A Facile and Novel Route to ^{99m}Tc-labeled Antimony Sulfide Nanocolloid

Sang Hyun Park,* Sang Mu Choi, Hui Jeong Gwon, and Kyung Bae Park
Division of Radioisotope Production and Application, HANARO Center,
Korea Atomic Energy Research Institute, 150 Deokjin-dong, Yuseong-gu, Daejeon 305-353, Korea

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^{99m}Tc-labeled antimony sulfide nanocolloid was prepared by a new labeling method that involves room temperature reconstitution with Na^{99m}TcO$_4$. Transmission electron microscope showed that the mean particle size was estimated to be mostly 7–15 nm. The labeling yield of ^{99m}Tc-Sb$_2$S$_3$ was more than 99%. The stability of the labeled product was studied at room temperature and it was found to be excellent.

^{99m}Tc-labeled antimony sulfide nanocolloid has been widely used for lymphoscintigraphy or bone marrow scintigraphy,[1–3] since nanocolloids are good carriers of radionuclides which can be used in diagnostic nuclear medicine.[4–6] ^{99m}Tc is an excellent candidate for radiodiagnosis because of its ready availability of carrier-free form from ^{99}Mo/^{99m}Tc generator. The 6 h half-life and gamma ray emission of 140 keV are appropriate for obtaining a gamma picture.[7,8] The preparation and radiolabeling of antimony sulfide nanocolloid was reported previously and the particle size of the nanocolloid has been determined. We have also studied the preparation of ^{99m}Tc labeled colloid such as ^{99m}Tc-Sb$_2$S$_7$, ^{99m}Tc$_2$S$_7$, and so forth.[9] It is also important and interesting to investigate the effect of the nature of reducing agents on radiolabeling of nanocolloids because many radiolabeling of nanocolloids occurs under harsh conditions. In this paper we report the influence of reducing agent on the radiolabeling of antimony sulfide nanocolloid. The investigation has developed a novel, simple, and efficient method for the ^{99m}Tc labeling of antimony sulfide nanocolloid with potential applications in nuclear medicine.

Antimony sulfide nanocolloid was prepared using a procedure as described in a previous work[9] with minor modifications. Hydrogen sulfide was added into 120 mL of distilled water that was cooled to about 0–5 °C. 20 mL of 1% antimony potassium tartrate was added and stirred for 5 min. 10 mL of 3.5% polyvinylpyrrolidone (PVP-mw 40000) was added into the saturated hydrogen sulfide solution, and then nitrogen gas was bubbled through the solution to get rid of the excess hydrogen sulfide. The absence of hydrogen sulfide was confirmed with lead acetate paper. The preparation was then sterilized by membrane filtration (0.22 μm) into sterile reaction vials for storage at 0 °C in a refrigerator. Then it was spotted on to plastic-coated (carbon-stabilized) copper grids (300 mesh) for characterization by transmission electron microscope (TEM). The electron micrographs revealed that the Sb$_2$S$_3$ nanocolloids were all spherical. The schematic structure of the nanocolloid is shown in Figure 1.

