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Research Article

Comparative evaluation of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor synthesized by conventional method and by using carbonyl kit

Drishty Satpati, Madhav Mallia, Kanchan Kothari* and M.R.A. Pillai
Radiopharmaceuticals Division, Isotope Group, Bhabha Atomic Research Centre, Mumbai 400085, India

Summary

A comparison of the preparation of Tc carbonyl complexes of a few ligands of interest through the conventional method as well as by using the recently introduced carbonyl kit method is described. Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor was carried out by the conventional method in which $^{99m}TcO_4^-$ was reduced with sodium borohydride and carbonylated by purging CO gas. Tricarbonyl complexes of MIBI, TBI, isoniazid and mebrofenin were prepared using the above precursor under optimized conditions. $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor was also synthesized by using the carbonyl kit and its utility for the synthesis of tricarbonyl complexes was studied by carrying out synthesis of complexes with the above ligands. For comparison, these complexes were first prepared under similar conditions optimized with conventional precursor in order to see the variation in the yields in the two different procedures. Characterization of the precursors as well as the respective tricarbonyl complexes was carried out by reverse phase HPLC using C18 column. It was observed that $[^{99m}Tc(OH_2)_3(CO)_3]^+$ precursor could be synthesized in high yields using the carbonyl kit, however, the reaction kinetics was found to be slow while using the kit precursor. In the case of TBI and MIBI, it was observed that increase in reaction time was essential to achieve good yields. A five-fold increase in the ligand concentration was needed while using the carbonyl kit in order to get good complexation yields with isoniazid and mebrofenin. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: ^{99m}Tc; ^{99m}Tc-carbonyl; carbonyl kit; boranocarbonate

*Correspondence to: K. Kothari, Scientific Officer, Radiopharmaceuticals Chemistry Section, Radiopharmaceuticals Division, Bhabha Atomic Research Centre, Mumbai 400085, India. E-mail: kanchan@apsara.barc.ernet.in

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Introduction

Recent advances in ^{99m}Tc radiopharmaceuticals chemistry is aimed at developing site-specific radiopharmaceuticals that take advantage of an *in vivo* biochemical reactions such as receptor binding.[1–4] One of the essential requirements for achieving this is to have very high specific activity radiotracers. The most common approach for the preparation of site-specific radiopharmaceuticals is to use the concept of the bifunctional chelating agent (BFCA) in which one functionality is used for attaching to the molecule of interest and the other is used for chelating with the radionuclide.[5,6] However, this approach often suffered from complicated labeling procedure, insufficient labeling yields, and the need for purification procedures. This was mainly attributed to the lack of stable substitution labile ^{99m}Tc(V) precursors. Generally, after reduction of ^{99m}Tc(VII) to ^{99m}Tc (V) a large excess of ligand is required to stabilize the complex in the $+5$ oxidation state.[7,8] Despite efforts there are no significant improvements to make high specific activity tracers using the Tc(V) chemistry and having improved ligand system.[9] Hence, the investigations are currently focused on technetium chemistry in lower oxidation state and use of substitution labile, oxidation stable and easily accessible precursors.

Organometallic technetium complexes in low oxidation states have gained considerable attention in the development of novel site-specific radiopharmaceuticals in the recent years.[10–14] Specially Tc (I) tricarbonyl complexes seem to be ideal candidates for labeling receptor avid biomolecules. The Tc-tricarbonyl core allows the labeling of small biomolecules to high specific activity and specificity without too much change in the structure.[15] Earlier approaches to produce Tc tricarbonyl complexes were not preferred due to the difficulty of performing the reaction in routine clinical environment as it needed to have high pressure, temperature and multiple synthesis steps.[16,17] Intensive investigations in the ^{99m}Tc carbonyl chemistry has made available an organometallic $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor in a single-step procedure from aqueous $^{99m}TcO_4^-$ in the presence of carbon monoxide gas and BH_4^- at normal pressure.[8] Various N-containing ligands such as histidine, histamine, imidazole, Schiffs bases[18,19] are able to react with the precursor to yield stable complexes and are being tried for functionalization of biomolecules for the development of site-specific radiopharmaceuticals.

