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Research Article

Role of protecting groups in the preparation of thiolate complexes of Technetium-99 m using cysteine as a model

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Summary

An attempt has been made to develop a suitable protecting group for the thiolate function for ^{99m}Tc binding ligands having such function and which could be deprotected automatically during ^{99m}Tc-chelation without the use of any additional reagents. As a model ligand a simple molecule like L-cysteine was selected. Seven S-protected derivatives of this amino acid were synthesized, radiolabelled with technetium-99 m under a variety of experimental conditions and the yield of the desired chelate was compared to that of ^{99m}Tc–L-cysteine, the authentic standard chelate, by HPLC. The corresponding ^{99}Tc chelate of cysteine from L-cystine and *S*-thiomethyl L- cysteine was also prepared. It was found that the ^{99}Tc chelates exhibited similar retention profiles to those of the corresponding ^{99m}Tc chelates in reverse phase HPLC. The results of the biodistribution studies after ^{99m}Tc chelation were likewise compared to those of ^{99m}Tc-L-cysteine. The effect of probenecid on renal excretion was studied only on the ^{99m}Tc chelate of S-thiomethyl-L- cysteine to determine whether tubular excretion was involved. The results suggest that the *S*-thiomethyl group could be used as an ideal protective group to mask the high reactivity of thiolate functions attached to different ^{99m}Tc binding ligands.

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Introduction

Protecting groups are used in different branches of organic chemistry to conduct a chemical reaction of a multifunctional compound selectively at one reactive site and to avoid undesirable by-product formation. In the preparation of inorganic chelate complexes, masking of heteroatoms of the ligands with easily removable protecting groups to produce specific chelates has been a standard practice

Technetium-99 m is the most widely used radioisotope in diagnostic nuclear medicine. However, in many cases of technetium chelation the ligands used are mostly unprotected and with such compounds very often more than one technetium chelate is formed under slightly different experimental conditions. Most frequently this problem is encountered with ligands that contain one or more thiolate function.[1,2] In ^{99m}Tc-MAG$_3$, the most important ^{99m}Tc chelate for renal function studies benzoyl protection on the sulphur atom of the ligand during chelation[3] was used. Whether the benzoyl group is the ideal protecting group in this experiment could be questioned since reproducible yields of the chelate could not be obtained in many cases and different methods, each claiming its superiority over that of others, have appeared in the literature over a long period of time.[4–6]

To provide information relating to the formation of undesired technetium chelates with thiolate containing ligands, one may look into the chemistry of chelation with the long-lived isotopes of technetium (^{99}Tc). In some cases the desired chelate can be produced in good yield only when the oxotechnetium derivative and the thiolate ligand are allowed to react in stoichiometric proportion, and a relatively pure ^{99}Tc-chelate can be obtained only when the thiolate group is derivatized with acetyl amino methyl, benzyl, benzamido-methyl, trityl etc.[7–9]

We observed that cysteine, a common and simple thiolate ligand, also shows this abnormal behaviour during chelation with technetium. It produced the desired oxotechnetium ^{99}Tc *bis* cysteinate in exellent yield only when the ligand and $^{99}TcO_{4-}$ is allowed to react in stoichiometric ratio, but the presence of any excess of cysteine in the reaction mixture furnished only polymeric products. However, this problem could be avoided by using the corresponding disulphide (cystine) as monosulphide precursor; use of large excess of the former produced the above chelate in excellent yield.[10] Therefore it may be concluded that the protection of monosulphide by disulphide formation, which is a useful tool in peptide chemistry, may be successfully applied in thiolate chelation with technetium.

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The objective of the present paper is to show how the aforementioned methods of chelation of thiolate compound with ^{99}Tc can be transferred to the preparation of ^{99m}Tc-radiopharmaceuticals where technetium is used at tracer level. Since, for clinical application, the required radiopharmaceutical needs to be available as a kit the chelation is best carried out, at near, neutral pH. Under such conditions it is inconvenient to use symmetrical disulphides like cystine because of poor aqueous solubilities of such compounds. We have observed that thiolated compounds, when coupled with thiomethyl chloride, produce the mixed disulphides which have a better solubility in aqueous conditions.

