Application of a Macromolecular Sn(II) Complex to the Preparation of ^{99m}Tc Radiopharmaceuticals

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SYNOPSIS

To develop insoluble Sn^{2+} complexes for the preparation of ^{99m}Tc radiopharmaceuticals, the adsorption of Sn^{2+} to macroreticular chelating ion exchange resins having various functional groups was investigated. Among them, a resin containing aminophosphonic acid groups showed a high adsorption capacity for Sn^{2+}, which was bound strongly to the resin by chelation. This macromolecular Sn^{2+} complex was very stable against hydrolysis and oxidation, and could be applied satisfactorily for the reduction of ^{99m}Tc.

INTRODUCTION

Technetium-99m (^{99m}Tc) is widely used as a radionuclide for radiopharmaceuticals because its physical and chemical properties are suitable for body scanning.[1] ^{99m}Tc is generally eluted from the ^{99}Mo–^{99m}Tc generator as $^{99m}Tc^{7+}$ pertechnetate ions ($^{99m}TcO_4^-$). The subsequent reduction of $^{99m}TcO_4^-$ from +7 to a lower level of oxidation in the presence of various ligands is essential for the preparation of ^{99m}Tc complexes for use as radiopharmaceuticals.

Despite its inherent disadvantages of being easily hydrolyzable and oxidizable,[2,3] it is generally recognized that stannous chloride ($SnCl_2$) is an appropriate reducing agent for the preparation of ^{99m}Tc complexes. A larger amount of $SnCl_2$ than that required for the reduction of ^{99m}Tc stoichiometrically is added to most of commercial kits for the preparation of ^{99m}Tc radiopharmaceuticals. However, it has been reported that a large excess of $SnCl_2$ in the labeling system affects not only the quality, purity, and stability of the radiopharmaceuticals but also the biological behavior of ^{99m}Tc complexes.[4-6] Insoluble macromolecular Sn^{2+} (R–Sn) complexes have been proposed as one way to resolve these problems in the preparation of ^{99m}Tc radiopharmaceuticals. In this study, we investigated chelating ion exchange resins suitable for the preparation of R–Sn complexes applicable to the reduction of ^{99m}Tc.

EXPERIMENTAL

Materials

The chelating ion exchange resins used in this study were obtained from various sources (Table I). Stannous chloride ($SnCl_2 \cdot 2H_2O$), diethylenetriaminepentaacetic acid (DTPA), and 8-hydroxy-2-methylquinoline (MeOx) were obtained from Nakarai Tesque Inc. $^{99m}TcO_4^-$ in a saline solution was eluted daily from a Daiichi Radioisotope Lab. generator, and 37 MBq dm^{-3} was used for the labeling reaction. Human serum albumin (HSA) was kindly supplied by the Chemo-sero-therapeutic Research Institute (Japan). All other reagents used were of analytical grade.

Preparation of Macroreticular (MR) Chelating Resins

Various MR chelating resins were synthesized at our laboratories (Fig. 1). MR chelating resins (RSP and RCSP) containing dihydroxyphosphino and phosphono groups were prepared from styrene–divinylbenzene (DVB, 10 mol %) copolymer beads synthesized by suspension polymerization in the presence of 2,2,4-trimethylpentane (100 vol % per

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Table I Details of the Commercially Available Chelating Resins Tested

Manufacturer	Commercial Name	Functional Group	Abbreviation
Rohm & Haas	Amberlite CG-50	$-COOH$	CG-50
Bayer	Lewatit ATP-202	$-COOH$	ATP-202
Bayer	Lewatit MP-62	$-N(CH_3)_2$	MP-62
Mitsubishi	Diaion CR-20	$-NH(C_2H_4NH)_nH$	CR-20
Mitsubishi	Diaion CR-10	$-N(CH_2COOH)_2$	CR-10
Dow Chemical	Dowex A-1	$-N(CH_2COOH)_2$	A-1
Diamond Shamrock	Duolite ES-467	$-NHCH_2PO_3H_2$	ES-467
Bayer	Lewatit OC-1060	$-NHCH_2PO_3H_2$	OC-1060
Diamond Shamrock	Duolite CS-346	$-C(NH_2)NOH$	CS-346

monomer).[7] MR type 4-vinylpyridine-DVB (10 vol %) copolymer beads (4VP) were synthesized by suspension polymerization in the presence of toluene (100 vol % per monomer).[8] An MR chelating resin (RSH) with triazolethiol as the functional group was prepared from ethyl acrylate-DVB (10 vol %) copolymer beads synthesized by suspension polymerization in the presence of 2,2,4-trimethylpentane (100 vol % per monomer).[9]

