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Research Article

Synthesis and characterization of ^{99m}Tc-labelled biologically active molecules using borohydride exchange resin as a reducing agent for the preparation of radiopharmaceuticals

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Summary

A novel and efficient method for preparing ^{99m}Tc-complexes of radiopharmaceuticals has been developed by reacting [^{99m}Tc]pertechnetate with a ligand in the presence of borohydride exchange resin (BER) as a reducing agent. The latter is stable over a wide range of pH (2–11) and thus can be used with biologically active compounds without the formation of insoluble $^{99m}TcO_2$ or SnO_2 colloids. Since the radiolabelled complexes are produced with high radiochemical purity and labelling efficiency under milder conditions than those required for the conventional reducing agents, the latter can be replaced. The method is expected to be applicable to the preparation of ^{99m}Tc-radiopharmaceuticals. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: radiolabelling; borohydride exchange resin (BER); a new reducing agent for $^{99m}TcO_4^-$; ^{99m}Tc-Complexes

Introduction

It is known that ^{99m}Tc, as a radionuclide, has desirable nuclear properties, for example, a short half-life of 6 h and 140 keV γ-ray emission energy suitable for obtaining gamma images as well as a low price and a general utility, thus it is commonly applied in nuclear medicine as radiopharmaceuticals for diagnosis and therapy.[1–4] ^{99m}Tc forms stable complexes with species having donor electron pairs such as isocyanate, amine, carboxyl or thiol groups, and various

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complexes are used as an imaging agents for organs including lungs, liver and brain.[5]

Since [^{99m}Tc]pertechnetate does not bind to most ligands, the technetium must first be reduced. The reduction of [^{99m}Tc]pertechnetate can be achieved electrolytically, or most commonly, by reducing agents such as stannous ion, ferrous ion, ferrous-ascorbate, sodium borohydride, hydrochloric acid, sodium dithionite and hypophosphorus acid. Therefore, in the labeling step with ^{99m}Tc, the choice of reducing agent is very important. Tin(II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$) is the most commonly used reducing agent for the rapid preparation of ^{99m}Tc-radiopharmaceuticals.[6–8] However, although tin chloride dihydrate is stable, precipitates can form in alkaline conditions. Also, the use of Sn(II), usually in excess, often leads to several problems such as the formation of insoluble $^{99m}TcO_2$ or SnO_2 colloids, which greatly diminishes the labelling efficiency of the ^{99m}Tc-complexes, and the dangers of toxicity from excess of reducing agent and its oxidation products. In contrast, sodium borohydride is stable in the pH range 2–11. These problems can be overcome by employing an anion exchange resin to which borohydride ion (BH_4^-) is bound, with which the metal Tc pertechnetate is reduced in a solid phase, and after reduction the excess of borohydride exchange resin (BER) can be removed by simple membrane filtration, regardless of the amount used.

Experimental

Materials and methods

Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. Sodium pertechnetate ($Na^{99m}TcO_4$) was obtained from $^{99}MoO_4^-$ by solvent extraction. The ^{99}Mo was produced at the 30 MW-HANARO research reactor, Korea Atomic Energy Research Institute (KAERI) by the neutron irradiation of ^{98}Mo (n, γ) ^{99}Mo. Methylene diphosphonate (MDP) was obtained from Radiopharmaceuticals R&D department, KAERI. Melting points were determined on a Mel-Temp (50/60 cycles, 110–120 V, 250 W) apparatus and are uncorrected. Reactions involving air or moisture sensitive reagents or intermediates were performed under an inert atmosphere of argon in glassware that had been oven and/or flame dried. Solvent removal was accomplished at aspirator pressure using a rotary evaporator. Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck) and all chromatographic separations were monitored by TLC analyses, performed using glass plates pre-coated with 0.25-mm 230–400-mesh silica gel impregnated with a fluorescent indicator (254 nm). IR spectra were recorded on a Bomen MB154 FTIR (KBr pellets or neat). ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 300-MHz NMR spectrometer (Korea Basic Science Institute,

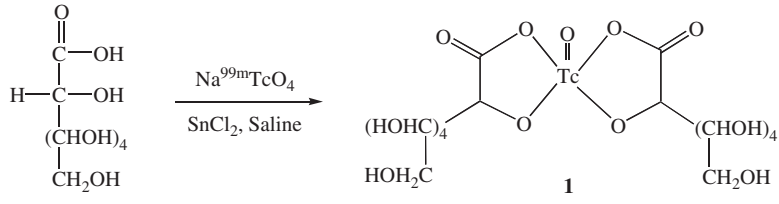
Daejeon, Korea) in $CDCl_3$, DMSO-d_6, or D_2O solution, and chemical shifts were recorded in ppm units using $SiMe_4$ as an internal standard. Mass spectra were measured on a Varian MAT 371 Mass Spectrometer at 70 eV. Instant thin-layer chromatography (ITLC) system consists of ITLC scanner (EC & G Berthold linear Analyzer, Germany) and one-dimensional analysis of Berthold chroma program. Reversed phase high performance liquid chromatography (HPLC) system was equipped with 2 Waters 501 pumps, μBondapak C-18 column (3.9×300 mm, 10 μm, Waters, USA), Waters automated gradient controller, ultraviolet detector, ray-test gamma ray detector, autochro data module, and autochro-WIN analysis program.