The procedure for the synthesis of the precursor relying on CO gas is unsuitable for use in commercial radiopharmaceutical kits. Synthesis of boranocarbonate, a reducing agent and a solid source of CO has made it feasible for the preparation of a kit for radiolabeling.[20] However, composition of the ingredients used in the synthesis of the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor by the existing method in which CO gas is used (conventional precursor) and

by carbonyl kit in which boranocarbonate is used as CO source (kit precursor) is not the same and this could lead to changes in the conditions needed for complexation. Hence, it is worthwhile investigating the matrix effect of the two $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursors on synthesis of tricarbonyl complexes.

Due to structural similarity of ^{99m}Tc-TBI and ^{99m}Tc -MIBI with ^{99m}Tc $(CO)_3$-TBI and ^{99m}Tc $(CO)_3$- MIBI, respectively, the latter complexes could be expected to accumulate in the myocardium.[21] Isoniazid is an effective drug used for treating tuberculosis[22] hence $^{99m}Tc(CO)_3$-isoniazid could have potential for imaging patients suffering from tuberculosis infection. ^{99m}Tc-mebrofenin is a hepatobiliary agent presently used.[23] Mebrofenin contains functional groups for complexation with $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor. We report the work carried out on the preparation of tricarbonyl complexes of MIBI, TBI, isoniazid and mebrofenin using the two different precursors. The difference in the reaction conditions needed to get good complexation yields using the kit precursor is arrived by optimizing reaction parameters.

Results and discussion

$[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor could be prepared in $>98\%$ yield by using the conventional method as well as with the carbonyl kit. HPLC of the precursors revealed single peak with retention time of 13.6 ($\pm$ 0.2) min with both the methods (Figure 1(a) and (b), respectively).

$^{99m}Tc(CO)_3$-TBI

$^{99m}Tc(CO)_3$-TBI could be prepared with conventional precursor in 95–98% yields under optimized conditions (i.e. at 50°C for 20 min at pH 8). The labeled