Measurement of Physical and Chemical Properties

The specific surface area for each resin was measured on a Yuasa surface area apparatus (BET method), pore volume, and radius of the resins were determined using a Carlo-Erba mercury porosimeter (Model 1520). Phosphor content was determined spectrophotometrically by the molybdenum blue method after decomposition with a mixture of hydroperchloric and nitric acids.[10]

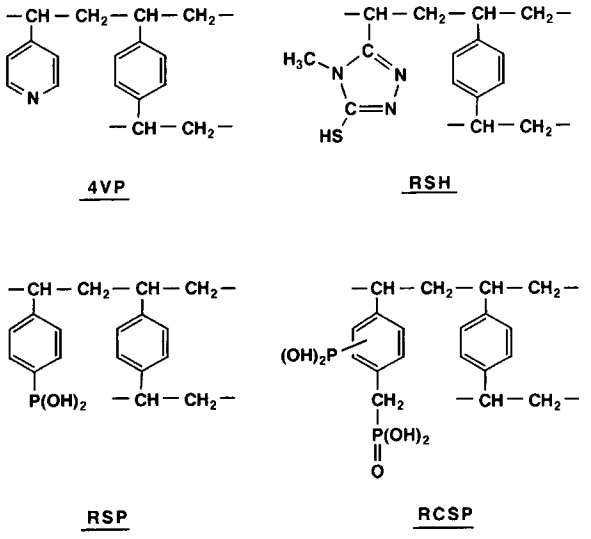

Figure 1 The structures of the functional groups of 4VP, RSH, RSP, and RCSP.

Preparation of R–Sn Complexes

All chelating resins were conditioned by washing them several times with alternating large excesses of hydrochloric acid and sodium hydroxide solution. Finally, the resins were immersed in 1 mol dm^{-3} hydrochloric acid and washed with distilled water until the pH of the washings reached 5–6. The resins were then dried in air and in an oven at 50°C before being stored.

After the addition of each chelating resin (100 mg) to 30 cm^3 of 0.1 mol dm^{-3} hydrochloric acid in a 50 cm^3 flask, the mixture was degassed *in vacuo* and purged with nitrogen gas for 10 min. A $SnCl_2$ solution (0.04 mol dm^{-3}) was carefully prepared in nitrogen-purged 0.1 mol dm^{-3} hydrochloric acid, and 10 cm^3 of this solution was immediately added to the flask. After shaking for 1 h, the resins were rapidly filtered off with a glass filter, washed with 0.1 mol dm^{-3} hydrochloric acid and methanol, and then dried *in vacuo*. The concentration of $SnCl_2$ in the supernatant was determined using a Hitachi polarized Zeeman atomic absorption spectrophotometer (Z-8000).

^{99m}Tc-Labeling of HSA, DTPA, and MeOx with R–Sn Complexes

HSA. In a 5 cm^3 vial, 3.5 mg of HSA was dissolved in 0.7 cm^3 of 0.01 mol dm^{-3} hydrochloric acid and then 3 mg of the R–Sn complex (1.8 mmol Sn adsorbed/g resin) was added to the solution. After shaking for 5 min, 0.1 cm^3 of ^{99m}Tc pertechnetate in saline solution was added to the vial and the mixture was shaken at room temperature.

DTPA and MeOx. In a 5 cm^3 vial, 3 mg of the R–Sn complex (1.8 mmol Sn adsorbed/g resin) was

mixed with 0.7 cm^3 of 0.01 mol dm^{-3} DTPA prepared with 0.05 mol dm^{-3} acetate buffer (pH 4) or 0.001 mol dm^{-3} MeOx containing 10% ethanol. After standing for 5 min, 0.1 cm^3 of ^{99m}Tc pertechnetate was added to the vial and the mixture was shaken at room temperature.

Thin Layer Chromatography (TLC) of ^{99m}Tc-HSA, DTPA, and MeOx

Solutions of ^{99m}Tc–HSA, DTPA, or MeOx were spotted onto Merck cellulose (Art 5577) or silica gel (Art 5748) strips or plates. A methanol–water mixture (85 : 15 v/v) was used as the development solvent and an Aloka chromatoscanner was used to measure the radioactivity of the strips.

