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Synthesis of *S*-thiomethyl MAG_3, radiolabelling with technetium-99m and biological evaluation

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Protection of the thiolate function of the mercaptoacetyltriglycine (MAG_3) by *S*-thiomethyl group allows automatic deprotection of the protecting group during technetium-99m radiolabelling by transchelation using stannous chloride dihydrate as reductant. Protection of the thiolate group with *S*-thiomethyl increases the stability of the ligand, desired complex of high radiochemical purity could be prepared under relatively mild labelling condition (at room temperature) omitting the aeration step. The complex prepared from the *S*-thiomethyl protected MAG_3 ligand were chromatographically (HPLC) and biologically compared with the corresponding complex prepared from the *S*-benzoylated MAG_3 precursor. This result suggests that technetium-99m complex of MAG_3 could be prepared from *S*-thiomethylated MAG_3 precursor in comparatively higher purity under relatively milder labelling condition and this method of radiolabelling could be used for the development of less cumbrous single vial MAG_3 kit.

Keywords: protecting group; *S*-thiomethyl MAG3; biodistribution; chelation

Introduction

The introduction of the technetium-99m complex of mercaptoacetyltriglycine (^{99m}Tc-MAG_3) into clinical practices as a potential replacement of ^{131}I-orthoiodohipuran (^{131}I-OIH) had opened up a new era in the field of renal radiopharmaceuticals. This most clinically successful renal tubular agent was first reported by Fritzberg *et al.* in 1986, where the thiol functionality of the MAG_3 ligand was protected by benzoylation.[1] This benzoyl functional group was removed during technetium-99m chelation of the ligand under alkaline condition in presence of dithionite as reductant. But in spite of using protective group, the yield was found to be >80% by reverse phase HPLC where apart from the desired peak, another chelate with slightly shorter retention time was observed which when isolated and heated was converted to the main product.[1] Fritzberg reported that the higher radiochemical yield could be achieved by developing kit where radiolabelling of benzoyl MAG_3 (Bz-MAG_3) with ^{99m}Tc was achieved by transchelation approach using sodium gluconate as labile ligand and stannous chloride as reductant. In both the cases to achieve desired radiochemical purity heating at elevated temperature (95 °C) was required. Immediately after this reporting, Muller-Suur *et al.*[2] conducted a detailed physicochemical and biological studies with HPLC purified and HPLC unpurified MAG_3 complexes and also MAG_3 complex prepared from commercial kits developed for routine clinical studies, where it was observed that all the aforementioned preparations exhibited some extra renal clearance approximated to be 10% of the total renal excretion in rat model depending on the purity of the complexes.

To understand the nature of the impurities, Brandau *et al.*[3] first initiated a thorough HPLC studies and biological investigations on ^{99m}Tc-MAG_3 and its impurities produced under different methods of complexation reaction. The aforementioned studies on this radiopharmaceutical were repeated by different workers too,[4,5] with the objective to determine whether those impurities could be eliminated during complex preparation. All these workers were observed apart from the desired ^{99m}Tc-MAG_3; there were four other non-MAG_3 components in varying proportion depending on the method of complexation and purity of the ligand. These radioactive by products exhibited hepatobiliary accumulation interfered with the desired renal imaging pattern of ^{99m}Tc-MAG_3. One of the minor components obtained by using HPLC analysis was postulated to be ^{99m}Tc(IV)-MAG_3, which on aerial oxidation furnished ^{99m}Tc-MAG_3. However, this by product could not be removed by HPLC purification. Although thorough investigations were conducted by many workers, no one could ascertain anything about the exact nature of different intermediates and by products formed during the preparation of ^{99m}Tc-MAG_3.[6,7] Probably, the use of an *S*-protected ligand during the preparation of the radiopharmaceutical may complicate the complexation process that requires a boiling step for the removal of the protective functionality and the resulting complex may not attain desired kind of purity, and it is assumed that all these discrepancies could be eliminated by using a suitable protecting group.