Preparation of borohydride exchange resin (BER)

The borohydride exchange resin (BER) was prepared by the reported method.[9] Chloride-form resin (Amberlite® ion exchange resin, 12.5 g) was slurry-packed with water into a 30-ml fritted glass funnel mounted on a filter flask. Then, aqueous sodium borohydride solution (200 ml, 0.25M) was slowly passed through the resin over a period of 30 min. The resulting resins were washed thoroughly with distilled water until free of excess, and finally with ethanol ($10 \text{ ml} \times 3$). The borohydride form anion exchange resin (BER) was then partially air-dried by removing the ethanol on the surface of BER. This resin was analyzed for borohydride content by hydrogen evolution upon acidification with 0.08M HCl, and the average capacity of BER was found to be 2.5 meq of tetrahydroborate ion per gram. The BER was stored under nitrogen at 4°C. The hydride content was constant over 5 weeks.

Preparation of ^{99m}Tc-glucoheptonate (1)

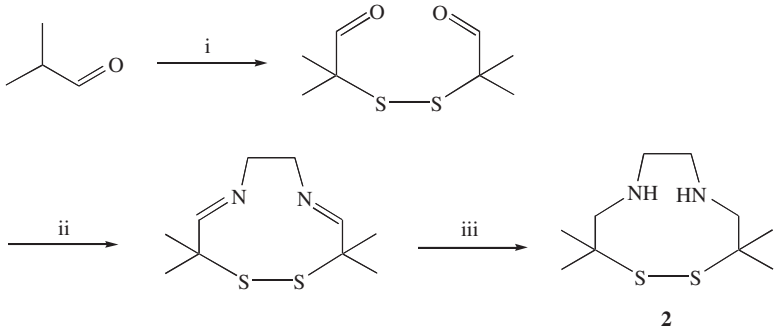
0.5 ml of $Na^{99m}TcO_4$ (925 MBq) was added to a vial containing lyophilized glucoheptonate. The reaction mixture was well stirred for about 20 s at room temperature under nitrogen atmosphere, and the reaction was carried out until the glucoheptonate powder was completely dissolved, thus forming ^{99m}Tc-glucoheptonate (**1**, ^{99m}Tc-GHA, 920 MBq) (Scheme 1). The title complex **1** was filtered using a 0.22 μm membrane filter and was used for transchelation.



Scheme 1. Preparation of ^{99m}Tc-glucoheptonate (1)

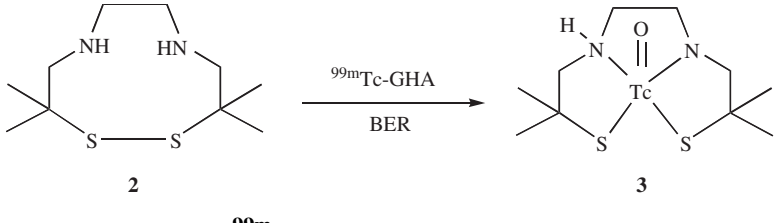
Preparation of ^{99m}Tc-DADS by transchelation (3)

3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (**2**, diamine disulfide, DADS) was synthesized by the reported method.[10] The synthesis is outlined in Scheme 2. Analytical data: m.p. 57–59°C; IR (cm^{-1}, KBr pellet) 3450, 2964, 2964, 2735, 2734, 2456, 1560, 1468, 1388; ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 6 H, 2 CH_3), 1.29 (s, 6 H, 2 CH_3), 1.75 (bs, 2 H, 2 NH), 2.4-3.0 (m, 8 H, 4 NCH_2).