RESULTS AND DISCUSSION

By the introduction of various functional groups to polymer beads, a large number of chelating ion exchange resins which showed a high affinity towards specific transition metal ions have been developed.[11] These chelating resins have been expected to be useful in the investigation of R–Sn complexes suitable for the preparation of ^{99m}Tc radiopharmaceuticals. However, there have been few reports so far of chelating resins that have selectivity and a high affinity for Sn^{2+}.

It is necessary to search for chelating resins having a high adsorption capacity and rate for Sn^{2+} in acidic media which can retard the hydrolysis of Sn^{2+}.[12] For this study, nine chelating ion exchange resins were chosen from among those commercially available, and four chelating resins were synthesized at our laboratories (Table I and Fig. 1). The physical and chemical properties of these resins are summarized in Table II. Specific surface area and pore volume data demonstrated that most of the resins tested were of MR type resin, for which it is known that the adsorption rate for metal ions is higher than that for gel-type resins.[13] Iminodiacetic acid-, aminophosphonic acid-, and amidoxime-type resins possess both oxygen and nitrogen as donor atoms, while the other resins contain oxygen, nitrogen, or sulfur as the donor atom. As described in Experimental, these resins were carefully placed in contact with Sn^{2+} in 0.1 mol dm^{-3} HCl. The chelating resins containing phosphinic and phosphonic acid groups showed a higher capacity for the adsorption of Sn^{2+} than the resins containing other functional groups (Table II). The adsorption capacity for Sn^{4+} was also determined under the same conditions, and the amount of Sn^{4+} adsorbed by the resins was found to be smaller than that of Sn^{2+}. These results suggested that most of the Sn adsorbed by the resins was in the lower oxidation state which is effective for the reduction of ^{99m}Tc. Figure 2 illustrates the time course of Sn^{2+} adsorption by five resins. With the ES-467, RSP, and RCSP resins, 1.0 mmol Sn^{2+}/g resin could be adsorbed within 15 min. It was found that the presence of phosphinic or phosphonic acid groups and the macroporous structure of the resin contributed to a high adsorption capacity and rapid rate of Sn^{2+} adsorption in acidic solution. In fact,

Table II Characteristics of the Chelating Resins Tested

Resin	Specific Surface Area (m^2/g)	Pore Volume (cm^3/g)	Average Pore Radius (nm)	N Content (mmol/g)	P Content (mmol/g)	Sn Adsorbed (mmol/g resin)	
						Sn^{2+}	Sn^{4+}
CG-50	0.6	—	—	—	—	0.1	0.1
ATP-202	30.7	0.749	33.2	—	—	0.1	0.1
MP-62	11.7	0.368	25.4	4.4	—	0.2	0.1
CR-20	50.1	0.991	56.0	3.2	—	0.2	0.1
CR-10	5.2	0.153	28.2	3.2	—	0.3	0.2
A-1	0.0	—	—	3.5	—	0.6	0.1
ES-467	12.7	1.101	147.8	2.5	3.3	1.9	0.3
OC-1060	16.0	0.852	82.7	2.9	3.1	1.8	0.4
CS-346	27.7	0.465	24.5	6.1	—	0.4	0.3
4VP	50.1	0.886	23.3	5.7	—	0.8	0.2
RSH	24.3	0.832	85.6	13.4	—	0.1	0.1
RSP	47.4	0.887	39.3	—	4.8	2.4	0.6
RCSP	33.5	0.630	36.5	—	4.2	2.8	0.9

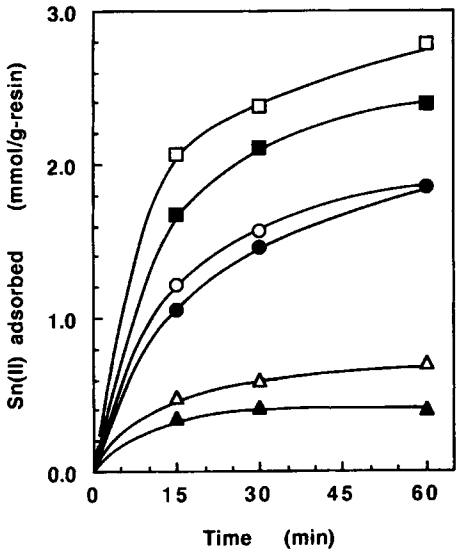

Figure 2 Adsorption of Sn^{2+} by various chelating resins: (□) RCSP; (■) RSP; (○) ES-467; (●) OC-1060; (△) A-1; (▲) CS-346.

