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Particle size analysis: ^{90}Y and ^{99m}Tc-labelled colloids

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Summary

Colloidal particle size is an important characteristic to consider when choosing a radiopharmaceutical for diagnosis and therapeutic purposes in nuclear medicine. Photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM) were used to determine the particle-size distribution of ^{90}Y- and ^{99m}Tc-labelled antimony trisulfide (Sb_2S_3) and tin colloids (Sn-colloid). ^{90}Y-Sb_2S_3 and ^{99m}Tc-Sb_2S_3 were found to have a diameter of 28.92 ± 0.14 and 35.61 ± 0.11 nm, respectively, by PCS. By TEM, ^{90}Y-Sb_2S_3 particles were measured to be 14.33 ± 0.09 nm. ^{90}Y-labelled Sn colloid were found to exist with a $d_{v(max1)}$ of 805 nm and a $d_{v(max2)}$ of 2590 nm, by PCS, whereas ^{99m}Tc-Sn colloid was shown to have more than 80% of radioactive particles of approximately 910 nm by PCS. For ^{90}Y-labelled Sb_2S_3 and Sn colloid, a comparison of TEM and PCS indicates that these techniques found significantly different mean diameters. TEM has an excellent resolution necessary for radiocolloid particle-sizing analysis, and it is a desirable size-measuring technique because it is more reliable than PCS.

Introduction

The interest for the application of radiopharmaceuticals in direct management of serious illnesses, especially cancer and rheumatism, has increased during the last decade. Yttrium-90 (^{90}Y) is a clinically acceptable β-emitting radionuclide useful for therapy. It is a pure β-emitter, with a high $E_{max}\beta^-$ 2.27 MeV and $t_{1/2}$ 64.4 h and lack of gamma radiation makes outpatient treatment feasible (Chinol & Hnatowich, 1987).

There is a class of ^{99m}Tc-radiopharmaceutical complexes of large molecules, colloids or microspheres that is used to assess disease state or function in patients by taking advantage of the biological distribution of these particles *in vivo*. The particle size, not molecular composition, determines the biological fate of these molecules.

The radiocolloid particles are taken up selectively by various organs of the reticuloendothelial system (lungs, liver, spleen, lymph, bone marrow) depending on the particle size and charge (Ikomi *et al*., 1999).

Particle sizes greater than 10–15 μm (but <100 μm) in size are trapped in the lung capillaries by a purely mechanical process, and thereby can be used to evaluate pulmonary lung perfusion. Particle sizes below 1–10 μm in size are taken up by the reticuloendothelial system of the liver (primarily 0.3–1 μm particles), spleen (mainly >1 μm particles) and bone marrow (predominantly <0.1 μm particles). In normal individuals, 80–90% of the reticuloendothelial (RE) cells are located in the liver, 5–10% in the spleen and the remainder in the bone marrow (Jurisson *et al*., 1993).

^{99m}Tc-antimony trisulfide colloid (^{99m}Tc-Sb_2S_3) has been used for bone marrow imaging, lymphedema assessment, scintigraphic mapping of lymphatic channels and sentinel nodes in melanoma and breast cancer. Its particles have been reported as ranging from 3 to 30 nm, an optimum size for imaging lymphatic channels in lymphoscintigraphy (Gang *et al*., 1995). ^{99m}Tc-Tin colloid (^{99m}Tc-Sn colloid) has been used for measuring liver perfusion by relative uptake in reticuloendothelial cells of the liver and spleen (Tsopelas *et al*., 2003). In the present study, ^{90}Y-labelled colloids were studied with an aim to prepare therapeutic radiopharmaceuticals.

Experiment

$^{90}YCl_3$ was purchased from Polatom, Poland, in a no-carrier-added form (29.64 GBq cm^{-3}, in 0.05 M HCl). ^{99m}Tc-pertechnetate was obtained from a ^{99}Mo/^{99m}Tc radionuclide generator (Vinca Institute of Nuclear Sciences, Belgrade, Serbia). Commercial kits (Vinca Institute of nuclear sciences) of Sb_2S_3 colloid and Sn colloid were used for investigation.

Antimony sulfide colloid was prepared by saturating 100 mL of boiled water for injection with hydrogen sulfide gas for 1 h. Twenty millilitres of 1% aqueous solution of antimonyl potassium tartrate (Merck, Darmstadt, Germany) was added and after mixing, an orange liquid was obtained. Then, 10 mL of 4.0% aqueous solution of polyvinylpyrrolidone (PVP, Fluka) of 44 000 mol. wt. were added, and the excess hydrogen sulfide was removed by purging with nitrogen gas for 30 min. The

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final pH of the mixture was adjusted at 1.5 using 0.15 mM HCl. The reagent was sterilized by membrane filtration (0.22 μm). Each vial contains 1.5 mg Sb_2S_3, 1.7 mg potassium bitartrate and 6 mg polyvinylpyrrolidon. It is a clear orange liquid. The total volume was 2 mL.