Scheme 2. Preparation of 3,3,10,10-1,2-dithia-5,8-diazacyclodecane (2). Reagents and conditions: i. S_2Cl_2, CCl_4, 50–55°C, 3 h; ii. $H_2NCH_2CH_2NH_2$, PTSA, toluene, 2 h, reflux; iii. $NaBH_3CN$, CH_3OH, 55–60°C, 15 min

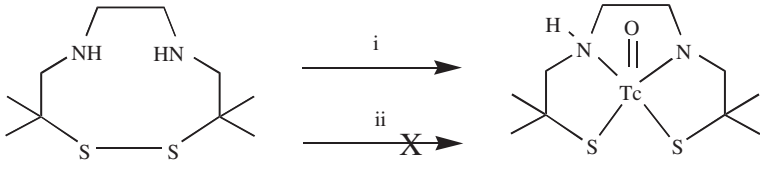
A solution of 0.1 mg of **2** in 0.1 ml of distilled water, 0.1 ml of ^{99m}Tc-glucoheptonate (185 MBq) and 0.8 ml of saline were added simultaneously to a vacuum vial containing 5.0 mg of BER, and transchelation reaction was then performed. The reaction mixture was well stirred for 30 min at room temperature under nitrogen atmosphere. After stirring, the reaction mixture was filtered using a 0.2 μm membrane filter, thus giving ^{99m}Tc-DADS (**3**) as a technetium-labelled compound of interest (Scheme 3).



Scheme 3. Preparation of ^{99m}Tc-DADS by transchelation (3) in the presence of BER

Preparation of ^{99m}Tc-DADS using BER (4)

To a vacuum vial containing 5.0 mg of BER were simultaneously added $^{99m}TcO_4^-$ and a solution of 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodecane (**2**, diamine disulfide, DADS) as a ligand, prepared by dissolving 0.1 mg thereof in 0.1 ml distilled water, and the radiolabelling was performed, resulting in formation of ^{99m}Tc-diamine disulfide (**4**, ^{99m}Tc-DADS) (Scheme 4).



2 **4**

Scheme 4. Preparation of ^{99m}Tc-DADS (4) using BER.
Reagents and conditions: i. Na$^{99m}TcO_4$, BER, saline, r.t., 30 min; ii. Na$^{99m}TcO_4$, $SnCl_2$, saline, r.t., 30 min

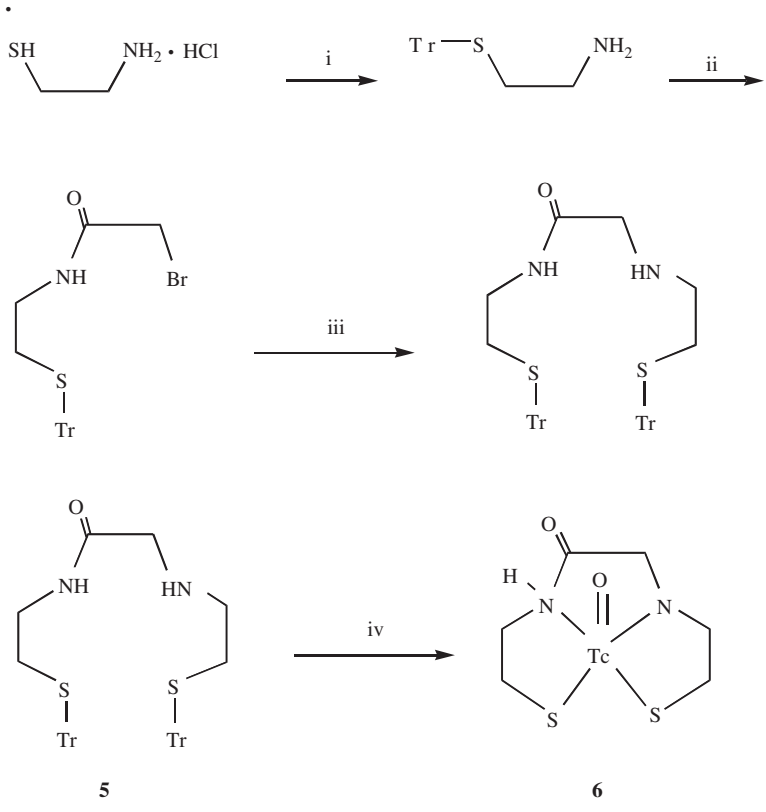
Preparation of ^{99m}Tc-MAMA (6)

N-[2-((2-((triphenylmethyl)thio)ethyl)amino)acetyl]-*S*-(triphenylmethyl)-2-aminoethanethiol (**5**, monoamine monoamide-Tr_2, MAMA-Tr_2) was synthesized by the reported method.[11] The synthesis is outlined in Scheme 5. Analytical data: ^{1}H NMR ($CDCl_3$) δ 2.37 (q, 4H, $2CH_2$), 2.46 (t, 2H, CH_2), 3.04 (s, 2H, CH_2CO), 3.08 (q, 2H, CH_2), 7.18-7.30 (m, 18H), 7.39-7.44 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 32.1, 32.4, 37.8, 48.4, 51.9, 66.8, 126.8, 128.2, 128.3, 129.6, 144.8, 171.2.