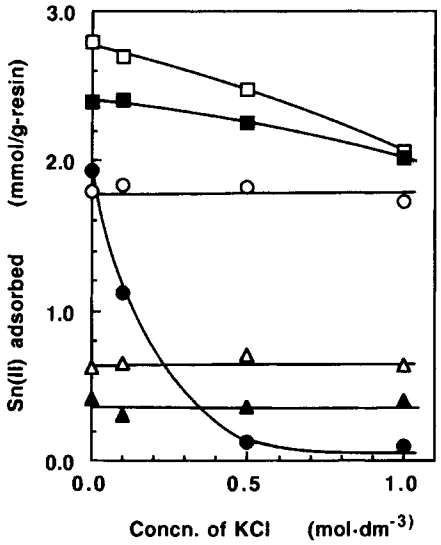

Figure 3 Effect of KCl on the adsorption of Sn^{2+} to chelating resin: (□) RCSP; (■) RSP; (○) ES-467; (△) A-1; (▲) CS-346; (●) Amberlite CG-120.

the capacity for Sn^{2+} adsorption was found to be proportional to the phosphor content of the resins. Although 4VP had a relatively high capacity for Sn^{2+}, this resin was excluded from subsequent experiments because $^{99m}TcO_4^-$ was also adsorbed rapidly by it.

To assess the stability of the Sn^{2+} adsorbed onto the resins, the capacity for Sn^{2+} adsorption in the presence of various concentrations of potassium chloride was determined for the RSP, RCSP, ES-467, A-1, and CS-346 (Fig. 3). A strong acidic cation exchange resin (Amberlite CG-120) with no donor atom was used for reference. Increasing the concentration of potassium chloride caused little decrease of the capacity of ES-467 for Sn^{2+}. Amberlite CG-120 also had a high capacity for Sn^{2+} in the absence of potassium chloride, but its adsorption of Sn^{2+} was markedly decreased as the concentration of potassium chloride increased because it was only based on ionic interactions. Although the adsorption capacity of A-1 and CS-346 was smaller than that of ES-467, the decrease in capacity with increasing potassium chloride concentrations was very slight. These results indicated that Sn^{2+} binds strongly by chelation to resins containing nitrogen and oxygen as donor atoms (Fig. 3). The effect of potassium

Table III Efficiency of ^{99m}Tc Labeling with R–Sn Complexes

R–Sn			Radioactivity	
Chelating Resin	Weight (mg)	Sn Content (μmol)	^{99m}Tc–HSA (%)	$^{99m}TcO_4^-$ (%)
ES-467	0	0.0	0.0	100.0
	1	0.2	96.2	3.8
	3	0.7	99.7	0.3
	5	1.2	97.9	2.1
	5	4.6	98.7	1.3
	5	9.6	99.8	0.2
	10	19.2	95.9	4.1
OC-1060	5	9.2	97.0	3.0
A-1	5	3.2	99.8	0.2
CR-10	5	1.3	99.8	0.2
CS-346	5	2.2	98.6	1.4

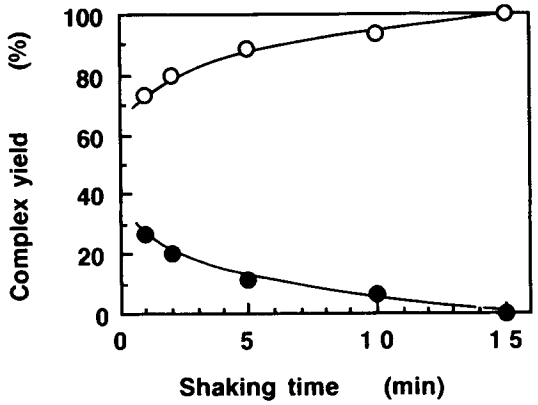

Figure 4 Labeling of HSA with ^{99m}Tc in the presence of R–Sn complexes: (○) ^{99m}Tc–HSA; (●) $^{99m}TcO_4^-$.

