Radiopharmacy

Effects of Al^{3+} Ion on Tc-99m Sulfur Colloid Preparations with Different Buffers

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The effect of aluminum ions on two Tc-99m sulfur colloid preparations, one with phosphate buffer and the other with acetate buffer, was studied by the filtration technique using Nuclepore filters of sizes 0.6 μm, 3 μm, and 10 μm. The aluminum concentrations were < 0.5, 7.5, and 15 μg/ml of the final preparation. A considerable number of large particles (3–10 μm) formed in the sulfur colloid preparation containing phosphate buffer when the Al^{3+} concentration was 7.5 μg/ml or more. This particle formation was not observed with sulfur colloid preparation containing acetate buffer.

Several reports have been published on the lung uptake of Tc-99m sulfur colloid during liver imaging (*1–6*). In some instances, it has been suggested that the macrophages in the lungs phagocytize the colloid particles, causing increased lung uptake of Tc-99m sulfur colloid (*2,3*). In some cases, the cause of these observations has been attributed to the formation of aggregated particles from colloids in the presence of aluminum ion in the Tc-99m eluate (*7*) and an increased plasma aluminum level (*6*). The aggregated particles are large enough to be trapped in the lungs. Cragin et al. (*8*) studied the effect of aluminum concentration in the Tc-99m eluate on the particle size of Tc-99m sulfur colloid, but could not establish any definite correlation between aluminum concentration and extent of aggregation of colloids. Haney et al. (*9*) added disodium edetate (Na_2 EDTA) to complex aluminum ions so that flocculation of colloid is prevented. They found no aggregates of colloid at a level of 10 μg Al^{3+}/ml of Tc-99m sulfur colloid preparation. Staum (*7*) found that when phosphate buffer was used, flocculation of sulfur colloid took place at aluminum concentration of 100 μg/ml of the final preparation, whereas with citrate and acetate buffer no precipitation was observed. However, these observations were made by visual estimation only.

We studied the effects of aluminum ions on the flocculation of Tc-99m sulfur colloids in the presence of phosphate buffer and of acetate buffer. We used a new technique: filtration of colloid preparations through Nuclepore filters to give the fraction of different size particles formed in Tc-99m sulfur colloid with different aluminum concentrations.

Materials and Methods

Two commercial sulfur colloid kits were used—one containing phosphate buffer (designated SC 1) and the other containing acetate buffer (SC 2). The composition of the two kits is shown in Table 1. The Tc-99m sulfur colloids were prepared according to the directions given in the package insert using approximately 10 mCi [^{99m}Tc] pertechnetate. Before adding [^{99m}Tc] pertechnetate to the reaction vial, 75 μg and 150 μg of aluminum ion (aluminum chloride in distilled water) were added separately to the Tc-99m eluate. The volume of the Tc-99m eluate varied from 3 to 6.3 ml. Since the final volumes of the Tc-99m sulfur colloid preparations were adjusted to 10 ml, the Al^{3+} concentration was either 7.5 μg/ml or 15 μg/ml of the final preparations. For comparison, a control sample of Tc-99m sulfur colloid was prepared without adding any aluminum. In the control sample, all components were tested for aluminum by the colored spot test. The sensitivity of the test was approximately 1 μg/ml. All components gave negative results. Only the Tc-99m eluate indicated 2 to 5 μg/ml of aluminum, which gave 0.2 to 0.5 μg/ml after dilution to 10 ml in the reaction vial. The control sample was designated as containing < 0.5 μg/ml.

After Tc-99m sulfur colloid preparation, the samples were allowed to cool for 1 hr. A 1-ml sample was drawn in a 3-ml syringe from the SC 1 preparation and its activity was measured in a dose calibrator. The sample was passed through a 0.6 μm Nuclepore filter. (Millipore filters were not used for particle sizing because their pores are tortuous and their thickness 100 μm, while the pores of Nuclepore filters are cylindrical and their thickness is about 10 μm (*10*). The choice of 0.6 μm filters was arbitrary; our aim was to size the colloid particles above the mean size of the colloids, which is about 100–200 nm in both kits.)

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TABLE 1. Composition of Two Commerical Kits

SC 1 (phosphate buffer)	SC 2 (acetate buffer)
Reaction vial—4-mg sodium thiosulfate 3-mg gelatin 8.5-mg dibasic potassium phosphate 0.93-mg sodium edetate	Reaction vial—0.5-ml 1N HCl Syringe A—1.9-mg sodium thiosulfate Syringe B—5.3-mg gelatin 177-mg sodium acetate
Syringe A—0.25-N HCl Syringe B—40-mg sodium biphosphate 10-mg NaOH	

The residual activity in the syringe was then measured to determine the activity that was actually passed through the filter. The filter was washed with 10-ml isotonic saline. The activities in the filtrate plus wash and the filter were separately measured in the dose calibrator. The percentage of activity retained by the filter gave the percentage of particles greater than 0.6 μm size. This filtration was repeated for two additional samples and the mean of three measurements was calculated along with its standard deviation. These same experiments were performed with SC 1 preparations containing different Al^{3+} concentrations and using 3 μm and 10 μm size Nuclepore filters. These filtration experiments were also performed for the SC 2 preparations containing the various Al^{3+} concentrations.

Results and Discussion

The mean values and their standard deviations of the activities retained by the different size Nuclepore filters for SC 1 and SC 2 preparations are shown in Table 2. The Tc-99m sulfur colloid preparation with phosphate buffer (SC 1) appeared as a milky suspension, although the particle size in the SC 1 preparation without aluminum was mostly less than 0.6 μm. The SC preparation with acetate buffer (SC 2) appeared almost clear.