To a vacuum vial containing 5.0 mg of BER were simultaneously added a solution of 1 mg of **5** in 0.1 ml of distilled water, 0.1 ml of sodium [^{99m}Tc]pertechnetate (185 MBq) and 0.8 ml of physiological saline. After being well mixed, the reaction mixture was heated at 100°C for 15 min and cooled to room temperature, then filtered with a 0.2 μm membrane filter, resulting in the formation of ^{99m}Tc-L-*N*-[2-((2-((triphenyl methyl)thio)ethyl)amino)acetyl)-*S*-(triphenylmethyl)-2-aminoethanethiol (**6**, ^{99m}Tc-monoamine monoamide, ^{99m}Tc-MAMA) (Scheme 5).

Preparation of ^{99m}Tc-N-(2,6-Diisopropylchloroacetanilide)-iminodiacetate (8, ^{99m}Tc- DISIDA)

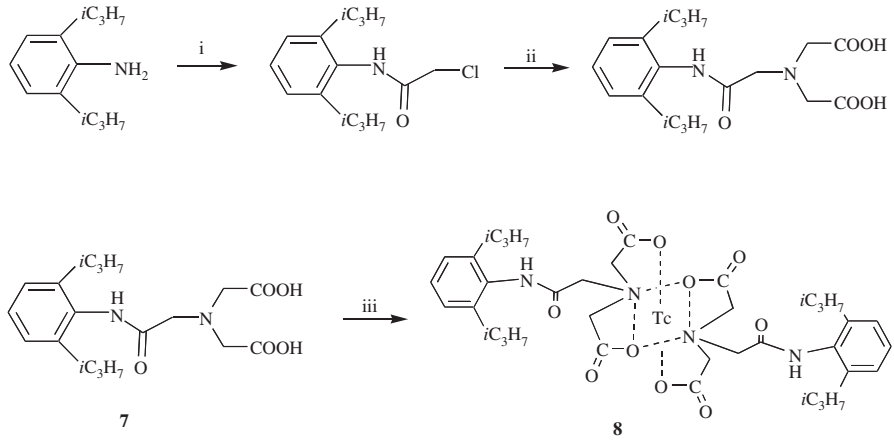
N-(2,6-Diisopropylchloroacetanilide)-iminodiacetate (**7**, DISIDA) was synthesized by the reported method.[12] The synthesis of **7** and its ^{99m}Tc-complex is



Scheme 5. Preparation of ^{99m}Tc-MAMA (6).
Reagents and conditions: i. Ph_3COH, CF_3CO_2H, r.t., 1 h; ii. $BrCOCH_2Br$, Et_3N, CH_2Cl_2, −20°C, 15 min; iii. $TrSCH_2CH_2NH_2$, Et_3N, CH_2Cl_2, r.t., 24 h; iv. $Na^{99m}TcO_4$, BER, water bath, 30 min

outlined in Scheme 6. Analytical data: m.p. 198–200°C; IR (cm^{-1}, KBr pellet) 3240, 2965, 2400, 2310, 1680, 1552, 1362, 1256; ^{1}H NMR (CD_3OD) δ 1.20 (d, J = 5.3 Hz, 12 H, 4 CH_3), 3.05-3.14 (m, 2 H, 2 ArCH), 3.66 (s, 2 H, $NHCOCH_2N$), 3.70 (s, 4 H, 2 NCH_2CO_2), 7.19-7.33 (m, 3 H, 4 ArH); ^{13}C NMR (CD_3OD) δ 17.3, 22.9, 28.9, 55.7, 57.3, 58.7, 123.3, 128.3, 146.5, 172.8, 173.7.

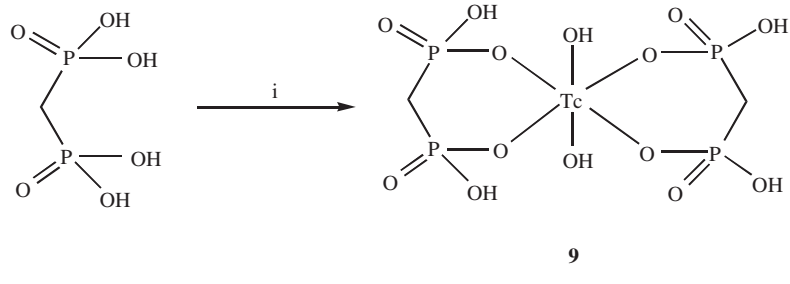
To a vacuum vial containing 5.0 mg of BER were simultaneously added a solution of 0.1 mg of **7** in 0.1 ml of distilled water and 0.1 ml of sodium [^{99m}Tc]pertechnetate (185 MBq). After being well mixed, the reaction mixture was stirred at room temperature for 15 min, and then filtered with a 0.2 μm membrane filter, resulting in the formation of ^{99m}Tc-*N*-(2,6-diisopropylchloroacetanilide)iminodiacetate (**8**, ^{99m}Tc-DISIDA) (Scheme 6).