Radiolabeling of the nanocolloid with ^{99m}Tc, the formation of ^{99m}Tc-Sb$_2$S$_3$ nanocolloid (**1**), was performed by conventional method with minor modifications.[9,10] To a vial of lyophilized antimony sulfide nanocolloid, 0.2 mL of 0.1 N HCl and 0.5 mL of Na^{99m}TcO$_4$ were added. After heating the mixture in boiling water for 20 min, it was cooled to room temperature. And 0.5 mL of sodium acetate buffer (0.35 M, pH 8) was added to adjust the pH to 6.5. The ^{99m}Tc-antimony sulfide nanocolloid prepared by the conventional method had a particle size ranging from 5–20 nm (mostly 7–15 nm) (Figure 1). Lin and co-workers have proposed the labeling mechanism of nanocolloid with ^{99m}Tc in which technetium is incorporated onto the surface of the colloid after redox reaction between Tc(VII) and Sb(II).[10] ^{99m}Tc should be reduced prior to the reaction of [^{99m}Tc]pertechnetate with Sb$_2$S$_3$ nanocolloid to form ^{99m}Tc-Sb$_2$S$_3$ nanocolloid. The reduction of [^{99m}Tc]pertechnetate can be achieved in the presence of a reducing agent including hydrochloric acid, stannous ion, sodium tetrahydroborate, and etc. Hydrochloric acid is the most commonly used reducing agent for the preparation of ^{99m}Tc-Sb$_2$S$_3$ nanocolloid as shown above. However, tin chloride dihydrate (SnCl$_2$·2H$_2$O) is stable in an acidic condition, but it often leads to several problems such as the formation of insoluble ^{99m}TcO$_2$ or SnO$_2$ colloid. The formation of colloids greatly diminishes the labeling efficiency of the ^{99m}Tc-Sb$_2$S$_3$ nanocolloid. In contrast, sodium tetrahydroborate is stable at high pH, while unstable at low pH. The reducing agents described above are typically utilized in a liquid phase. When they are used excessively, impurities such as colloids can be generated, in addition to a danger of potentially residual toxicity. These problems can be overcome by employing a tetrahydroborate exchange resin (BER)[11] to which tetrahydroborate ion (BH$_4^-$) is bound. After that, unreacted tetrahydroborate ion can be removed by a simple membrane filtration, regardless of its used amount. Since the reduction of [^{99m}Tc]pertechnetate has been achieved in either acidic or basic condition as described above, there is a need for developing methods capable of reducing [^{99m}Tc]pertechnetate in a wide range of pH 2 to 14 for the preparation of ^{99m}Tc-Sb$_2$S$_3$ nanocolloid.

Radiolabeling of the nanocolloid with ^{99m}Tc, the formation of ^{99m}Tc-Sb$_2$S$_3$ nanocolloid (**2**), was achieved by the new method as follows. To a vial of lyophilized antimony sulfide nanocolloid, 5 mg of tetrahydroborate exchange resin and 0.5 mL of Na^{99m}TcO$_4$ were added. After stirring the mixture at room temperature for 20 min, it was filtered by membrane filter (0.22 μm). The ^{99m}Tc-antimony sulfide nanocolloid prepared by the new method had a particle size ranging from 5–20 nm (mostly 7–15 nm) (Figure 1). The assay for formation of ^{99m}Tc-Sb$_2$S$_3$ nanocolloids, ^{99m}TcO$_2$ colloid, and [^{99m}Tc] pertechnetate ion can be achieved by investigating their positions using an instant thin-layer chromatography (ITLC). In this experiment, labeling efficiency of ^{99m}Tc-Sb$_2$S$_3$ nanocolloids thus prepared was determined by performing the ITLC. Table 1 shows the results of thin-layer chromatography for ^{99m}Tc-Sb$_2$S$_3$ nanocolloids (**1**, **2**) by performing ITLC on silica gel-impregnated glass fiber sheets using acetone and physiological saline as a developing solvent, respectively. In case of ^{99m}Tc-Sb$_2$S$_3$ nanocolloid (**2**), prepared by the new method, ITLC-SG (silica gel) was performed using

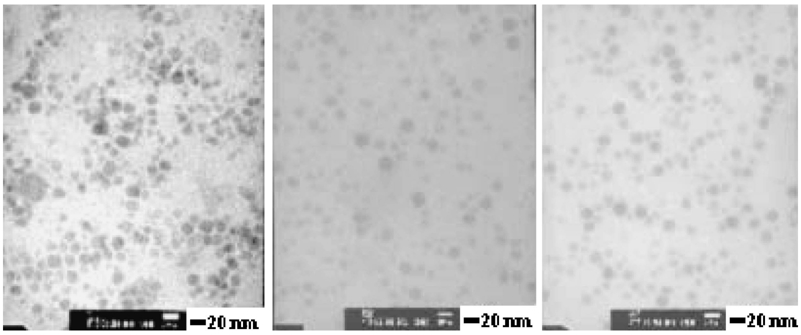

Figure 1. TEM images of nanocolloids. Left photograph shows Sb_2S_3 nanocolloid. Middle photograph shows ^{99m}Tc-Sb_2S_3 nanocolloid (**1**) prepared by the conventional method. Right photograph shows ^{99m}Tc-Sb_2S_3 nanocolloid (**2**) prepared by the new method.

Table 1. ITLC analysis of ^{99m}Tc-Sb_2S_3 nanocolloids (**1**, **2**)

Chromatographic System		^{99m}Tc species at	
Support	Solvent	Origin	Solvent Front
ITLC-SG	Acetone	100% of ^{99m}Tc-Sb_2S_3	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	100% of ^{99m}Tc-Sb_2S_3	0% of $^{99m}TcO_4^-$

acetone and physiological saline as a developing solvent, and the results are given in Table 1.