Preparation of the desired ^{99m}Tc-cysteine complex from cystine is a well-known procedure.[10–12] Using this chelate preparation as standard we undertook the comparative HPLC studies of different technetium–L-cysteine preparations using several *S*-protected L-cysteine derivatives which include *S*-thiomethyl and *S*-thiophenyl cysteine as disulphide precursor as well as *S*-benzyl, *S*-acetamidomethyl cysteine etc. as proclaimed by different groups.[13,14] From such a study we expect the derivative best suitable for efficient use in ^{99m}Tc radiopharmaceutical preparation to emerge. The results are discussed in the following sections.

Experimental

General

S-t butyl L-cysteine (III) was purchased from Aldrich Chemical Company, USA. Proton NMR spectra were recorded on a 300 MHz Bruker DPX 300 spectrometer. Elemental analysis was performed in the microanalytical laboratory of the Indian Association for Cultivation of Science, Kolkata. 99MoO4 was purchased from the Bhabha Atomic Research Centre and ^{99m}TcO4 was obtained by 2-butanone extraction from a 5(N) NaOH solution of MoO_4^-. Ammonium ^{99}Tc-pertechnetate was purchased as an aqueous solution (0.38 mmol ml) from Amersham. γ-Counting was performed using a well type counter from Electronic Corporation of India Model LV4755. HPLC analyses were performed on a reverse phase C-18 column (4.6 × 250 mm particle size 5 μm) fitted to Water Associates (USA) solvent delivery system consisting of two M515 pumps driven by a M680A automatic gradient controller and Rheodyne 77251 injector. Radioactivity in the eluate was monitored using a Beckman Model 170 detector and integrated by a Water M746 data Module. For ^{99}Tc chelates the UV detector (Waters Model 481) was set at 425 nm.

Synthesis

In most cases the thiol protection of L-cysteine was performed by literature methods leading to the preparation of *S*-benzyl-L-cysteine(I),[13] *S-p*-methoxy

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benzyl-L-cysteine (II),[15] S-acetamido methyl-L-cysteine (IV),[14] L-thiazolidine-4-carboxylic acid (V)[16] and *S*-thiophenyl-L-cysteine (VI).[17] The synthesized compounds (Figure 1) had comparable melting points with those reported in the literature and were further characterized by TLC analysis and ^{1}HNMR spectroscopy. Methyl sulphenyl chloride was prepared by the reported method[18] with some modifications. *S*-thiomethyl L-cysteine (VII) was prepared by the following two step synthesis:

(a) *Preparation of methyl sulphenyl chloride*: A solution of chlorine (15 g) in dry carbon tetrachloride (70 ml) was added to a solution of dimethyl disulphide (6.76 g) in carbon tetrachloride (15 ml) at −20°C. A white solid (5 g) appeared which was filtered and was added to dimethyl disulphide (2.9 ml) pre-cooled at –20°C. A deep orange liquid was formed which was stored at −20°C.

(b) *Preparation S-thiomethyl-L-cysteine (VII)*: An aliquot of the above solution having the theoretical amount of methyl sulphenyl chloride (1.07 g, 0.013 mol) was added dropwise to a suspension of L-cysteine hydrochloride monohydrate (1.89 g, 0.010 mol) and sodium bicarbonate (1.09 g, 0.13 mol) in ethanol (34 ml) under nitrogen. The reaction mixture was allowed to stand at room temperature until cysteine was no longer detectable by nitroprusside. Pyridine (1.45 ml) was added when the reaction mixture showed a neutral pH and the solution was concentrated to half of the original volume; the precipitate was then filtered and washed with ethanol. The crude material was purified from its ammoniacal aqueous solution by adding acetic acid to pH-5 to furnish the pure material (1.56 g, 69%). TLC single spot R_f, 0.65 EtOH:H_2O (7:2); and R_f, 0.70 (BuOH:Py:H_2O:AcOH;5:3:4:1), cellulose plate; m.p. 180°C; ^{1}HNMR (D_2O) 2.44 (s, 3 H, CH_3), 3.12–3.64(t, 2 H, S-CH_2-C), 4.40–4.64 (t, 1 H, -CH-). Calculated for $C_4H_9NS_2O_2$ C, 28.71; H, 5.42; N, 8.37; found C, 28.61; H, 5.09; N, 8.94.