Scheme 6. Preparation of ^{99m}Tc-DISIDA (8).
Reagents and conditions: i. $ClCOCH_2Cl$, glacial acetic acid, 10–15°C, 30–60 min; ii. iminodiacetic acid disodium salt, ethanol, reflux, 8 h; iii. $Na^{99m}TcO_4$, BER, 30 min

Preparation of ^{99m}Tc-methylene diphosphonate (9, ^{99m}Tc-MDP)

A solution of methylene diphosphonate (MDP) prepared by dissolving 1.0 mg in 0.1 ml of distilled water, 0.1 ml of sodium [^{99m}Tc]pertechnetate (185 MBq) and 0.8 ml of physiological saline were simultaneously added to a vacuum vial containing 5.0 mg of BER, resulting in the formation of ^{99m}Tc-MDP (**9**) (Scheme 7).



Scheme 7. Preparation of ^{99m}Tc-MDP (9).
Reagents and conditions: i. $Na^{99m}TcO_4$, BER, 30 min

Preparation of ^{99m}Tc-human serum albumin (10, ^{99m}Tc-HSA)

The same procedure as shown above was carried out, except that a solution of human serum albumin (HSA) prepared by dissolving 1.0 mg in 0.1 ml distilled water and then adjusting to pH 2.5 using 0.5M HCl, and 0.1 ml of a solution of

sodium [^{99m}Tc]pertechnetate (185 MBq) were simultaneously added to a vacuum vial containing 5.0 mg of BER, resulting in the formation of ^{99m}Tc-human serum albumin (**10**, ^{99m}Tc-HSA).

Animal experiments

To examine the *in vivo* retention of ^{99m}Tc-complexes, male New Zealand white rabbits (6 week-old) were used. The animals were kept in individual cages at 22 + 1°C with a relative humidity of 60 + 10% and a 12 h-light/dark cycle. The animals were allowed free access to food and water, and left to acclimatize for 1 week. ^{99m}Tc-complexes were administered intravenously to rabbits via an ear vein for imaging tests such as dynamic kinetics and serial images scans using a gamma camera (Orbiter, Simens, USA).

Dynamic acquisition and analysis

Six week-old New Zealand white male rabbits (2887.6 + 101.5 g, $n = 3$) which were anesthetized with ketamine and xylazine were used for imaging studies. Each rabbit was injected with ^{99m}Tc-complex via the left ear vein with 111 MBq/0.5 ml. All rabbits were placed in a posterior position. To confirm the dynamic kinetics of ^{99m}Tc-complex, whole body dynamic images for 30 min and 16 static images at the predetermined time intervals were obtained using a gamma camera fitted with a low energy all-purpose collimator. A 20% window was centered around 140 KeV. Image data were analyzed under dynamic procedure of the Microdelta system (Simens, USA). The static images were taken at 1.52, 3.45, 5.37, 7.30, 9.22, 11.45, 13.07, 15.00, 16.52, 18.45, 20.37, 22.30, 24.22, 26.15, 28.07, and 30 min post administration with a Microdot imager (Simens, USA).

Results and discussion

Assay for labelling efficiency and radiochemical purity of ^{99m}Tc-DADS (2) prepared by transchelation

The assay for formation and structure of radiolabelled compounds with [^{99m}Tc]pertechnetate ion, $^{99m}TcO_2$ and technetium can be achieved by investigating their position using an instant thin-layer chromatography (ITLC) and HPLC. In this experiment, the labelling efficiency and radiochemical purity of the ^{99m}Tc-glucoheptonate used for transchelation was determined by performing ITLC and reverse phase HPLC.

Table 1 shows the results of thin-layer chromatography for ^{99m}Tc-glucoheptonate (**1**), by performing ITLC on silica gel impregnated glass fiber sheets, using acetone and physiological saline as a development solvent, respectively.

In case of ^{99m}Tc-DADS (**3**), prepared by transchelation, ITLC-SG (silica gel) was performed using acetone and methanol/HCl (99.5:0.5 v/v) as a development solvent, and the results are given in Table 2 and Figure 1. As apparent from Table 2, the ITLC-SG of ^{99m}Tc-DADS using acetone as a development solvent gave no peak at the solvent front where $^{99m}TcO_4^-$ would be expected. As some $^{99m}TcO_2$ was observed at the origin, after elution of ^{99m}Tc-GHA and $^{99m}TcO_4^-$ with MeOH/HCl. These results indicate that ^{99m}Tc-DADS having 97% of labelling efficiency was formed.