As apparent in Table 1, showing a result of ITLC-SG of **2** using acetone as a development solvent, there was no observation of a peak of $^{99m}TcO_4^-$ at the solvent front, which is expected to migrate with the solvent front. These results indicate that **2** having more than 99% of labeling efficiency was formed. In order to estimate stability of the radiolabeled compound, the ^{99m}Tc-Sb_2S_3 nanocolloid solution in closed vacuum vials were stored at room temperature and the radiolabeling efficiency was determined at 1, 2, 4, 6, 8, 12, 16, 20, 24 h, respectively. The results are given in Figure 2. It was found to be over 98% till 24 h.

In conclusion, attempts to improve the overall efficiency of this process have focused on use of tetrahydroborate exchange resin (BER) as a solid phase reducing agent. Contrary to the conventional usage of 0.1 N hydrochloric acid, heating, and pH adjustment, ^{99m}Tc-antimony sulfide nanocolloid was prepared efficiently employing tetrahydroborate ion, room temperature, and a simple membrane filtration. The stability of ^{99m}Tc-Sb_2S_3 nanocolloid was also studied and it was found to be excellent. Lymphoscintigraphy of **2** shows faster deposition than **1** in lymph nodes in rabbits. The present report describes the first use of BER as a new reducing agent for the facile preparation of ^{99m}Tc-labeled antimony sulfide nanocolloid and a new labeling method. The new labeling method is expected to be amenable to the preparation of ^{99m}Tc-labeled nanocolloids that are expected to have diagnostic potential in nuclear medicine.

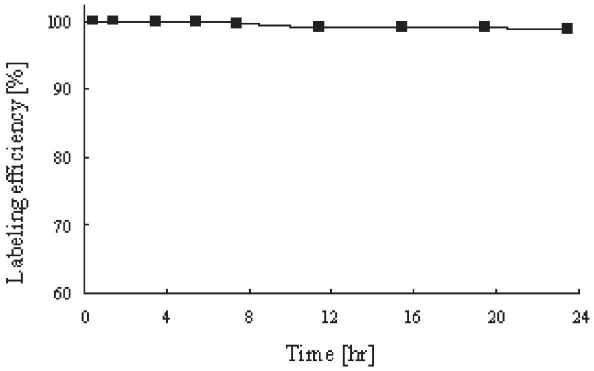

Figure 2. Stability of ^{99m}Tc-Sb_2S_3 nanocolloid.

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References

1 C. Tsopelas, *Appl. Radiat. Isot.*, **59**, 321 (2003).
2 M. Mitterhauser, W. Wadsak, L. K. Mein, H. Eidherr, S. Roka, G. Zettinig, P. Angelberger, H. Viernstein, K. Kletter, and R. Dudczak, *Appl. Radiat. Isot.*, **59**, 337 (2003).
3 K. Nagai, Y. Ito, N. Otsuka, and A. Muranaka, *Eur. J. Nucl. Med.*, **7**, 3966 (1982).
4 J. Hauspy, L. Verkinderen, C. D. Pooter, L. Y. Dirix, and P. A. Dam, *Gynecol. Oncol.*, **86**, 358 (2002).
5 F. W. C. Ent, R. A. M. Kengen, H. A. G. Pol, and A. G. M. Hoofwijk, *Eur. J. Surg. Oncol.*, **25**, 24 (1999).
6 L. Ren and G. M. Chow, *Mater. Sci. Eng., C*, **23**, 113 (2003).
7 S. S. Jurisson and J. D. Lydon, *Chem. Rev.*, **99**, 2205 (1999).
8 W. A. Volkert and T. J. Hoffmann, *Chem. Rev.*, **99**, 2269 (1999).
9 K. B. Park, O. D. Awh, J. R. Kim, S. M. Lim, and S. W. Hong, *Kor. J. Nucl. Med.*, **23**, 71 (1989).
10 Y. Lin, X. Zhang, J. Li, D. Yin, and Y. Wang, *Appl. Radiat. Isot.*, **58**, 347 (2003).
11 S. H. Park, H. J. Gwon, Y. H. Kim, and K. B. Park, *Bull. Chem. Soc. Jpn.*, **76**, 1977 (2003).

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