Radiolabelling of ligands

^{99m}Tc-chelation of S-benzyl-L-cysteine (I), S-p-methoxy benzyl-L cysteine (II) and S-thiophenyl-L-cysteine (VI). The appropriate amount of either (I) or (II) was suspended in nitrogen purged water (0.2 ml) and sodium hydroxide solution was added to just dissolve the ligands. In the case of *S*-thiophenyl-L-cysteine (VI) was dissolved in EtOH (0.2 ml), water (0.1 ml) was added and the pH adjusted to 12 with alkali. To each of the ligand solutions $^{99m}TcO_4^-$ solution (0.1 ml, 74–185 MBq) was added followed by the addition of freshly prepared stannous chloride solution (50 µl; 50 µg). The pH of the solution was then adjusted to 7 and after 30 min the chelate solution was centrifuged. The supernate, which contained most of the radioactivity, was used in subsequent experiments.

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*^{99m}Tc Chelation of S-t-butyl-*L*-cysteine (*III*), s-acetamido-methyl-*L*-cysteine (*IV*) and* L*-thiazolidine-4-carboxylic acid (*V*).* To a solution of the appropriate amount of the ligand in water (0.4 ml), acetate buffer (pH 4, 0.2M, 0.4 ml) was added followed by the addition of aqueous $^{99m}TcO_{4-}$ (0.1 ml, 74–185 MBq) and stannous chloride solution (50 µl containing 20 µg of $SnCl_2$). The chelate solution was allowed to stand for 30 min at room temperature before using for subsequent experiment.

^{99m}Tc Chelation of S-thiomethyl L*-cysteine (*VII*).* The desired amount of ligand was dissolved in water (0.4 ml) and pH of the solution was adjusted to 8; to this solution was added $^{99m}TcO_{4-}$ (0.1 ml, 74–185 MBq), followed by the addition of stannous chloride solution (50 µg in 50 µL) prepared as described above, and allowed to stand at room temperature before use.

*Preparation of ^{99m}Tc-*L*-cysteine chelate.* The chelate was prepared as reported[11] method from L-cystine and ^{99m}TcO4.

*Preparation of ^{99m}Tc-*L*-cysteine kits from S-thiomethyl-*L*-cysteine.* A kit preparation of L-cysteine was chosen using a 5 ml vial. To the solution (0.5 ml, pH 8.0) of ligand (VII, 5 mg), Na_2HPO_4 solution (6.75 mg; 300 µl), 100 µl of a solution containing ascorbic acid (1.5 mg) and NaCl (5.5 mg) was added followed by the addition of freshly prepared stannous chloride dihydrate solution (50 µl, 0.225 mg). The vial was lyophilized and stored for a specified period. The kit was reconstituted by the addition of 2.0 ml of sodium pertechnetate containing the required activity to the lyophilized vial, and mixed well and heated in a boiling water bath for 2–3 min.

*^{99}Tc-chelation of S-thiomethyl-*L*-cysteine.* The chelation was carried out as reported in our method for the preparation of oxotechnetium (^{99}Tc) –L-cysteinate. To an aqueous solution (1 ml, pH 8.5) of *S*-thiomethyl-L-cysteine (135 mg, 1 mmol), NH_4TcO_4 solution (0.22 ml, 0.085 mmol) and a freshly prepared solution (0.5 ml) of $Na_2S_2O_4$ (33 mg, 0.185 mmol) was added and incubated at room temperature for 4 h. The pH was adjusted to 7.0. The reaction mixture was then passed through celluloseCM and sephadex-LH columns to furnish pure chelate. UVλmax:425 nm.(4255), 307(1147), IR (KBr) ν; 942 cm^{-1}(Tc = O).

Aeration of the above chelates

An aliquot of each chelate (0.5 ml) was aerated by bubbling air (20 cm^3) and subsequently heating (95°C) for 3 min.

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Radioanalytical purification studies

HPLC studies of the radiochemicals were performed on a C-18 μ bondapak column. The column was eluted isocratically with tetrabutylammonium chloride (0.005 M): MeOH in 1:1 ratio (volume) at a flow rate of 1 ml/min. In each case the retention time and per cent purity were recorded.