The particle size distribution obtained from the data in Table 2 is presented in Table 3. The particle size in SC 1 increased with increasing aluminum concentration; in SC 2 there was little change in particle size distribution. It should be noted that when the aluminum ion concentration was increased from < 0.5 μg/ml to 7.5 μg/ml, the fraction of particles in SC 1 increased from 1.5% to 44% in the 3–10 μm range, while no increase in size was observed in SC 2. However, a further increase in Al^{3+} concentration from 7.5 μg/ml to 15 μg/ml changed the particle size only slightly in SC 1.

The Student t-test was applied to corresponding sets of data for SC 1 and SC 2 preparations. With no aluminum, there was a significant difference in 0.6 μm filter retention in SC 1 and SC 2 preparations (at a confidence level of 95%); 3 μm and 10 μm filter activities did not demonstrate any significant difference. At the Al^{3+} concentration of 7.5 μg/ml, there was a significant difference in the 0.6 and 3.0 μm filter retentions but no difference in 10 μm filter retentions. The SC 1 preparation with phosphate buffer showed almost ten times higher retention than the SC 2 preparation with acetate buffer. Similar results were obtained with the Al^{3+} concentration of 15 μg/ml in both preparations.

The USP limit for Al^{3+} in the Tc-99m eluate is 10 μg/ml for fission-produced Mo-99. In the package inserts of both SC kits, the maximum allowable Tc-99m eluate volume to prepare Tc-99m sulfur colloid is 5 ml and, therefore, the maximum allowable Al^{3+} ion in both preparations is 50 μg. Since the total volume of both preparations is 10 ml, the permissible Al^{3+} concentration in each final preparation is 5 μg/ml. Our values of 7.5 μg/ml and 15 μg/ml are well above these limits. Evidently, a good fraction of particles are formed in SC 1 in the size range of 3–10 μm at these concentrations of Al^{3+}. However, in SC 2 preparation, no such flocculation occurs even at such high concentration of aluminum.

Another interesting point is that the SC 1 (phosphate buffer) kit had EDTA to form a chelate with Al^{3+} to prevent flocculation of colloids. SC 1 contains 2.5 μmole of edetate, and 2.9 μmole of aluminum was added at a concentration of 7.5 μg/ml. Assuming that Al^{3+} and EDTA form a chelate at a molar ratio of 1:1, 0.4 μmole of excess aluminum would be available to form flocculation. We did a separate experiment with SC 1 using 0.5 μg/ml Al^{3+} concentration, which corresponds to 1.9 μmole of Al^{3+} in solution, all of which reacts with EDTA. Under these circumstances, the 0.6 μm filter retention was only 7.5%.

The results indicate that sulfur colloids with phosphate buffer are more amenable to flocculate formation in the presence of Al^{3+}, whereas the colloids with acetate buffer form no flocculate with aluminum. This finding is in agreement with that of Staum and is most likely due to the coprecipitation of colloid with aluminum phosphate (*7*). The fraction of particles with size greater than 10 μm with higher Al^{3+} concentrations may partly account for the increased lung uptake of sulfur colloids. Even though a high Al^{3+} concentration may not be present in the Tc-99m eluate, an increased intake of aluminum

TABLE 2. Retention of Activity by Nuclepore Filtration

Al^{3+} concentration (μg/ml)	Pore size of filter (μm)	Activity retained by different filters (%) SC 1 (phosphate buffer)	SC 2 (acetate buffer)
< 0.5	0.6	15.19 ± 2.77	9.82 ± 1.00
	3.0	5.90 ± 0.65	6.31 ± 5.48
	10.0	4.36 ± 1.70	3.24 ± 0.27
7.5	0.6	86.00 ± 2.81	8.98 ± 0.27
	3.0	50.73 ± 3.63	6.25 ± 0.16
	10.0	7.22 ± 3.85	6.74 ± 0.69
15.0	0.6	90.57 ± 3.87	8.07 ± 1.60
	3.0	50.16 ± 14.07	3.78 ± 0.06
	10.0	12.81 ± 6.50	3.85 ± 0.85

compounds such as antacids may result in in vivo flocculation of colloids and hence an increased lung uptake, especially in patients with renal diseases (*11*) or obstructive bowel diseases (*6*). This, however, does not rule out the possibility of macrophagic uptake of colloids in the lungs.

In conclusion, it has been demonstrated by the particle sizing technique using Nuclepore filters that a fraction of sulfur colloid aggregates to form large particles in the presence of Al^{3+} ions, when the phosphate buffer preparation is used. This does not occur in sulfur colloid preparations with acetate buffer.

TABLE 3. Particles Size Distribution in SC 1 and SC 2 Preparations with Different Al^{3+} Concentrations

SC preparation	Al^{3+} concentration (μg/ml)	Particle size distribution (%)			
		< 0.6μm	0.6–3.0μm	3.0–10μm	> 10μm
SC 1 (phosphate buffer)	< 0.5	84.81	9.29	1.54	4.36
	7.5	14.00	35.27	43.51	7.22
	15.0	9.43	40.41	37.35	12.81
SC 2 (acetate buffer)	< 0.5	90.18	3.51	3.07	3.24
	7.5	91.02	2.73	0.00*	6.4
	15.0	91.93	4.29	0.00*	3.85

*These values turn out negative because of statistical variations and have been assumed to be zero. Therefore, the values across these lines do not total 100%.

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