Several attempts were undertaken to develop this radiopharmaceutical in well standardised kit form.[8] Commercial kits of ^{99m}Tc-MAG_3 were developed in USA and Europe. Extensive comparative studies of the kits with ^{131}I-OIH were carried out in experimental animals,[1,3] human volunteers[9] and patients with renal diseases[10] in different nuclear medicine centres. It was accepted as the agent of choice for routine renal imaging and

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function studies.[11,12] Because of the poor chemical stability of the thiol group, mercaptoacetyltriglycine was synthesised as the *S*-benzoyl protected precursor and in this form, the ligand was present in the various commercial labelling kits. Moreover, when compared with ^{131}I-OIH, the renal clearance of ^{99m}Tc-MAG$_3$ was 50–60% of that of ^{131}I-OIH and often exhibited sporadic uptake in liver and gall bladder.[13,14]

Researchers from all over the world performed extensive research to minimise this unwanted physicochemical behaviour of this radiopharmaceutical. The replacement of the benzoyl group by other protective functionality is an important approach towards the improvement of the physicochemical properties of ^{99m}Tc-MAG$_3$ kit. Attempts were also undertaken in modifying the chemical structure of the ligand.[15–18] The benzoyl group as an *S*-protected precursor is used to prolong the shelf life of MAG$_3$ ligand during storage and also used as *S*-protective functionality in commercially available kits for routine clinical application. The heating step included in the preparation of ^{99m}Tc-MAG$_3$ by transchelation can be considered as the most inconvenient step in routine clinical practice. To avoid the inconvenience, attempts were undertaken to replace the benzoyl with other different protective functionalities, for example, benzyl, benzamidomethyl, acetamidomethyl, acetyl, *p*-methoxy benzyl and others.[19] The labelling efficiency obtained by the direct labelling method of the aforementioned different MAG$_3$ precursors varied from 32–94%. In all the mentioned cases, radiolabelling efficiency was sufficiently increased by exchange labelling and on heating the complexes at elevated temperature. None of the aforementioned protective groups appeared to be superior than benzoyl.

In the course of our study, *S*-thiomethyl (–S-CH$_3$) has been developed as a sulfhydryl protecting group and was well studied on different technetium binding –SH containing ligands, for example, cysteine, L,L′-EC, Dimercaptosuccinic acid and others.[20–22] In this study, we report the protection of the thiol function of MAG$_3$ by *S*-thiomethylation that could be deprotected automatically during technetium-99m labelling at relatively mild reaction condition. The ^{99m}Tc-complexes prepared from protected and unprotected precursors have been compared with HPLC and biological studies. The results are discussed in the following section.

Materials and methods

Molybdate ion ($^{99}MoO_4^-$) was purchased from the Bhabha Atomic Research Centre (Mumbai), and $^{99m}TcO_4^-$ was obtained by using 2-butanone extraction of a 5 (N) NaOH solution of $^{99}MoO_4^-$. *S*-benzoyl mercaptoacetyltriglycine (**1**) was prepared as per the reported method.[1] Methylsulphenyl chloride was prepared by following the procedure described by us.[20] HPLC analysis were performed on a reverse phase C-18 column (4.6 × 250 mm, particle size 5 μm) fitted to a Waters Associates HPLC system (Waters Corporation, MA, USA). Radioactivity in the eluate was monitored using Beckman model 170 detector and integration was carried out using a Waters M-746 data Module. All ^{1}H NMR spectra were recorded on a 400-MHz Bruker VM-400 spectrometer (Bruker Biospin, MA, USA).

Synthesis of *S*-thiomethylmercaptoacetyltriglycine (2)

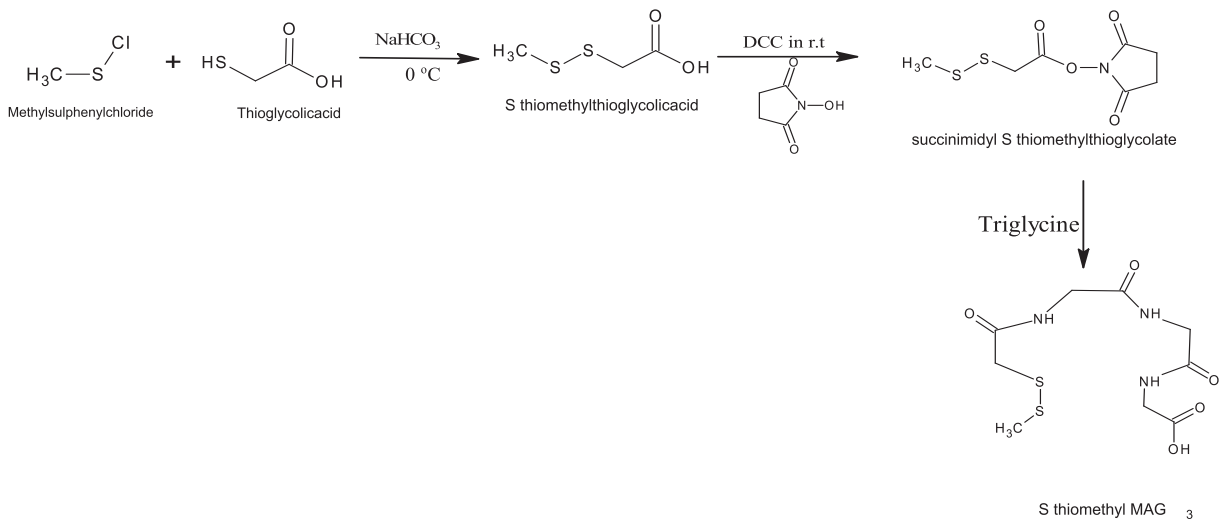
The synthesis comprises following three steps (Scheme 1)

