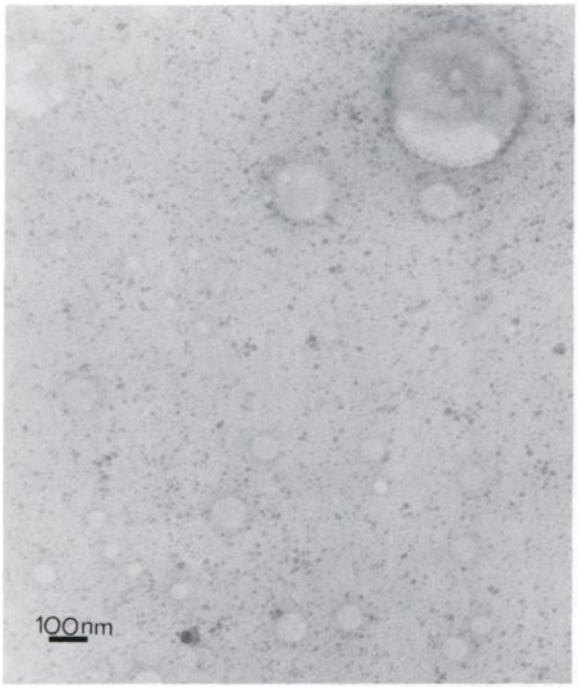

FIG. 5. Electron micrograph of technetium-99m antimony sulfide colloid.

tions are not in agreement with the sizing studies of Garzon et al. (*29*) and Patomaki et al. (*30*). Both groups reported the colloid to be larger than 100 nm.

Since Garzon used Millipore filtration to size antimony sulfide, the charged cellulose membrane probably caused particle retention and resulted in a mean particle size for the distribution far greater than the actual particle size (*13*). Patomaki, using the electron microscope and ultracentrifugation to measure the colloid§ reported a size range of 200–400 nm for antimony sulfide colloid. Figure 5 indicates that this preparation is identical to the colloid prepared by us. The particle size distribution is shown in Fig. 6. It appears likely that Patomaki mistook the large PVP particles for antimony sulfide. This stresses the importance of identifying individual particles in the dispersion, especially when the radiocolloid is polydisperse or the stabilizer is colloidal.

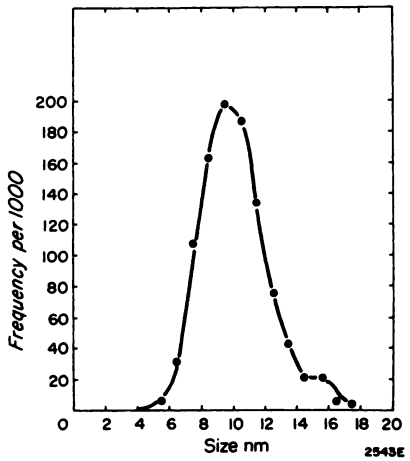

FIG. 6. Particle-size distribution of technetium-99m antimony sulfide colloid.

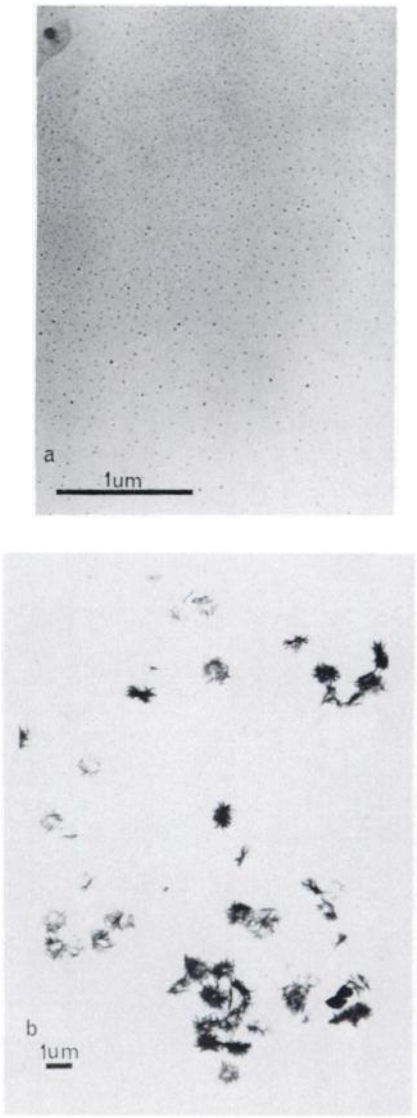

FIG. 7. (A) Electron micrograph of indium-113m colloid. (B) Electron of spiny artifacts present on electron microscope grids of indium-113m colloid.

INDIUM-113m COLLOID

Figure 7 shows an electron micrograph of indium-113m colloid. The distribution is log normal and

the majority of the particles lie between 10 and 20 nm. Our size observations agree with the results of French (*27*) with one exception. We observed spiny-shaped particles 1000 nm in size, that were present in addition to the uniform spherical particles.

X-ray fluorescence analysis revealed that the smaller particles contained indium and possibly some tin. The larger particles were artifacts due to contraction of the gelatin stabilizer during drying. These larger structures were observed by French but were not reported (personal communication, P. H. S. Smith, The Royal Marsden Hospital, Surrey, England).

CONCLUSION

A comparison and evaluation of various sizing techniques has shown that electron-microscopic analysis in conjunction with x-ray fluorescence analysis is an accurate technique for the determination of the size, shape, and chemical nature of radiocolloids. The practical application of Nuclepore filters in measuring colloid particle size is limited at present by large steps in the size ranges available and the difficulty of filtering the smaller sizes. In comparison with ultracentrifugation, electron microscopy is a simpler and quicker technique. The preparation of samples for it is easy and the technique is reliable and reproducible. In addition, x-ray fluorescence analysis for chemical identification requires no further preparative procedures. At present we are using this technique to screen radiocolloids with possible applications in lymphoscintigraphy.

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FOOTNOTES

* Nuclepore Corp., Pleasanton, Calif.

† LKB Wallac 8000, Fisher Scientific Co., Ltd., Don Mills, Ontario, Canada.

‡ Siemens Elmiskop 102, Iselin, N.J.

|| EDAX International Inc., Prairie View, Ill.

§ Orion Pharmaceutical Co., Helsinki, Finland.

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