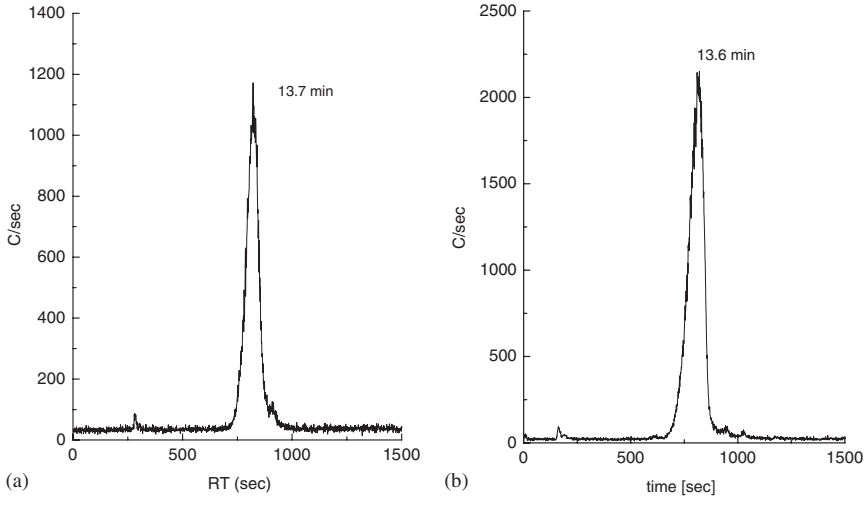

Figure 1. Synthesis of precursor by (a) conventional method, (b) carbonyl kit

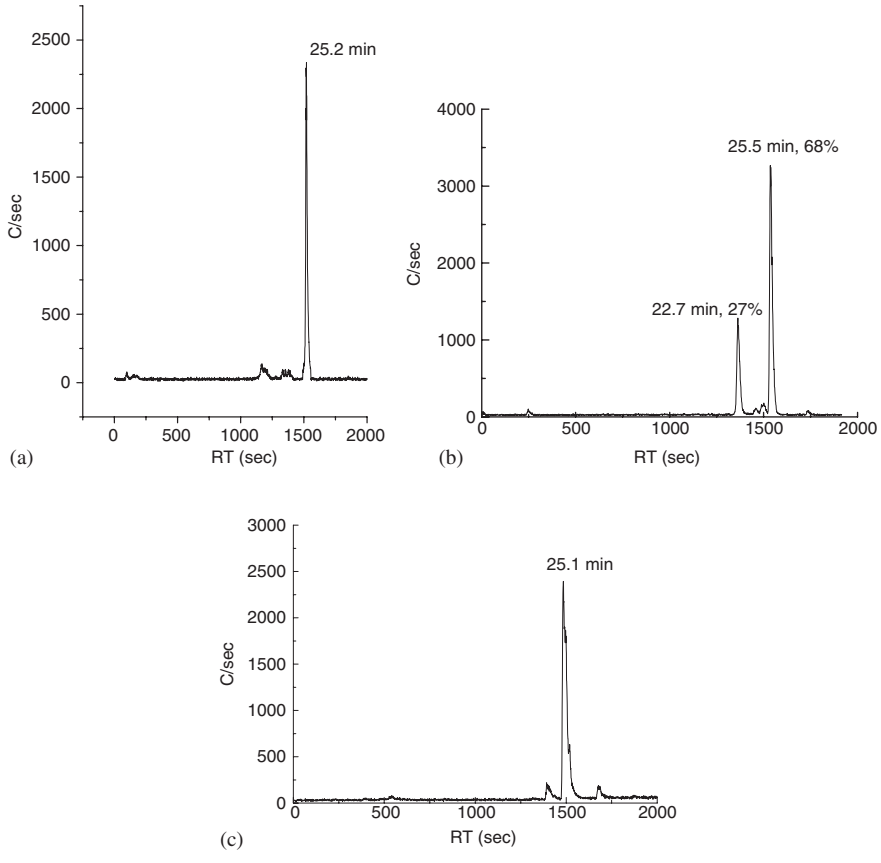

Figure 2. Synthesis of $^{99m}Tc(CO)_3$-TBI with (a) conventional precursor, (b) kit precursor, (c) kit precursor and at 1 h reaction time

compound was eluted as a single species with HPLC retention time of 25.2 ± 0.3 min (Figure 2(a)). However, two species (27% at 22.7 min, 68% at 25.5 min) were obtained when complex was prepared under identical reaction conditions with the kit precursor (Figure 2(b)). It was observed that increasing the reaction time from 20 min to 1 h could convert the complex into a single species (Figure 2(c)).

$^{99m}TC(CO)_3$-MIBI

^{99m}Tc tricarbonyl complex of MIBI could be prepared with conventional precursor in 96% yield under optimized conditions of heating at 100°C for 5 min at pH 9. The labeled compound was eluted as a single species with retention time of 24.3 ± 0.3 min (Figure 3(a)). The results were not reproducible when the complex was prepared while using the kit precursor (Figure 3 (b)). Two species with HPLC retention times of 21.8 min (23%) and 24.2 min (77%) were formed. Increasing the concentration of MIBI in the