Table 1. ITLC analysis of ^{99m}Tc-GHA (1)

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-GHA & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	2% of $^{99m}TcO_2$	98% of ^{99m}Tc-GHA & $^{99m}TcO_4^-$

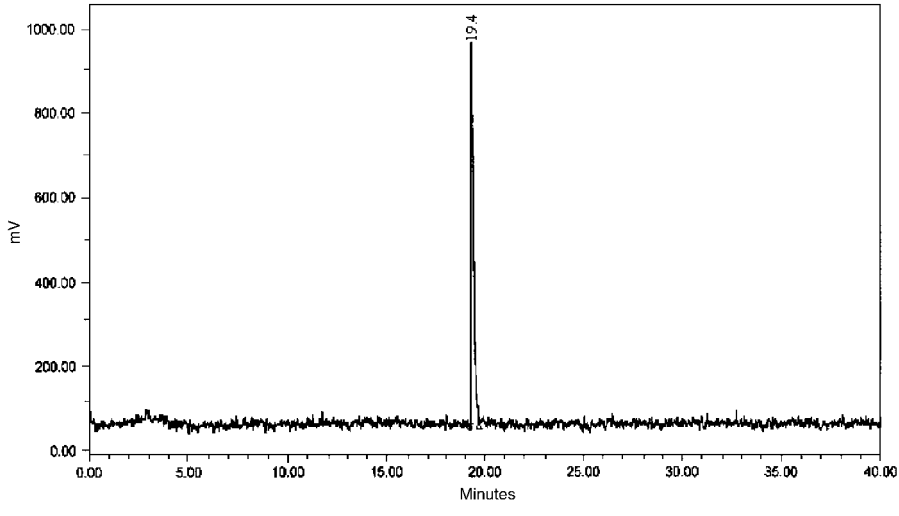


Figure 1. Elution profile of ^{99m}Tc-DADS (3) prepared by transchelation at 30 min post labelling from a C_{18} RP-HPLC column

Table 2. ITLC analysis of ^{99m}Tc-DADS (3) by transchelation

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-GHA & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Methanol/HCl (99.5:0.5 v/v)	4% of $^{99m}TcO_2$	96% of ^{99m}Tc-GHA & $^{99m}TcO_4^-$

Radiochemical purity of the complex ^{99m}Tc-DADS was determined using HPLC involving a C-18 reverse-phase column as a stationary phase and a phosphate-buffered triethylammonium solution/methanol as a mobile phase, while maintaining a flow rate of 1 ml/min. The result is given Figure 1.

As shown in Figure 1, only one peak was seen at a retention time of 19.4 min due to the compound of interest, indicating formation of ^{99m}Tc-DADS complex with more than 99% of radiochemical purity.

An active site of the complex is demonstrated to be $[Tc^{v}=O]^{3+7}$. Based on this finding, production of technetium-labelled complex and its structure can be identified by performing a transchelation reaction with another ligand having an affinity higher than the bound ligand, glucoheptonate, and then by confirming a peak at a homologous position to the complex containing the complex containing $[Tc^{v}=O]^{3+}$ through thin layer chromatography (TLC) or reverse-phase high performance liquid chromatograph (HPLC). It is directed at improving the preparation of known pharmaceuticals which happen to contain $[Tc^{v}=O]^{3+}$.

Assay for labelling efficiency and radiochemical purity of ^{99m}Tc-DADS (4) using BER

Labelling efficiency and radiochemical purity of ^{99m}Tc-diamine disulfide (**4**, ^{99m}Tc-DADS) were evaluated according to the same method using ITLC-SG and reverse-phase HPLC as in the preparation of **3**.

ITLC-SG was performed using acetone or methanol/HCl (99.5:0.5 v/v) as a developing solvent, and the result is given Table 3. In addition, reversed-phase HPLC was carried out using a C-18 reverse-phase column as a stationary phase and a phosphate-buffered triethylammonium solution/methanol as a mobile phase, while maintaining a flow rate of 1 ml/min, and the result was the same as for ^{99m}Tc-DADS prepared by transchelation as shown in Figure 1.

The single peak with the same retention time of 19.4 min demonstrates the formation of ^{99m}Tc-DADS having over 99% of radiochemical purity by the new method.

The BER carries borohydride ion (BH_4^-) bound to cation supported on the resin. The preferred cation is a quaternary ammonium functionality. The resin

Table 3. ITLC analysis of ^{99m}Tc-DADS (4) by BER

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-GHA & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Methanol/HCl (99.5:0.5 v/v)	3% of $^{99m}TcO_2$	97% of ^{99m}Tc-GHA & $^{99m}TcO_4^-$

used in the present study includes polystyrene, high-density polyethylene and amberlite containing quaternary ammonium groups.