(a) *Synthesis of S-thiomethylthioglycolic acid*

To the dry thioglycolic acid (5.69 g, 0.06 mol) taken in dry THF (30 ml), sodium bicarbonate (11 g, 0.1 mol) was added. To the aforementioned stirring mixture, 7.2 ml of freshly prepared methylsulphenyl chloride was added drop by drop at 0 °C under nitrogen atmosphere. Stirring at room temperature was continued until the reaction mixture became negative to nitroprusside. It was then filtered, and the filtrate was neutralised with few drops of pyridine and concentrated in vacuum, the oil obtained was extracted with ethyl acetate (20 ml × 3). Ethyl acetate layer on evaporation furnished white solid. It was dissolved in ethyl acetate, concentrated, dropwise addition of diethylether resulted crystalline material (1.21 g, 14.18%), mp 96 °C. Thin layer chromatography (TLC) single spot, R_f 0.85 (chloroform: methanol: acetic acid; 7:3:1). ^{1}H NMR ($CDCl_3$) δ (ppm): 2.49 (s, 3H, -SCH_3), 3.52 (s, 2H, -S-CH_2).

(b) *Synthesis of succinimidyl-S-thiomethylthioglycolate*

To the solution of *S*-thiomethylthioglycolic acid (1.21 g, 0.008 mol) in dry THF (5 ml), *N*-hydroxysuccinimide (0.92 g, 0.008 mol) in absolute THF (15 ml) was added followed by the drop



Scheme 1. Synthesis of the ligand *S*-thiomethylmercaptoacetyltriglycine.

wise addition of *N,N′*-dicyclohexylcarbodiimide (1.70 g,0.008 mol) dissolved in absolute THF (5 ml) , at −5 °C with constant stirring. The reaction mixture was stirred at room temperature for 18 h. Acetic acid (0.2 ml) was added and stirring was continued for one more hour. It was filtered, and filtrate was concentrated and extracted with petroleum ether. Evaporation of the solvent created colourless residue, which was finally recrystallised from isopropanol to produce pure solid product (1.13 g, 55%) mp 76 °C. TLC single spot, R_f 0.79 (chloroform: methanol: acetic acid; 7:3:1).[1]H NMR ($CDCl_3$) δ (ppm): 2.53(s, 3H, -SCH_3), 2.86 (s, 4H, -CO-CH_2-CH_2-CO-), 3.71 (s, 2H, -S-CH_2-COO-).

(c) *Synthesis of S-thiomethylmercaptoacetyltriglycine* (2)

To the solution of succinimidyl-*S*-thiomethylthioglycolic acid (0.500 g, 0.002 mol) in ethanol (20 ml), aqueous solution (5 ml; pH 8.0) of triglycine (0.397 g, 0.002 mol) was added. The two mixtures were mixed and stirred for 3 h. After removal of the solvent, the crude material obtained was dissolved in water (0.2 mL) and few drops of 1(N) HCl was added to bring the pH between 2 and 3. A white solid which precipitated out on cooling was separated by filtration. Further purification was carried out by dissolving the precipitate in aqueous ammonia (0.1 mL) and reprecipitation was carried out by adding 1(N) HCl (few drops). Finally, it was recrystallised from isopropanol to produce white solid (0.30 g, 46%) mp 210 °C. TLC single spot, R_f 0.12 (chloroform: methanol: acetic acid 7:3:1). ^{1}H NMR at DMSO-d_6/D_2O shake), 2.50 (s, -SCH_3), 3.52 (s, 2H, -S-CH_2-CO-), 3.72 (s, 2H, -N-CH_2-CO), 3.78 (s, 2H, -N-CH_2-COO-).