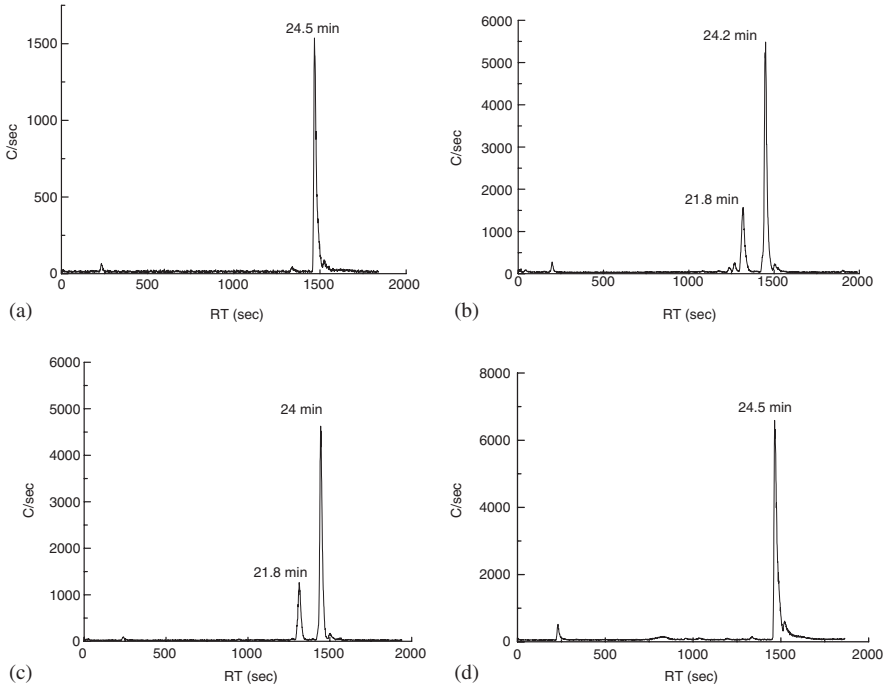

Figure 3. Synthesis of $^{99m}Tc(CO)_3$-MIBI with (a) conventional precursor, (b) kit precursor, (c) kit precursor and with 2×10^{-2} M MIBI, (d) kit precursor and with reaction time of 30 min

reaction (2×10^{-3} to 2×10^{-2} M) did not help to increase the yield (Figure 3(c)). However, single species formation was seen when reaction time was increased from 5 to 30 min and the complex eluted as single species with a retention time of 24.3 ± 0.3 min (Figure 3(d)).

$^{99m}Tc(CO)_3$-Mebrofenin

$^{99m}Tc(CO)_3$-Mebrofenin was obtained in 95–98% yield with conventional precursor while using 0.7×10^{-5} M of mebrofenin, at pH 7 and carrying the reaction at 75°C for 1 h. Complex eluted as single species with retention time of 19.3 ± 0.3 min (Figure 4(a)). However, when prepared with the kit precursor under similar conditions two species were obtained with HPLC retention time of 18 min (5.2%) and 19 min (85%) along with 9% of unreacted precursor (Figure 4(b)). Reaction conditions were further optimized to get reproducible results with kit precursor. Since the reaction time is already long, the reaction was attempted at higher temperature. Increase in reaction temperature to 100°C did not help to get complex with single species (Figure 4(c)). However, by increasing the mebrofenin concentration five times (3.5×10^{-5} M), kit precursor reacted completely giving a single species with HPLC retention time of 19 min (Figure 4(d)).