Assay for labelling efficiency of ^{99m}Tc-MAMA (6)

In order to investigate labelling efficiency of ^{99m}Tc-MAMA (**6**), ITLC-SG was performed using acetone as a development solvent, and the result is given in Table 4. As shown in Table 4, no peak of $^{99m}TcO_4^-$ was observed at the solvent front, which is expected to migrate with the solvent front, indicating the formation of the complex **6** with good labelling efficiency.

Assay for labelling efficiency, radiochemical purity and imaging of ^{99m}Tc-DISIDA (8)

Labelling efficiency and radiochemical purity of ^{99m}Tc-DISIDA (**8**) were evaluated according to the same method using ITLC-SG and reverse-phase HPLC. The ITLC-SG was performed using acetone or methanol/HCl (99.5:0.5 v/v) as a developing solvent, and the result is given Table 5. In addition, reversed-phase HPLC was carried out using a C-18 reverse-phase column as a stationary phase and a distilled water/acetonitrile as a mobile phase, while maintaining a flow rate of 1 ml/min, and the result is given in Figure 2.

As shown in Figure 2, there was only one peak with a retention time of 8.9 min, which is the retention time of the compound of interest, demonstrating the formation of ^{99m}Tc-DISIDA (**8**) with over 98% of radiochemical purity. As shown in Figure 3, the serial static image scans of rabbit

Table 4. ITLC analysis of ^{99m}Tc-MAMA (6)

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-MAMA & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	1% of $^{99m}TcO_2$	99% of ^{99m}Tc-MAMA & $^{99m}TcO_2$

Table 5. ITLC analysis of ^{99m}Tc-DISIDA (8)

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-DISIDA & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	2% of $^{99m}TcO_2$	98% of ^{99m}Tc-DISIDA & $^{99m}TcO_2$

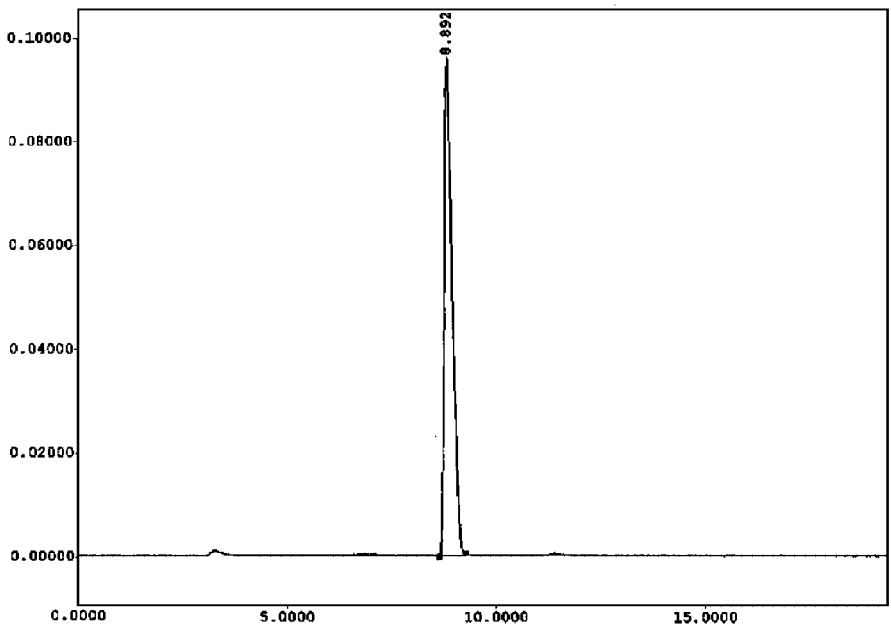


Figure 2. Elution profile of ^{99m}Tc-DISIDA at 30 min post labelling from a C18 RP-HPLC column