Biodistribution studies

Experiments using mice were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation. Male swiss albino mice (20–25 g) were hydrated by intraperitoneal administration of saline (0.9%, 3 ml) for 1 h. After another 1 h, the ^{99m}Tc-chelate of ligands I–VII or L-cysteine (185–259 kBq) premixed with ^{131}I-OIH (74–111 kBq) in a total volume of 0.03 ml was injected via a tail vein. The mice were sacrificed either at 30 or 10 min (for probenecid study) post-injection and the desired organs were collected. The radioactivity in each organ is expressed as the percentage of injected dose. Blood was assumed to be 7% of the body weight. In the probenecid experiment mice were pre-treated with probenecid (50 mg Kg^{-1}), 10 min prior to radiopharmaceutical injection.

Results

S-benzyl –L-cysteine (I), *S*-*p*-methoxy benzyl-L-cysteine (II) and L-thiazolidine-4-carboxylic acid (V) were prepared using reported methods. *S*-Acetamido methyl L-cysteine (IV) was prepared by reacting *N*-hydroxy methylacetamide with L-cysteine under strongly acidic conditions. The two

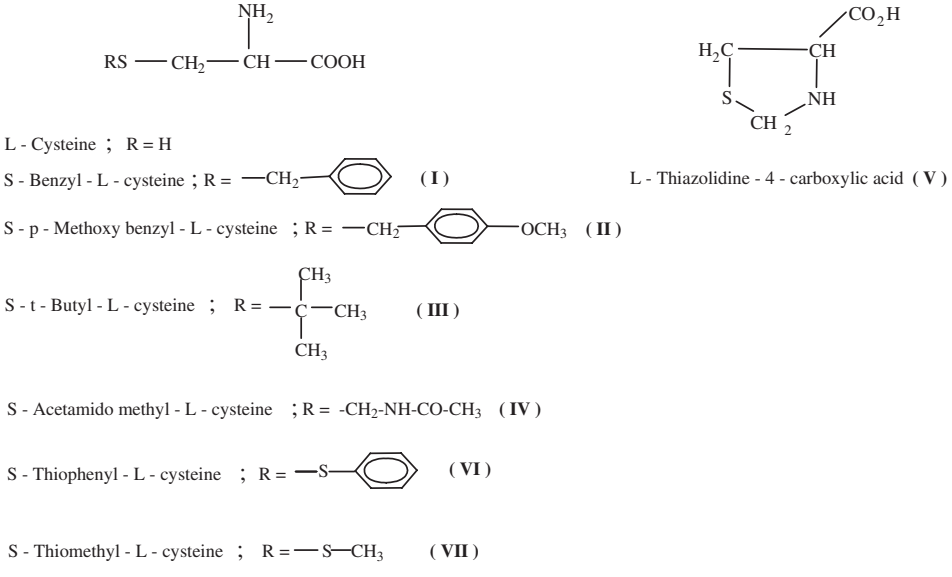

Figure 1. Chemical structures of thiol protected L-cysteine derivatives (I–VII)

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Table 1. HPLC analysis of different ^{99m}Tc-L-cysteine preparations using compounds I–VII as metal binding ligands along with ^{99m}Tc-L-cysteine

Sl. no.	Ligand	Amount (mg)	HPLC (ret. time in min)	(i) HPLC (percentage of labelling yield) before air pass & boiling	(ii) HPLC (percentage of labelling yield) after air pass & boiling
1	S-benzyl-L-cysteine (I)	(a) 5	5.16	86.25	86.41
		(b) 10		81.25	84.63
2	*S*-*p*-methoxy benzyl-L-cysteine (II)	(a) 10	5.27	81.73	85.66
3	*S*-*t*-butyl–L-cysteine (III)	(a) 5	5.14	82.06	83.47
		(b) 10		81.25	89.48
4	S-acetamido-methyl-L-cysteine (IV)	(a) 2	5.19	82.70	88.00
		(b) 5	5.22	76.30	87.15
		(c) 10	5.26	61.88	70.72
5	L-thiazolidine-4-carboxylic acid (V)	(a) 5	5.20	85.59	93.53
		(b) 10	5.17	87.59	92.76
6	*S*-thiophenyl-L-cysteine (VI)	(a) 2	5.15	92.32	96.09
		(b) 5	5.18	92.47	96.02
7	*S*-thiomethyl-L-cysteine (VII)	(a) 3	5.19	77.190	90.25
		(b) 5	5.18	97.60	97.71
		(c) 10	5.18	98.92	97.28
		(d) 20	5.22	96.24	96.97
8	^{99m}Tc-L-cysteine (std.)	(a) 5	5.16	97.60	97.71
9	^{99m}Tc-L-cysteine (kit)	(a) 5	5.20	96.25	—