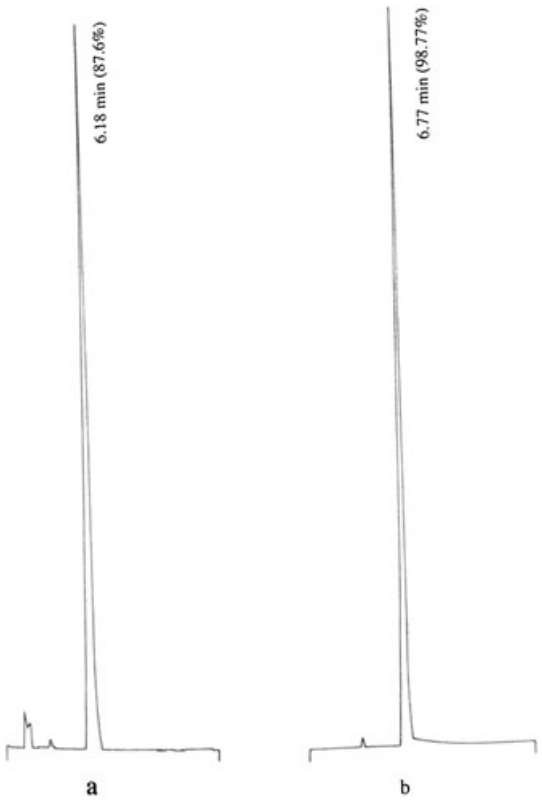


Figure 1. HPLC analysis of ^{99m}Tc-MAG_3 chelate prepared from (a) *S*-benzoyl MAG_3 ligand and (b) *S*-thiomethyl MAG_3 ligand.

Preparation of complexes

Preparation of ^{99m}Tc-MAG3 (from S-benzoyl MAG_3) (^{99m}Tc-1)

The complex was prepared by glucoheptanoate transchelation process as per the reported method[1].

Preparation of ^{99m}Tc-MAG_3(from S-thiomethyl MAG_3) (^{99m}Tc-2)

To the aqueous solution of MAG_3 (1.5 m, 0.2 mL) adjusted to pH 8.5 by N or N/10 NaOH, freshly prepared glucoheptanoate solution (25 mg, in 0.6 mL water) was added, followed by the addition of aqueous $^{99m}TcO_4^-$ (0.1 mL, 74–185 MBq) and freshly prepared stannous chloride dihydrate solution (50 μL, 25 μg) under nitrogen atmosphere. The mixture was incubated for 30 min at room temperature.

Chromatography

Instant thin layer chromatography was performed on precoated silica-gel (SG) strips 1 × 12 cm (Merck) in acetone and saline to determine the purity of the ^{99m}Tc-MAG_3 complexes (^{99m}Tc-1, ^{99m}Tc-2).

Technetium-99m complex of mercaptoacetyltriglycine complexes (^{99m}Tc-1 and ^{99m}Tc-2) were subjected to HPLC analysis on C-18 reverse phase column. The column was eluted at a flow rate of 1 ml/min with an isocratic mixture of 0.01 M phosphate buffer (pH 6.5):ethanol (95:5) as per the reported method.[1]

Paper electrophoresis

Paper electrophoresis of ^{99m}Tc-chelates of 1 and 2, along with $^{99m}TcO_4^-$ was run on a Whatman No. 1 paper (26 × 60 cm) in bicarbonate buffer (0.01 M, pH 7.0) across which a potential difference of 3 kV was applied at a current strength of 10 mA for 1 h.