Tin colloid was prepared from tin(II) fluoride (0.125 mg SnF_2; Cerac Micropure, Milwaukee, WI) and 1 mg sodium fluoride (NaF; Cerac Micropure) in a freeze-dried form.

Eighteen to 37 MBq of ^{99m}Tc in 1.0 mL saline was added to 2.0 mL of the pre-formed antimony trisulfide reagent in a 10-mL reaction vial. The mixture was heated for 30 min in a boiling-water bath, and after cooling to room temperature, 2 mL of a 0.1 M citrate buffer was added to adjust the pH value to 5.1–5.9. To prepare ^{99m}Tc-Sn colloid, 5 mL of $^{99m}TcO_4^-$ solution (37 MBq) was added to a lyophilized kit. This reaction vial was shaken and left at room temperature for 30 min. Its pH was 4.5–5.5. Five to 10 μL of $^{90}YCl_3$ stock solutions (approximately 185 MBq) were added to shielded vials containing Sb_2S_3 colloid and Sn colloid. ^{90}Y-labelled colloids were prepared in the same manner as reported for ^{99m}Tc-labelled colloids. Particle size was analyzed in undiluted samples, at 20°C, using a light-scattering photon correlation spectroscopy (PCS) instrument Zetasizer Nano ZS (Malvern Instruments Ltd., England, U.K.). It can measure particles in the size range 0.6 nm to 6 μm. Transmission electron microscopy (TEM) measurements of investigated samples were carried out using a Philips EM 400T instrument (Philips Electronics Co., Eindhoven, the Netherlands), which has an operating voltage of 120 kV. For TEM measurement, the prepared samples (mentioned earlier) were diluted six times with double-distilled water. The samples of radiocolloids were applied onto carbon-coated copper grids. The colloids were allowed to dry in air partially on the surface of the grids, which were then washed in distilled water to remove any water-soluble salts. After the samples were dried in air, the colloid sizing on each grid was examined by TEM.

The particle-size distribution and the stability of the ^{90}Y- and ^{99m}Tc-colloid particles, formed during preparation, were also studied at 72 h post-reconstitution.

Results and discussion

The principal d_v values of ^{90}Y- and ^{99m}Tc-labelled antimony trisulfide colloids and tin colloids were determined by PCS. The d_v is defined as the hydrodynamic diameter of a sphere having the same volume as the particle. For ^{90}Y-Sb_2S_3 and ^{99m}Tc-Sb_2S_3 particles, the mean d_vs were found to be 28.92 ± 0.14 nm and 35.61 ± 0.11 nm, respectively, of the total population. Their curves of particle-size distribution have a single definite peak (Fig. 1). ^{90}Y-Sb_2S_3 particles were visualized as spheric by TEM, and size was described as d_a (the diameter of a circle). The electron micrograph of ^{90}Y-antimony sulfide colloid (Fig. 2) shows both large and small particles. The larger particles were PVP, the stabilizer in the preparation as revealed by X-ray fluorescence analysis. The smaller particles were of ^{90}Y-antimony sulfide colloid. From Fig. 2, the $d_{a(max)}$ of the highest particle frequency in the total population of ^{90}Y-Sb_2S_3 was 14.28 nm, and the range of the total particle population was 7.13–21.43 nm. Of the total population of ^{90}Y-Sb_2S_3 particles, 96.5% were less than or equal to 23 nm. A comparison of TEM (14.28 nm) and PCS (28.92 nm) indicates that these techniques found significantly different mean diameters. It is assumed that ^{90}Y-Sb_2S_3 particles were

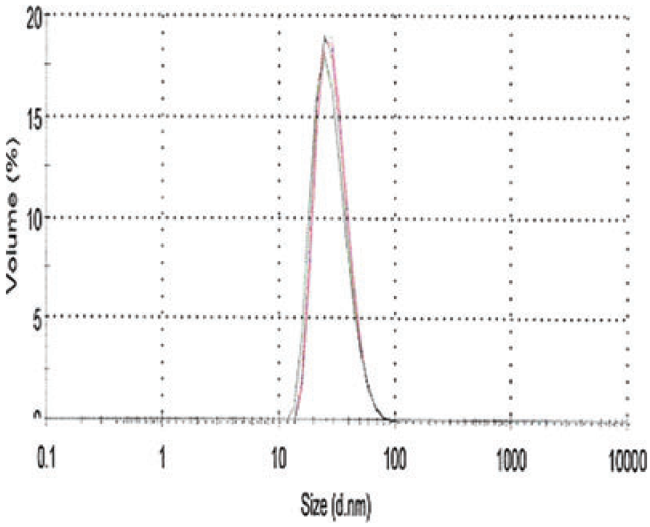

Fig. 1. Particle-size distribution of ^{90}Y-antimony trisulfide colloid by PCS.