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disulphides *S*-thiophenyl-L-cysteine (VI) and *S*-thiomethyl-L-cysteine(VII) were prepared by condensing L-cysteine with either benzene sulphenyl chloride or with methane sulphenyl chloride, respectively. Benzene sulphenyl chloride was prepared by chlorinating thiophenol and similar reaction using dimethyl disulphide under careful reaction condition produced the methane analogue. All these ligands (I–VII) were characterized by TLC analysis and ^{1}HNMR spectroscopy.

^{99m}Tc radiolabelling of the ligands I–VII was carried out using the standard stannous chloride reduction procedure under different experimental conditions involving the amount of ligand, pH of the solution and aeration of the chelate solution with subsequent heating in boiling water bath for 2 min to maximize the yield. The analysis was done by HPLC, which revealed that all the chelates consisted of a single component. The results of complexation studies ($n = 3$) at various ligand concentrations are shown in Table 1.

S-benzyl-L-cysteine(I), *S*-*p*-methoxy benzyl-L-cysteine(II) and *S*-thiophenyl –L-cysteine(VI) exhibited a maximum complexation yield at pH-12. For *S*-*t*-butyl–L-cysteine (III), *S*-acetamido methyl–L-cysteine(IV) and L-thiazolidine-4-carboxylic acid (V) the optimum pH for maximum yield was between pH 4–4.8. The labelling yield of *S*-thiomethyl–L-cysteine(VII) reached an optimum at pH 8. *S*-thiophenyl-L-cysteine (VI) is not soluble in water so it was complexed in an alcoholic medium.

It was evident from the HPLC result (Table 1) that in most of the cases the labelling yield was higher with 5 mg of ligand concentration than that for 1 or 2 mg of ligand concentration. In most cases the aeration step increased the stability of the desired chelate.

Of all the chelates studied it is evident from Table 1 that L-thiazolidine-4-carboxylic acid (V), *S*-thiophenyl-L-cysteine (VI) and *S*-thiomethyl-L-cysteine (VII) after ^{99m}Tc labelling produced the corresponding ^{99m}Tc-complexes in the best yields (93–98%). The yield of the corresponding ^{99m}Tc-chelates from other ligands (I–IV) was comparatively low (70–88%) (Table 1) and the product was contaminated with other impurities. The radiochemical yield of the ^{99m}Tc–L-cysteine chelate prepared from VII was 96% when it was formulated as single vial lyophilized kit.

Biodistribution studies in mice at 30 min post-injection were undertaken only with those preparations where the yield of the desired chelate (HPLC; Table 1) was maximum. The biodistribution results of ^{99m}Tc-I to ^{99m}Tc-VII, ^{99m}Tc-L-cysteine and ^{131}I-OIH is depicted in Table 2 as per cent dose per organ. From the table it is evident that the chelates ^{99m}Tc-I to ^{99m}Tc-IV which were contaminated with radioacative artefacts, exhibited elevated hepatobiliary uptake (10.76–14.72%) and decreased urinary excretion (51–55%) whereas ^{99m}Tc-VI and ^{99m}Tc-VII exhibited comparatively high urinary excretion of 58.58, and 60.38%, respectively with decreased hepatobiliary uptakes of 8.01

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Table 2. Biodistribution results of different ^{99m}Tc-L-cysteine preparations using compounds I–VII as metal binding ligand along with ^{99m}Tc-L-cysteine coinjected with ^{131}I-orthoiodohippurate (results in parenthesis) at 30 mins time point expressed as per cent dose/organ