Table 1. Biodistribution results of ^{99m}Tc-MAG_3 complex prepared from *S*-thiomethyl MAG_3 (^{99m}Tc-2) and S-benzoyl MAG_3 (^{99m}Tc-1) coinjected with ^{131}I-orthoiodohippuran (results in parenthesis) at 30-min post injection expressed as percent dose/organ in mice

	^{99m}Tc-MAG_3 (ligand *S*-thiomethyl MAG_3)	^{99m}Tc-MAG_3 (ligand *S*-benzoyl MAG_3)
Blood	1.86 ± 0.80 (1.79 ± 0.50)	2.45 ± 0.31 (1.69 ± 0.72)
Liver	2.00 ± 0.33 (1.67 ± 0.13)	3.83 ± 0.58 (1.23 ± 1.09)
Intestine	2.26 ± 0.45 (1.98 ± 0.13)	4.70 ± 0.49 (1.45 ± 0.99)
Kidney	2.30 ± 0.35 (1.64 ± 0.16)	4.25 ± 0.96 (1.99 ± 0.39)
Urine	68.55 ± 3.08 (77.56 ± 1.18)	66.53 ± 2.15 (76.89 ± 0.52)

Results are expressed as mean ± SD of six animals.

Biodistribution studies

Experiments using well hydrated Swiss Albino mice (20–25 g) were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation. ^{99m}Tc-MAG3 complexes prepared from the ligands 1 and 2 (8–12 MBq/Kg) premixed with ^{131}I-OIH (2–4 MBq/Kg) were injected via tail vein in mice. The animals were sacrificed either at 30 min or at 10 min post-injection, the desired organs were collected, rinsed with saline, blotted dry and weighed. Counting were carried out in ECIL, NaI (Tl) well counter (model LV4755) and the results were expressed as the percentage of injected dose per organ.

Inhibition studies

To hydrated mice an aqueous solution of probenecid (50 mg/Kg), pH 7.4 was introduced via tail vein 10 min prior to the radiopharmaceutical injection. The animals were sacrificed at 10 min post-injection, and biodistribution studies were carried out following exactly the previously described procedure.

Results

Methane sulphur trichloride was produced on chlorination of dimethyl disulfide under careful reaction condition at −15 °C. This reacted with dimethyl disulphide to give methyl sulphenyl chloride used for *S*-thiomethylation without further purification The preparation of *S*-thiomethyl MAG_3 involved three-step reaction processes. Attempted preparation of *S*-thiomethyl MAG_3 from commercially available thioglycolic acid and freshly prepared methyl sulphenyl chloride resulted in very poor yield of the desired product. This was improved by the purification of the commercial thioglycolic acid through Dean–Stark distillation process. After purification, thioglycolic acid was treated with methyl sulphenyl chloride at 0 °C under nitrogen atmosphere to give *S*-thiomethylthioglycolic acid. The product was crystallised from diethylether and ethyl acetate and characterised by ^{1}H NMR spectroscopy. *S*-thiomethylthioglycolic acid thus obtained was reacted with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide at room temperature. The prepared succinimidyl-*S*-thiomethylthioglycolate was recrystallised from isopropanol and characterised by ^{1}H NMR spectroscopy. Succinimidyl-*S*-thiomethylthioglycolate was then reacted with triglycine at room temperature to produce *S*-thiomethyl MAG_3, which was purified by recrystallisation from isopropanol and analysed by ^{1}H NMR spectroscopy.

Technetium-99m complex labelling of the *S*-thiomethyl MAG_3 (2) was carried out using stannous chloride reduction method in presence of a labile ligand sodium glucoheptonate. Unlike the ^{99m}Tc labelling of the *S*-benzoyl MAG_3, the complexation was allowed to proceed at room temperature. The analysis was carried out with TLC, HPLC and electrophoresis. In acetone, $^{99m}TcO_4^-$ moved to solvent front, whereas reduced hydrolysed technetium (Sn-Tc) and both MAG_3 chelates were stayed at the origin. In saline MAG_3 chelates and $^{99m}TcO_4$- moved to the solvent front leaving (Sn-Tc) at the origin. In all the cases, impurities accounted to be less than 2% of the total activity in the instant thin layer chromatography plates. In electrophoretic field, ^{99m}Tc-MAG_3 chelates prepared from the ligand 1 and the thiomethylated precursor 2 exhibited sharp and rapid movement of 12 cm (Fig. 2).