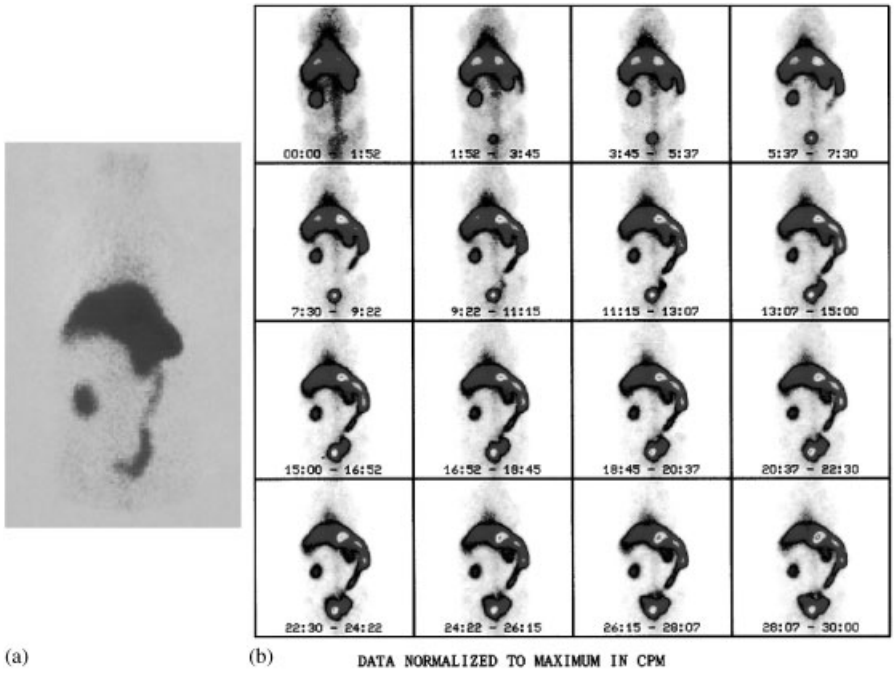


Figure 3. (a) Image at 30 min and (b) image scans for 30 min of rabbit after intravenous administration of ^{99m}Tc-DISIDA (8) showing hepatobiliary system

administered with **8** are essentially identical to those obtained with ^{99m}Tc-DISIDA formed by using tin chloride as a reducing agent, in the higher hepatobiliary accumulation of ^{99m}Tc. The radioactivity curve in hepatobiliary system of rabbit administered with **8** via an ear vein is shown in Figure 3.

Assay for labelling efficiency and imaging of ^{99m}Tc-MDP (9)

In order to investigate the labelling efficiency of ^{99m}Tc-methylene diphosphonate (**9**, ^{99m}Tc-MDP) prepared using BER, ITLC-SG was performed, using acetone or physiological saline as a development solvent, and the results are given in Table 6. As shown in Table 6, there was no peak of $^{99m}TcO_4^-$ at the solvent front, where it is expected. As shown in Table 6, $^{99m}TcO_2$ was not observed at the origin. These results indicate that the complex **9** was formed with good labelling efficiency.

As shown in Figure 4, the bone image of **9** in a rabbit is essentially identical with that of ^{99m}Tc-MDP formed by using tin chloride as a reducing agent in the higher bone accumulation of ^{99m}Tc.

Table 6. ITLC analysis of ^{99m}Tc-MDP (9)

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-MDP & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	2% of $^{99m}TcO_2$	98% of ^{99m}Tc-MDP & $^{99m}TcO_2$

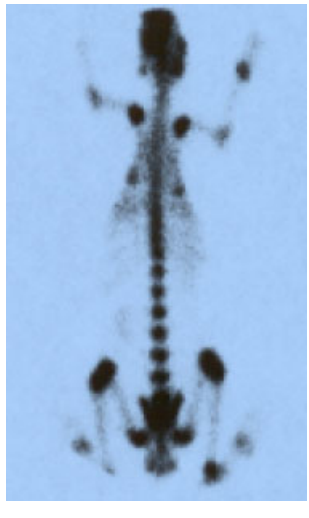


Figure 4. Whole-body image of rabbit at 30 min after intravenous administration of ^{99m}Tc-MDP (9)

Table 7. ITLC analysis of ^{99m}Tc-HSA (10)

Chromatographic system		^{99m}Tc species at	
Support	Solvent	Origin	Solvent front
ITLC-SG	Acetone	100% of ^{99m}Tc-HSA & $^{99m}TcO_2$	0% of $^{99m}TcO_4^-$
ITLC-SG	Saline	0% of $^{99m}TcO_2$	100% of ^{99m}Tc-HSA & $^{99m}TcO_2$

Assay for labelling efficiency of ^{99m}Tc-human serum albumin (10)

In order to investigate labelling efficiency of ^{99m}Tc-human serum albumin (**10**), ITLC-SG was performed using acetone as a development solvent. As shown in Table 7, no peak of $^{99m}TcO_4^-$ was observed at the solvent front, where it would be expected, indicating the formation of **10** with good labelling efficiency.

Conclusion

According to the present study, employing the borohydride exchange resin (BER) as a novel reducing agent, ^{99m}Tc-complex having high radiochemical purity as well as high labelling efficiency can be prepared by reacting [^{99m}Tc]pertechnetate with a ligand. The BER is advantageous in terms of being stable in most pH ranges including extreme acidic and alkaline pH conditions and applicable to biologically active molecules, as well as being easily removable through filtration when being administrated, thus providing the potential to economically and effectively produce ^{99m}Tc-radiopharmarceuticals without the formation of insoluble $^{99m}TcO_2$ or SnO_2 colloids by replacing the conventional reducing agent requiring very stringent condition for preparation. Therefore, the conventional reducing agents can be replaced by the BER of the present study. Since technetium and rhenium have similar chemistry, it is possible that the BER method will be useful in the preparation of rhenium-labelled pharmaceuticals.[13–15]

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