Compounds	Blood	Urine	Kidney	Liver	Intestine
^{99m}Tc-*S*-benzyl-L-cysteine (^{99m}Tc-I)	3.39 ± 0.52 (1.57 ± 0.38)	55.11 ± 1.62 (85.4 ± 5.77)	5.82 ± 0.28 (1.15 ± 0.46)	4.14 ± 0.19 (1.23 ± 0.16)	6.97 ± 0.76 (1.43 ± 0.17)
^{99m}Tc-*S*-*p*-methoxy-benzyl-L-cysteine (^{99m}Tc-II)	4.57 ± 0.42 (2.8 ± 0.71)	54.29 ± 1.07 (77.27 ± 0.99)	6.51 ± 0.52 (1.68 ± 0.57)	7.10 ± 0.50 (1.08 ± 0.07)	7.61 ± 0.70 (1.48 ± 1.88)
^{99m}Tc-*S*-*t*-butyl-L-cysteine (^{99m}Tc-III)	3.84 ± 0.65 (1.14 ± 0.32)	51.18 ± 1.89 (76.13 ± 3.13)	7.18 ± 0.84 (0.96 ± 0.42)	5.24 ± 0.41 (0.69 ± 0.20)	5.86 ± 0.73 (0.87 ± 0.27)
^{99m}Tc-*S*-acetamido methyl-L-cysteine (^{99m}Tc-IV)	3.89 ± 0.56 (1.39 ± 0.39)	52.84 ± 1.15 (81.31 ± 5.87)	7.13 ± 0.76 (1.25 ± 0.05)	4.31 ± 0.37 (0.81 ± 0.40)	6.46 ± 0.99 (0.76 ± 0.14)
^{99m}Tc-L-thiazolidine-4-carboxylic acid (^{99m}Tc-V)	3.63 ± 0.83 (0.7 ± 0.09)	57.60 ± 2.60 (84.27 ± 4.79)	7.01 ± 0.45 (1.72 ± 0.70)	4.44 ± 0.96 (0.79 ± 0.05)	5.83 ± 0.75 (1.26 ± 0.56)
^{99m}Tc-*S*-thiophenyl-L-cysteine (^{99m}Tc-VI)	3.81 ± 0.66 (1.19 ± 0.31)	58.58 ± 1.13 (83.31 ± 3.47)	5.33 ± 1.27 (0.88 ± 0.23)	3.88 ± 0.45 (0.70 ± 0.02)	4.13 ± 0.35 (1.27 ± 0.27)
^{99m}Tc-*S*-thiomethyl-L-cysteine (^{99m}Tc-VII)	3.75 ± 1.15 (1.49 ± 0.69)	60.37 ± 3.10 (85 ± 3.60)	4.77 ± 0.65 (0.82 ± 0.18)	3.31 ± 0.62 (0.79 ± 0.28)	4.33 ± 0.89 (1.11 ± 0.35)
^{99m}Tc-L-cysteine	4.46 ± 0.25 (1.72 ± 0.25)	58.48 ± 2.09 (79.49 ± 0.35)	5.80 ± 0.69 (1.10 ± 0.27)	3.45 ± 1.09 (1.21 ± 0.37)	3.71 ± 0.64 (1.17 ± 0.26)

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Table 3. Effect of probenecid on the biodistribution of ^{99m}Tc-*S*-thiomethyl-L-cysteine and ^{99m}Tc-cysteine coinjected with ^{131}I-orthoiodohippurate (results in parenthesis) in mice at 10 min time point expressed as per cent dose/organ

Organs	^{99m}Tc-*S*-thiomethyl-L-cysteine		^{99m}Tc-L-cysteine	
	Control	Probenecid	Control	Probenecid
Blood	3.87 ± 0.76	8.49 ± 3.17	4.41 ± 0.08	7.65 ± 1.42
	(3.91 ± 1.50)	(4.53 ± 1.15)	(3.81 ± 0.37)	(5.20 ± 0.25)
Urine	33.94 ± 2.38	21.35 ± 2.38	32.64 ± 2.10	16.33 ± 5.46
	(48.31 ± 4.94)	(42.81 ± 3.75)	(52.41 ± 1.78)	(42.39 ± 6.07)
Kidney	10.64 ± 1.63	2.97 ± 1.00	13.85 ± 0.17	4.86 ± 0.12
	(11.55 ± 1.63)	(7.64 ± 1.04)	(5.12 ± 1.20)	(3.64 ± 0.61)
Liver	3.82 ± 0.73	4.24 ± 1.00	3.34 ± 1.17	5.44 ± 0.70
	(3.81 ± 0.14)	(2.98 ± 0.75)	(2.29 ± 0.03)	(2.81 ± 0.42)
Intestine	4.03 ± 0.79	3.64 ± 0.49	4.72 ± 0.62	7.45 ± 1.13
	(5.00 ± 0.19)	(4.25 ± 1.36)	(2.66 ± 0.55)	(5.11 ± 0.50)