Technetium-99m complex of mercaptoacetyltriglycine chelate prepared from *S*-benzoyl MAG_3 ligand when subjected to HPLC

Table 2. Effect of probenecid on the biodistribution of ^{99m}Tc MAG_3 complex prepared from *S*-thiomethyl MAG_3 (^{99m}Tc-2) and *S*-benzoyl MAG_3 (^{99m}Tc-1) coinjected with ^{131}I-orthoiodohippuran (results in parenthesis) in mice at 10-min post injection

	99mTc-MAG_3 (ligand *S*-thiomethyl MAG_3)		99mTc-MAG_3 (ligand *S*-benzoyl MAG_3)	
	Control	Probenecid	Control	Probenecid
Blood	2.50 ± 1.12 (3.08 ± 1.62)	6.54 ± 0.37 (4.32 ± 0.33)	3.88 ± 1.09 (3.21 ± 0.51)	6.98 ± 1.62 (4.52 ± 1.81)
Liver	1.50 ± 0.26 (2.25 ± 0.31)	4.76 ± 1.72 (4.33 ± 1.34)	2.99 ± 0.79 (2.91 ± 1.24)	4.15 ± 2.42 (4.44 ± 1.55)
Intestine	2.29 ±0.44 (2.23 ± 0.33)	2.69 ± 0.58 (2.05 ± 0.16)	3.87 ± 0.84 (3.62 ± 0.59)	2.75 ± 1.93 (2.66 ± 1.64)
Kidney	3.07 ± 0.85 (2.48 ± 0.74)	5.47 ± 0.85 (5.32 ± 1.75)	3.21 ± 0.78 (2.53 ± 0.75)	6.73 ± 2.05 (5.39 ± 1.54)
Urine	51.92 ± 1.94 (61.03 ± 2.32)	40.52 ± 1.78 (51.21 ± 0.64)	48.87 ± 1.45 (59.95 ± 2.79)	37.64 ± 2.55 (50.24 ± 2.03)

Results are expressed as mean ± SD of six mice. In the control set, radiopharmaceuticals were injected to the animals not treated with probenecid.

analysis exhibited a major component (87.6% pure) at a retention time of 6.18 min, whereas under the similar condition, ^{99m}Tc-MAG3 complex prepared from *S*-thiomethyl MAG_3 as starting ligand exhibited a major component of much higher purity (≥98%) at retention time 6.77 min. The HPLC chromatograms were depicted in Figure 1.

The biodistribution studies in mice at 30-min post injection (Table 1) of ^{99m}Tc-MAG_3 complex prepared either from *S*-benzoyl MAG_3 or *S*-thiomethyl MAG_3 precursor exhibited appreciably high urinary excretion, which was in the range of 66.53 ± 2.15 (^{99m}Tc-1) and 68.55 ± 3.08 (^{99m}Tc-2). Although their hepatobiliary excretion was not pronounced, but still, it was little higher in ^{99m}Tc-MAG_3 complex prepared from the benzoylated precursor (8.53 ± 0.58) in comparison with that of the ^{99m}Tc-MAG_3 complex prepared from the thiomethylated precursor (4.26 ± 0.40). All the experiments were carried out under ^{131}I-OIH co administration. To determine the exact pathway of renal elimination, ^{99m}Tc-MAG_3 complex prepared from 1 and 2 were subjected to probenecid depression studies (Table 2) in mice pre-treated with probenecid (pH 7.4, 50 mg/kg) 10 min prior to radiopharmaceutical injection. In this study also, ^{131}I-OIH was used as standard renal agent. The renal excretion of all MAG_3 complexes was reduced by 11% with a concomitant rise in blood, liver, intestine and kidney uptake compared with that of the control.

Discussion

In the field of organic synthesis, the high sensitivity of the thiolate function for chemical reaction causes concern and often, the reactants bearing free sulphhydryl groups are masked. Technetium chelation of thiol-based ligands also resulted artefact formation, chelate denaturation and subsequent polymerization. The high reactivity of the thiolate donor therefore needs to be masked while chelating with technetium.[23] In the course of our study, *S*-thiomethyl (S-CH_3) has been developed as a sulphhydryl protecting group and well studied using different technetium binding ligand systems, for example, cysteine, ethylene dicysteine, DMSA and others. All these thiomethylated precursor on radiolabelling produced the desired technetium complex in quantitative yield.