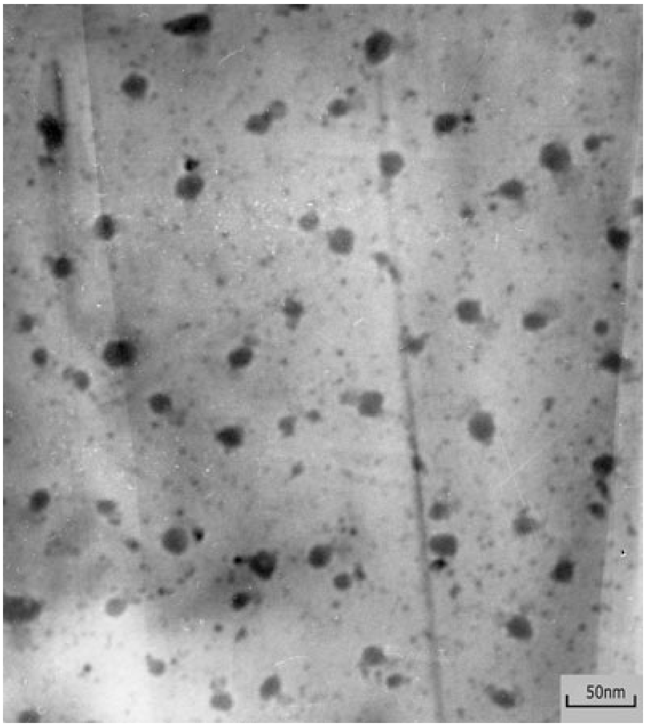

Fig. 2. Electron micrograph of ^{90}Y-antimony trisulfide colloid.

not large enough to exceed the size-threshold limitation or a lower accuracy for the PCS instrument, which resulted in an overestimation of particle size as a consequence of added stabilizer. The particle size obtained is the hydrodynamic diameter, that is any added stabilizer or solvent moving with the particle is included in the particle size. These results are in accordance with literature data for ^{99m}Tc-antimony trisulfide [Tsopelas (2001)].

The particle size of tin colloid labelled by ^{90}Y and ^{99m}Tc was determined by PCS. The results indicate that 97 and 3% of the colloidal particles in the samples of ^{90}Y-Sn colloid were characterized by a mean $d_{v(max1)}$ value of 805 nm and a mean $d_{v(max2)}$ of 2590 nm, respectively (Fig. 3). For ^{99m}Tc-Sn colloid, 83.4 and 16.6% of the colloidal particles in the samples were characterized by a mean $d_{v(max1)}$ value of 909 nm and a mean $d_{v(max2)}$ of 5320 nm, respectively. ^{90}Y-Sn colloid particles were visualized as spheric by TEM, and their size was labelled as d_a. The $d_{a(max)}$ of the highest particle frequency in the total population of ^{90}Y-Sn colloid was 94.0 nm (Fig. 4). Under the well-standardized conditions of the ^{90}Y-Sn colloid, the reproducibility of the particle size and its distribution is very good. A more rapid technique for measuring radioactive particle size distribution is filtration analysis. The principle of size measurement for this technique is based on d_v. These experiments (Table 1) showed that in the case of antimony trisulfide 94% of the total ^{90}Y radioactivity is associated with the colloidal particles smaller than 20 nm, while more than 90% of ^{99m}Tc radioactivity is associated with the particles retained on the filter with pore size of 20 nm. Almost 80% of the total ^{90}Y radioactivity was associated with tin-colloid particles smaller than 100 nm. ^{99m}Tc-tin colloid particles could be filtered through a 1 μm, but not through a 450 nm filter. The filteration analysis results correlate much better with the TEM than with the PCS ones.

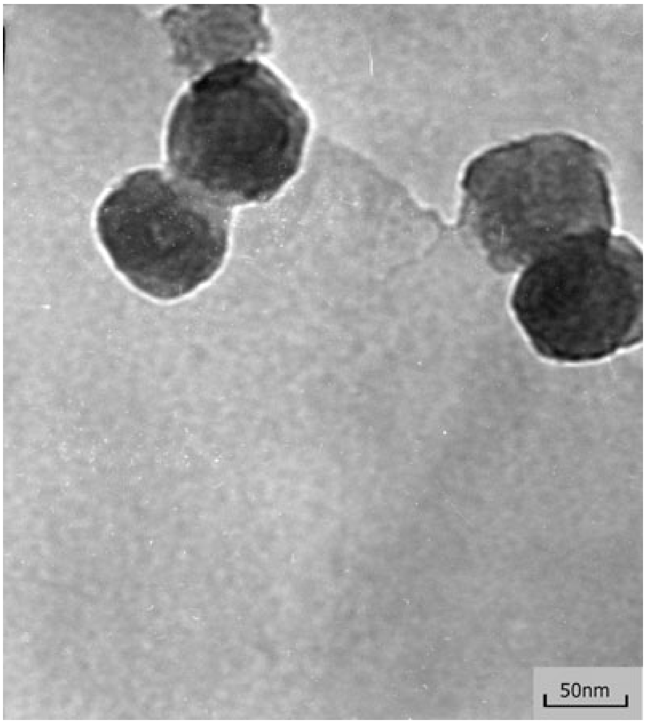

Fig. 4. Electron micrograph of ^{90}Y-tin colloid.