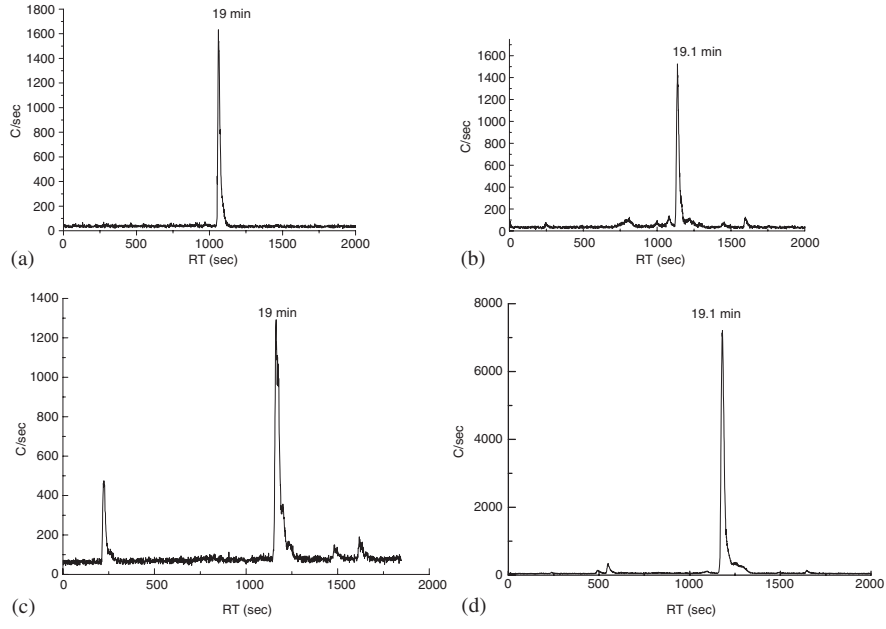

Figure 4. Synthesis of $^{99m}Tc(CO)_3$-mebrofenin with (a) conventional precursor, (b) with kit precursor, (c) with kit precursor and reaction time 1 h, (d) kit precursor and at 2.7×10^{-3} M mebrofenin

$^{99m}Tc(CO)_3$-isoniazid

$^{99m}Tc(CO)_3$-isoniazid was formed in 98–99% yields when prepared using conventional precursor. However, HPLC analysis revelled formation of two species with retention time of 8.0 ± 0.1 min (25%) and 9.0 ± 0.1 min (75%) (Figure 5(a)). Optimization of reaction conditions did not help in getting the complex as a single species. Complex with the kit precursor under identical reaction conditions was formed in lower yields (90%). Though the species formed had same retention times, proportion of the species formed was not the same as the one obtained with the conventional precursor. Radiochemical impurities in the complex were un-reacted precursor (5%) and pertechnatate (5%) (Figure 5(b)). Increase in reaction time to 45 min did not help to reduce radiochemical impurities (Figure 5(c)). However, increase in isoniazid concentration five fold to 7.3×10^{-3} M, resulted in increase in complexation yields to 99% with the formation of two species and in yields similar to that obtained using the conventional precursor (Figure 5(d)).

The use of potassium boranocarbonate $K_2BH_3CO_2$ was a major breakthrough in Tc carbonyl chemistry especially for kit formulation as this reagent serves as a solid source of CO as well as a reductant for reducing Tc from $+7$ to $+1$ state. This development has helped in the formulation of freeze dried kits for the synthesis of precursor which is more safe and convenient than the