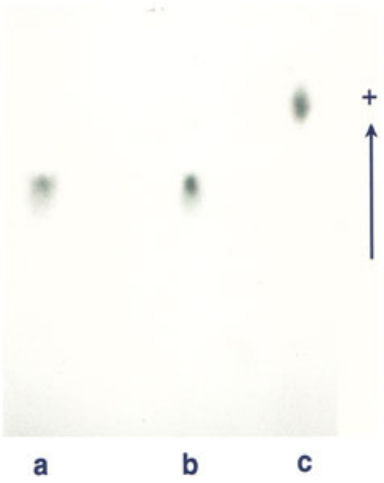


Figure 2. Autoradiograph showing the electrophoretic movement of (a) 99mTc-MAG3 chelate prepared from *S*-benzoyl MAG3, (b) 99mTc-MAG3 chelate prepared from *S*-thiomethyl MAG3 and (c) $99mTcO_4^-$ + sign indicates the anode, and the arrow indicates the direction of movement.

In this work, thiolate functionality of mercaptoacetyltriglycine was masked with thiomethyl group. Succinimidyl-*S*-thiomethylthioglycolic was coupled with triglycine under mild reaction condition to give *S*-thiomethyl MAG_3. After synthesising, the ligand was purified and radiolabelled with technetium-99 m by transchelation method using stannous chloride dehydrate as reductant. *S*-benzoyl-MAG_3 was synthesised as per reported method and radiolabelled with technetium-99 m by transchelation method. Both the complexes were subjected to TLC analysis, presence of radiochemical impurities, for example, $^{99m}TcO_4^-$ and reduced hydrolysed technetium were negligible (< 2%). HPLC elution profile of (Figure 1) ^{99m}Tc-MAG_3 complexes prepared from *S*-thiomethyl MAG_3 under transchelation method is similar to that of ^{99m}Tc-MAG_3 complex prepared from *S*-benzoylated precursor. As per HPLC chromatograms, ^{99m}Tc-MAG_3 complex prepared from *S*-benzoylated precursor exhibited a single radioactive component (yield 87.6%). Above yield was much improved (98.7%) when the same complex was prepared from thiomethylated precursor. However, the other workers in this area have reported that the yield of the ^{99m}Tc-MAG_3 complex prepared from *S*-benzoylated precursor could be increased (>90%) by boiling the complex for 10 min, after addition of pertechnetate to the kit vial.[24] We have here attempted to prepare ^{99m}Tc-MAG_3 complex of high radiochemical purity from *S*-thiomethyl-MAG_3 under relatively mild labelling condition (at room temperature) omitting both the heating and aeration step. The ^{99m}Tc-MAG_3 complex prepared routinely in different radiochemical clinics from benzoylated precursor necessitate the heating of the chelate at elevated temperature to increase the labelling yield that cause much inconveniency and may reduce the yield by forming some radioactive artefacts. This could be avoided if thiomethylated precursor could be used instead of benzoylated one during preparation of ^{99m}Tc-MAG_3 complex. Biodistribution studies of the complexes ^{99m}Tc-1 and ^{99m}Tc-2 showed almost similar uptake of the complexes by different organs of interest. The hepatobilliary excretion of ^{99m}Tc-2 was little less than that of ^{99m}Tc-1. During competitive inhibition studies, renal excretion of the complex prepared from the thiomethylated precursor was inhibited almost 11% by probenecid, which was comparable with the ^{99m}Tc-MAG_3 complex prepared from *S*-benzoyl MAG_3. All these studies were carried out under ^{131}I-OIH co-injection method and these confirmed a common renal excretory pathway for both.

Technetium-99m complex of mercaptoacetyltriglycine complexes prepared from *S*-benzoylMAG_3 and *S*-thiomethylMAG_3 also exhibited similar electrophoretic movement (12 cm) towards anode, indicating their identity and purity (Figure 2).

Conclusion

From this discussion, it is clear that thiomethyl group (–SCH_3) could be used for efficient protection of thiol functionality present in mercaptoacetyltriglycine ligand to avoid by product formation during complexation. *S*-thiomethylated MAG_3 precursor resulted in desired MAG_3 complex under relatively mild condition (incubation at room temperature), with considerably high radiochemical yield, leading to the simplification of the preparative method of the radiopharmaceuticals. The extrarenal uptake of ^{99m}Tc-MAG_3 complex prepared from thiomethylated precursor was considerably less than that prepared from *S*-benzoylated MAG_3. Lyophilised single vial kit for ^{99m}Tc-MAG_3 can also be developed using thiomethylated precursor as starting ligand.

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Conflict of Interest

The authors did not report any conflict of interest.

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