and 7.64%, respectively. In this respect these two chelates exhibited more similarity to ^{99m}Tc-L-cysteine, the authentic standard, than others. Although ^{99m}Tc-VI exhibited pronounced renal excretion and less hepatic uptake due to its water insolubility further study with this complex was discontinued. However, *S*-thiomethyl-L-cysteine is soluble in water at pH-8 and this could be used for easily reconstitutable kit formulation consisting of a lyophilized mixture of *S*-thiomethyl-L-cysteine and $SnCl_2$, $2H_2O$ at pH 8. The HPLC elution pattern of the above radiolabelled kit was also comparable to that of the ^{99m}Tc–L-cysteine standard.

To determine the exact pathway of elimination ^{99m}Tc-VII was subjected to probenecid depression studies and the results (Table 3) were compared with ^{99m}Tc-L-cysteine and ^{131}I-OIH. It was observed that both urinary excretion and kidney retention of ^{99m}Tc-VII at 10 min post-injection in mice pre-treated with probenecid was significantly reduced. This significant change in the biodistribution pattern proved that both compounds were eliminated via the renal tubular excretory pathway.

We prepared the ^{99}Tc chelate of VII from the *S*-thiomethyl-L-cysteine ligand following exactly the same method adopted earlier in this laboratory for the preparation of ^{99}Tc-L-cysteinate.[10] ^{99}Tc-chelate of VII exhibited UV -max at 425 nm and IR stretching bands at 942 cm^{-1} which were comparable to those of ^{99}Tc-L-cysteinate (λmax 426 nm and IR band 940 cm^{-1}) reported earlier.[10] The reverse phase HPLC retention time of ^{99}Tc and ^{99m}Tc-L-cysteinate prepared from L-cystine as starting ligands were 5.03 and 5.15 respectively, whereas the ^{99}Tc and ^{99m}Tc chelates prepared from *S*-thiomethyl-L-cysteine (VII) exhibited retention times of 5.08 and 5.18, respectively on the same reverse phase HPLC system (Figure 2).

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Discussion

Among the different protecting groups that are used in peptide chemistry, and also for thiol protection of different SH containing ligands in technetium chelation, we selected benzyl, *p*-methoxy benzyl, acetamidomethyl and *t*-butyl. They could mask the high reactivity of the thiolate function and could be removed instantaneously and quantitatively during ^{99m}Tc-chelation. The radiochemical yield of the corresponding ^{99m}Tc-chelates of these ligands were between 70 and 88% (Table 1). This variation in yield of the desired chelate is dependent on various factors like the amounts of ligand, pH of the ligand solution and oxidation of the chelate solution by aeration and subsequent heating. These variations in HPLC profile are also reflected in the biodistribution results. Many of these chelates exhibited appreciable amount of hepatobiliary uptake with less urinary excretion than that of ^{99m}Tc-L-cysteine under comparable experimental conditions.

After the establishment of the molecular structure of technetium cysteine[10] it was apparent that disulphide could be the appropriate precursor of monosulphide towards the preparation of desired technetium chelates. On this basis we further selected *S*-thiophenyl and *S*-thiomethyl as two other protective groups. It is expected that both *S*-thiophenyl-L- cysteine(VI) and *S*-thiomethyl-L-cysteine (VII) being mixed disulphide, could be more appropriate for use as a precursor for the preparation of desired technetium cysteinate chelate in quantitative yield because they may readily undergo metal induced disulphide cleavage.