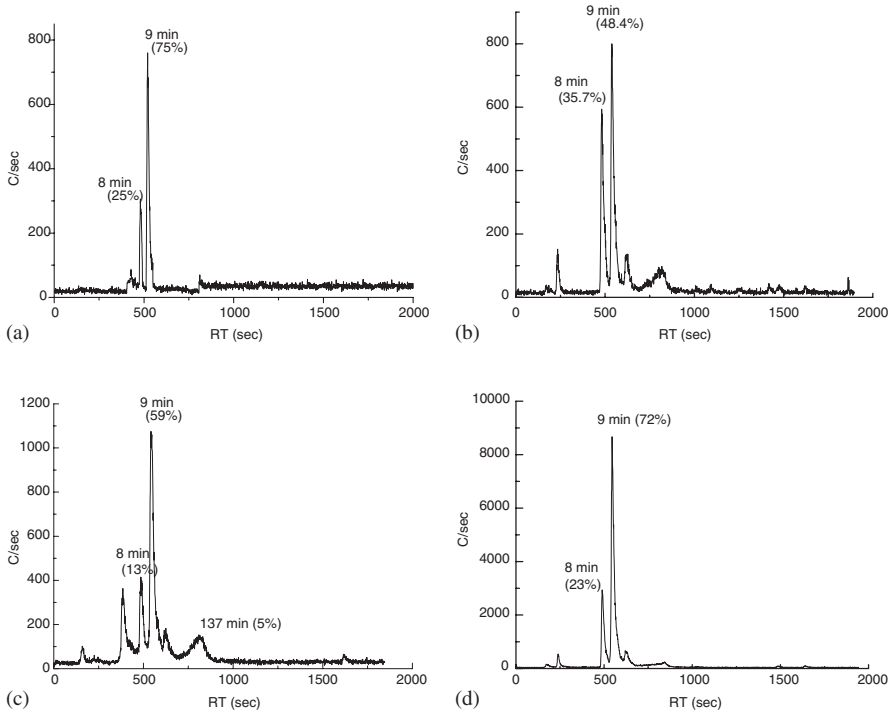

Figure 5. Synthesis of $^{99m}Tc(CO)_3$-isoniazid with (a) conventional precursor, (b) kit precursor, (c) kit precursor and reaction at 70°C for 45 min, (d) kit precursor and at 7.3×10^{-3} M isoniazid

conventional procedure of bubbling CO gas. Preliminary investigations using HPLC have confirmed the formation of Tc carbonyl core while using the freeze dried kit.[24] It has shown that the formulation is reproducible, robust and simple for the preparation of $^{99m}Tc(OH_2)_3(CO)_3$ precursor. Complexation studies of histidine with kit precursor have been carried out earlier to confirm its utility for synthesis of tricarbonyl complexes. The amount of histidine used in the reaction for complexation is given as 50 μg/ml (3×10^{-4} M).[20] Systematic studies are also reported on synthesis of $^{99m}Tc(CO)_3$-histidine with the conventional precursor. In this case the complex is reported to be formed with histidine in high yields and also with high specific activity (10^{-6} M).[9] However, the specific activity of $^{99m}Tc(CO)_3$-histidine made with the conventional precursor was found to be higher than the one made with the kit precursor.

We have earlier prepared Tc-carbonyl complexes with MIBI, TBI, mebrofenin and isoniazid (Figure 6) by using the conventional precursor to investigate the utility of these complexes as radiopharmaceuticals. Reaction conditions were optimized to achieve complexes with high yields and as single species wherein we expected all the three water molecules are replaced by CO

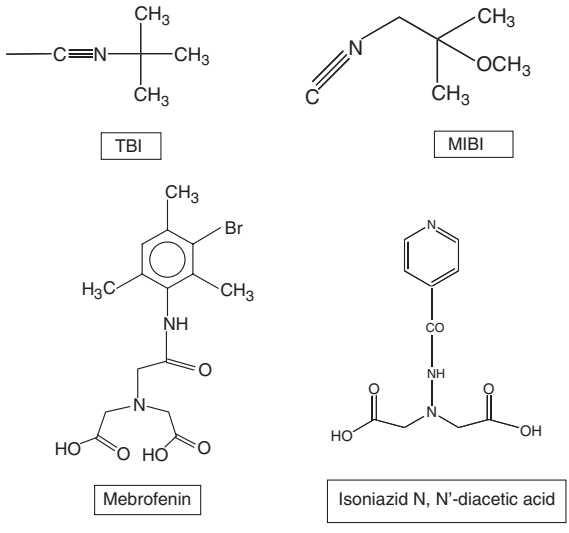

Figure 6. Structure of TBI, MIBI, mebrofenin and isoniazid derivative used the synthesis of tricarbonyl complexes

molecules. However in the case of isoniazid, though the complex was prepared in high yields, two species were obtained even after trying several reaction conditions. These species had retention times of 8.0 ± 0.1 min (25%) and 9.0 ± 0.1 min (75%). The formation of two species could be due to the possibility of coordination of isoniazid with precursor via the pyridine nitrogen also.