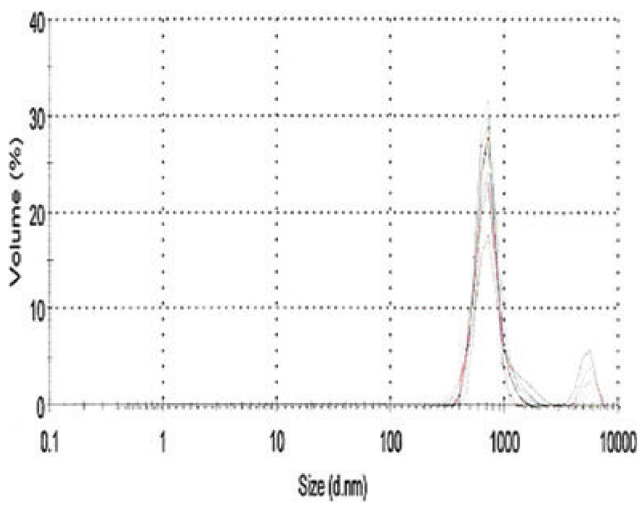

Fig. 3. Particle-size distribution of ^{90}Y-tin-colloid by PCS.

A comparison of TEM (94.0 nm) and PCS (805 nm) showed that these techniques found significantly different mean diametric sizes for the ^{90}Y-Sn colloid. The Sn colloid does form weakly bonded agglomerates up to 2–3 μm in the preparation vial. A number of factors have a great influence on puzzling growth in the particle size such as salt concentration, pH, surface charge and exposure of tin colloid to oxygen. The weakly bonded agglomerates undergo the re-dispersion, and it was found that gently shaking the light-scattering cell, when the measured size was 1.3 μm, reduced the particle diameter to only 550 nm (Whateley & Steele, 1985). As PCS determines the hydrodynamic diameter as the particle size, an overestimation of particle size at the size level indicates that PCS is not reliable for ^{90}Y-Sn colloid because of the nature of its particles (its growth and agglomeration properties).

PCS has the advantage of measuring particles in the prepared sample without any further processing. If the size distribution of the samples is broad, the use of this method, presuming spheric particles, is more problematic (Tsopelas, 2001). The typical colloidal sol is sensitive to the concentrations and charges of its particles (Moore, 1972). Surface-charge density and/or zeta potential of the sol particles influences its stability. Decrease of the surface-charge density and/or zeta potential leads to decrease of sol stability and particle agglomeration. The agglomerates have bigger sizes, and the limitations of PCS cause it to show erroneous particle-size data. In undiluted samples that were used for PCS analysis, it was supposed that the total number of particles was high

Table 1. Radioactive particle-size distribution.

	Filter pore size					
	% Activity retained on filter (mean ± SD)					
Colloids	1 μm	0.45 μm	0.22 μm	0.1 μm	0.05 μm	0.02 μm
^{99m}Tc-Sb_2S_3	—	—	—	—	5.3 ± 0.8	90.7 ± 2.2
^{99m}Tc-Sn colloid	18.8 ± 1.9	81.2 ± 3.8	—	—	—	—
^{90}Y-Sb_2S_3	—	—	—	—	—	5.9 ± 2.1
^{90}Y-Sn colloid	—	—	3.3 ± 1.2	18.0 ± 2.5	78.7 ± 3.7	—

enough so that agglomeration could occur. In the next experiment, to avoid the agglomeration of the particles, a series of diluted samples will be prepared and particle size and charge of the particles will be measured. TEM is a desirable size-measuring technique because the individual particles could be observed and measured.

The particle-size stability studies demonstrated that there were no significant changes in particle-size distribution over the 72-h study period ($P > 0.05$).

Conclusion

Biological behaviour of the radio-labelled colloidal particles is directly related to their particle size. A comparison of various sizing techniques has shown that TEM analysis is an accurate technique for determining the size, shape and chemical nature of the radiocolloids. It has the excellent resolution necessary for particle analysis at the nanometre level. The most rapid and simplest technique for measuring radioactive particle distribution is PCS, but the conditions of colloid preparation need to be optimized for good reproducibility of particle-size results.

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