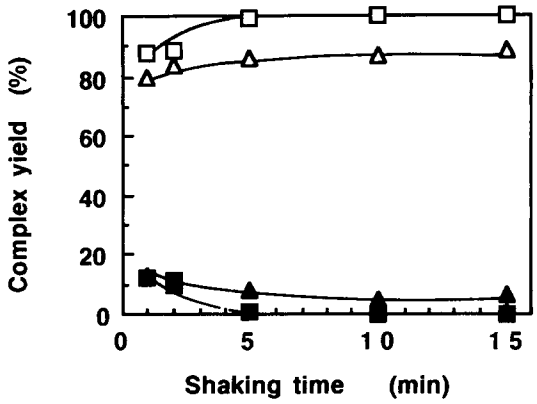

Figure 5 Labeling of DTPA and MeOx with ^{99m}Tc in the presence of R–Sn complexes. Labeling of DTPA: (□) ^{99m}Tc–DTPA; (■) $^{99m}TcO_4^-$. Labeling of MeOx: (△) ^{99m}Tc–MeOx; (▲) $^{99m}TcO_4^-$.

chloride on the resins indicated that the stability of Sn^{2+} binding to RSP and RCSP (only oxygen as donor atom) was lower than that for ES-467.

Reduced ^{99m}Tc ions can readily bind with HSA in a pH 2–4 solution to form ^{99m}Tc–HSA complexes, which is used as a radiopharmaceutical for blood pool imaging. The ES-467, A-1, and CS-346 resins containing various amounts of Sn^{2+} were applied to the ^{99m}Tc labeling of HSA, and the reducing activity of the R–Sn complexes was evaluated on the basis of the yield of labeled HSA. The radiochemical purity of ^{99m}Tc–HSA complexes is generally assayed by paper or thin layer chromatography.[14,15] Under the conditions described in Experimental, ^{99m}Tc–HSA (Rf = 0.0) was clearly resolved from nonreduced $^{99m}TcO_4^-$ (Rf = 0.5–0.6) and the yield of labeled HSA was expressed as a proportion of the total radioactivity on the cellulose plate (Table III). ^{99m}Tc–HSA was formed with a high yield using the R–Sn complexes, and the yield changed very little when these R–Sn complexes were stored for 6 months before use.

These results show that aminophosphonic acid-, iminodiacetic acid-, and amidoxime-type resins can strongly bind Sn^{2+} and are desirable polymer supports for use in the reduction of ^{99m}Tc. The introduction of an aminophosphonic acid group into the polymer matrix was found to be particularly effective for the adsorption of Sn^{2+}. As shown in Figure 4, ^{99m}Tc labeling at greater than 90% yield was performed simply by the short-term mixing of HSA and a $^{99m}TcO_4^-$ solution with ES-467 that had adsorbed Sn^{2+} (1.8 mmol/g resin). The R–Sn complexes were used for the formation of ^{99m}Tc–DTPA and ^{99m}Tc–MeOx. The former is an anionic and hydrophilic complex that is used as a kidney imaging radiopharmaceutical. The structure of the later compound has been established by single-crystal X-ray analysis.[16] As shown in Figure 5, the ^{99m}Tc-labeling reaction of low molecules such as DTPA and MeOx proceeded more rapidly than that for HSA. The R–Sn complexes prepared from aminophosphonic acid-type resin had a high reducing ability like that of $SnCl_2$. Since the complex yield of ^{99m}Tc–DTPA was decreased very little by the use of R–Sn complexes which had been let stand for 1 week in neutral aqueous solution, the Sn^{2+} adsorbed to the resins was very stable against hydrolysis and oxidation (Fig. 6). It can be easily understood that the Sn^{2+} complexes on the insoluble polymer surface play an important role in the preservation of the reducing ability of R–Sn compounds by preventing the hydrolytic polymerization of Sn^{2+}.

CONCLUSIONS

R–Sn complexes prepared by using a chelating resin containing aminophosphonic acid groups could be

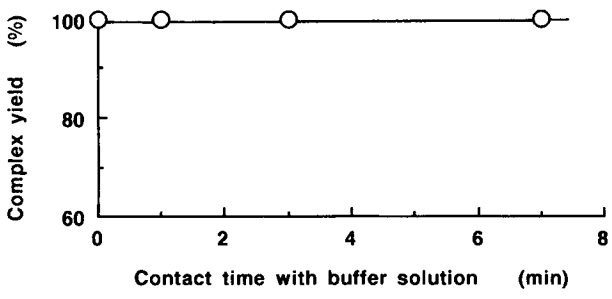

Figure 6 Stability of R–Sn complexes: resin (3 mg). Sn^{2+} was adsorbed onto ES-467 (1.8 mmol/g resin).

simply and effectively applied to the reduction of ^{99m}Tc in the preparation of ^{99m}Tc radiopharmaceuticals. The ^{99m}Tc radiopharmaceuticals obtained by using the R–Sn complexes were almost free of Sn^{2+} contamination.

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