Tricarbonyl complexes of TBI, MIBI, mebrofenin and isoniazid derivative were also prepared with the kit precursor for comparative evaluation. In all the cases it was observed that the results were not same while using the two different precursors. The reaction kinetics was found to be slower when complexation was carried out using the kit precursor resulting in additional peaks in the HPLC profile due to incomplete substitution reaction. These species were also observed during the optimization process with conventional precursor. By carefully optimizing the reaction conditions, we could get single species for TBI, MIBI and mebrofenin. However, using these optimized reaction conditions with kit precursor did not result in the same high complexation yields. It was essential to increase the reaction time with the kit precursor to obtain similar results with TBI and MIBI suggesting slower kinetics of reaction.

Additional peak observed with kit precursor in the case of mebrofenin and isoniazid was due to un-reacted carbonyl precursor. Attempts to increase the reaction time did not result in good yields. With mebrofenin prolonged heating dissociated unreacted precursor to TcO_4^- while with isoniazid apart from

dissociation to TcO_4^- additional hydrophilic species was also observed which could be due to the decomposition of $^{99m}Tc(CO)_3$-isoniazid. High complexation yields could be obtained with mebrofenin and isoniazid only after increasing the ligand concentration. One of the reasons for slower substitution reaction kinetics observed while using kit precursor could be due to the presence of traces of boranocarbonate in the reaction which would inhibit the substitution by other ligands. Hence, the need for higher ligand concentration or longer reaction times to get good yields.

The ability to get high specific activity tracers is the major advantage of ^{99m}Tc radiopharmaceuticals prepared via $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor. The introduction of the boranocarbonate kit for preparation of Tc-carbonyl complex is yet another step towards the use of Tc-carbonyl complexes in clinical nuclear medicine. However, preliminary investigations suggest that use of boranocarbonate in kit formulation for the synthesis of the precursor slows down the reaction kinetics needing more optimization studies. In the case of two of the ligands tried, mebrofenin and isoniazid, higher amount of ligands was needed resulting in the reduction of the specific activity of the preparation.

Experimental

Sodium borohydride and Na/K tartrate were obtained from Aldrich Chemicals. All chemicals and solvents were of reagent grade and used without further purification. Carbon monoxide in 0.5 l refillable canisters was obtained from M/s Alchemie Gases & Chemicals Pvt Ltd. Mumbai, India. $^{99m}TcO_4^-$ was eluted from $^{99}Mo/^{99m}Tc$ column generator using normal saline as eluant. Carbonyl kit for the synthesis of precursor was obtained as a gift from Mallinckrodt Medical, Netherland. TBI was obtained from Fluka Chemie GmbH, Switzerland. MIBI and mebrofenin were obtained from BRIT, Vashi, Mumbai, India. Isoniazid *N*, *N*′-diacetic acid derivative synthesized in house was used for the preparation of ^{99m}Tc tricarbonyl isoniazid complex. HPLC analysis of tricarbonyl precursor and tricarbonyl complexes with different ligands was performed on a Jasco PU 1580 system with Jasco low-pressure gradient valve (LV-1580-03), Jasco mixing module (HG-980-30) and Jasco 1575 tunable absorption detector along with indigenously developed radiometric detector system. For radiochemical purity (RCP) analysis a C-18 reversed phase HiQ Sil (5 μm, 4 × 250 mm) column supplied by KYA TECH corporation (Japan) was used.

Synthesis of conventional precursor

Briefly $NaBH_4$ (5.5 mg), Na_2CO_3 (4 mg) and Na/K tartrate (15 mg), were dissolved in 0.5 ml double-distilled water in a glass serum vial. The vial was sealed and carbon monoxide was purged through the solution for 5 min. After

the addition of 1 ml of the generator eluate containing 37–74 MBq of $^{99m}TcO_4^-$, the vial was heated at 80°C for 15 min. After cooling the vial for 10 min and re-equilibration to atmospheric pressure, pH of the reaction mixture was adjusted to 7 with 300 µl of 1:3 mixture of 0.5 M phosphate buffer (pH 7.5): 1 M HCl. The precursor was characterized by HPLC.