It was observed that both ^{99m}Tc-VI and ^{99m}Tc-VII produced the desired chelate in almost quantitative yield. Their biodistributive properties were comparable to ^{99m}Tc-L-cysteine in every respect. It was further observed that in the presence of probenecid the renal excretion of ^{99m}Tc-VII was inhibited to a greater extent and this behaviour was also comparable to ^{99m}Tc-L-cysteine. However, VI is insoluble in water and its chelation could be done only in alcoholic medium. This may result in inconvenience during kit formulation and kit reconstitution so further study with this ligand was discontinued.

Therefore, it appears that amongst the series of *S*-protected derivatives of cysteine studied *S*-thiomethyl-L-cysteine (VII) produced the desired ^{99m}Tc-chelate in quantitative yield, which excreted exactly in the same way as ^{99m}Tc-L-cysteine through the renal pathway. Moreover, *S*-thiomethyl-L-cysteine is soluble in water at pH 8 so it is easily adaptable for kit formulation and kit reconstitution. The 99-technetium chelate obtained from *S*-thiomethyl L-cysteine exhibited a virtually identical retention time in HPLC to that of oxotechnetium (^{99}Tc)-L-cysteinate (Figure 2) whose structure was already established from this laboratory. The thiomethylated precursor of L-cysteine,

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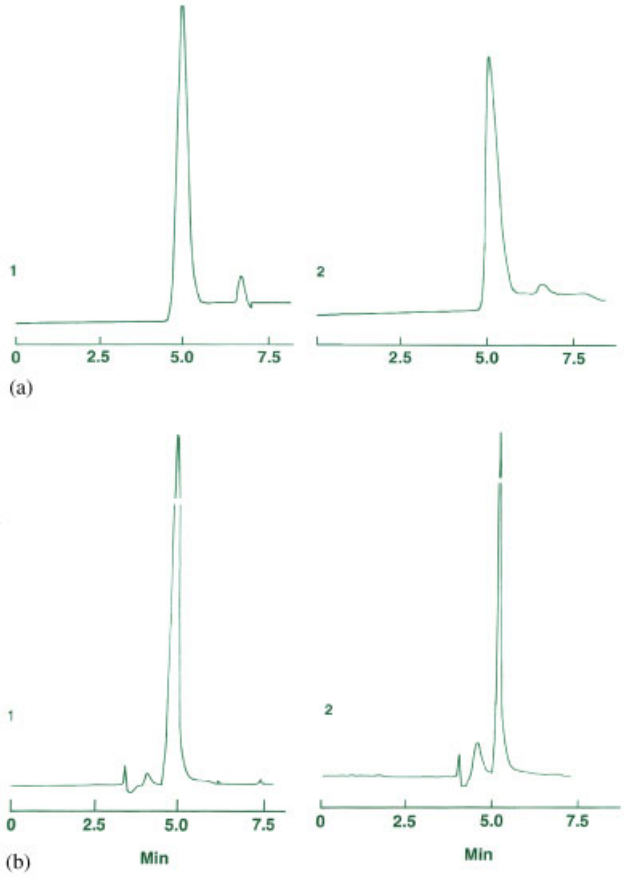

Figure 2. HPLC profiles for (a) ^{99m}Tc and (b) ^{99}Tc chelate of L-cysteine prepared from L-cystine (1) and *S*-thiomethyl-L-cysteine (2)

after technetium (99/99 m) labelling, produced the desired technetium (99/99 m)-L-cysteine chelate in quantitative yield.

Conclusion

It can be concluded that *S*-thiomethyl (*S*-CH_3) is an ideal protective group that can be used to mask the high reactivity of thiolate function attached to different ^{99m}Tc-binding ligands and can be removed instantaneously and quantitatively during ^{99m}Tc-chelation. An attempt to use this protective group for masking thiolate function of other clinically important ^{99m}Tc-radiopharmaceuticals like ^{99m}Tc-L,L'EC, ^{99m}Tc-L,L'-ECD, ^{99m}Tc-MAG_3 and ^{99m}Tc-DMSA is also underway. This type of modification of the ligand molecules may lead to the development of a single vial kit for the aforementioned radiopharmaceuticals and therefore improve the diagnostic capability of various nuclear medicine centres.

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