Synthesis of kit precursor

The kit precursor was prepared as per the procedure mentioned in kit protocol. To a 10 ml sealed vial containing 8.5 mg sodium tartrate, 2.85 mg $K_2B_4O_7.10H_2O$, 7.15 mg of sodium carbonate and 4.5 mg sodium boranocarbonate, 1 ml of $^{99m}TcO_4^-$ (37–74 MBq) from generator was added. The vial was kept in a boiling water bath for 15 min. After cooling the vial to room temperature, 175 µl of 1 N HCl was added to neutralize the solution and decompose any residual boranocarbonate. Radiochemical purity of the precursor was checked by reverse phase HPLC.

Synthesis of tricarbonyl complexes of TBI, MIBI, mebrofenin and isoniazid with conventional precursor

$^{99m}Tc(CO)_3$-TBI. Synthesis of ^{99m}Tc tricarbonyl TBI was carried out by the procedure standardized in our laboratory. 70 µl of methanolic solution of TBI (3 mg) was added to 400 µl of the conventional precursor (7×10^{-2} M of TBI). pH of solution was adjusted to ~8 and the reaction mixture was heated at 50°C for 20 min.

$^{99m}Tc(CO)_3$-MIBI. Synthesis of ^{99m}Tc(CO_3-MIBI was carried out by the procedure standardized in our laboratory. 100 µl of the methanolic solution of MIBI (100 µg) was added to 400 µl of conventional precursor (2×10^{-3} M of MIBI). pH of the solution was adjusted to 9 and heated at 100°C for 15 min.

$^{99m}Tc(CO)_3$-mebrofenin. Synthesis of $^{99m}Tc(CO)_3$-mebrofenin was carried out by reacting 100 µl (25 µg) of methanolic solution of mebrofenin with 400 µl of conventional precursor (5.4×10^{-4} M of mebrofenin) at pH 7 and at 75°C for 1 h.[25]

$^{99m}Tc(CO)_3$-isoniazid. Isoniazid functionalized at NH_2 end with iminodiacetic acid was used for complexation studies (Figure 6). $^{99m}Tc(CO)_3$-isoniazid was synthesized by reacting 50 µl aqueous solution of isoniazid derivative (1.46×10^{-3} M of isoniazid) with 500 µl of conventional precursor. The solution was adjusted to pH 5 and the reaction mixture was heated at 75°C for 15 min.

Synthesis of tricarbonyl complexes of TBI, MIBI, mebrofenin and isoniazid with kit precursor

Tricarbonyl complexes of MIBI, TBI, mebrofenin and isoniazid were synthesized using kit precursor under identical reaction conditions as

mentioned above for comparison. Reaction conditions were further optimized to get higher yields.

Characterization of the complexes

Characterization of tricarbonyl complexes of different ligands mentioned above was carried out by C 18 reverse phase HPLC. Eluting solvents consisted of H_2O containing 0.1% TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B). The HPLC gradient system for analysis of the product started with 90%A/10%B with a linear gradient to 10%A/90%B from 0 to 28 min with no change in the eluent composition from 28 to 30 min. The flow rate was 1 ml/min. 25 µl of the sample was used for analysis. Recovery of the activity from the column was determined by summing the counts in the eluted fractions and comparing it to the total injected activity.

Conclusion

Use of potassium boranocarbonate $K_2BH_3CO_2$ is a major breakthrough for formulation development in the synthesis of carbonyl precursor which will bring the carbonyl complexes close to clinical nuclear medicine. However, it is observed from our studies that complexation of ligands with the $[^{99m}Tc(H_2O)_3(CO)_3]^+$ precursor prepared using kit formulation slows down the reaction kinetics. The complexes in good yield could be prepared by increasing the reaction time, temperature or by increasing the ligand concentration. However, increasing ligand concentration will result in complexes with lower specific activity than the one prepared with the conventional precursor method.

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