*2005 International Nuclear Atlantic Conference - INAC 2005*
*Santos, SP, Brazil, August 28 to September 2, 2005*
*ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN*
*ISBN: 85-99141-01-5*

# PREPARATION OF THE PRECURSOR $^{99m}$TC-CARBONYL FOR LABELING BIOMOLECULES: OPTIMIZATION AND RADIOCHEMICAL EVALUATION

**Bluma L. Faintuch[1], Rodrigo L. S. R. Santos[1], Alessandro Pinheiro[1], Vivian Borges[1] and Tania Dantas[1]**

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
faintuch@ipen.br

## ABSTRACT

**Introduction**: The use of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ as a radiopharmaceutical precursor opens new routes in the labeling of biomolecules. The $^{99m}$Tc-tricarbonyl core allows the labeling of even the smallest biomolecules with high specific activity and without damaging the biological activity and specificity. This precursor is not only readily water soluble but reveals good stability in aqueous solutions over a broad pH range, for several hours. The three water molecules coordinated to the highly inert $[^{99m}Tc(CO)_3]^+$ core are readily substituted by a variety of functional groups such as amines, thioethers, thiols and phosphines. The aim of this study was the optimization and radiochemical evaluation of the preparation of $^{99m}$Tc-carbonyl using monoxide carbon gas. **Methods**: The organometallic precursor was prepared according to a published procedure (Alberto et al. 1998). Briefly, 4.4 mg $Na_2CO_3$, 15 mg Na/K-tartrate and 5.5 mg $NaBH_4$ were purged for 40 min with monoxide carbon gas. Then, 1 mL of $Na^{99m}TcO_4$ (185 MBq) eluted from a generator was added. The vial was heated at 75°C for 30 min. The reaction was stopped in ice bath. Then pH was adjusted to 7 using 0.2 mL of 1M HCl / 1M phosphate buffer solution (2:1). Optimization of this preparation was done by varying parameters such as radioactivity of isotope (185-3700 MBq), time to flushing with CO gas (30-60 min), time of reaction (20-60 min), $NaBH_4$ mass (4.0-5.5 mg) and Na/K-tartrate (15-20 mg). **Results**: The best radiochemical yield (97.1 ± 1.2%) was obtained when the parameters used were: activity till 1110 MBq, time to flushing with CO gas 60 min, reaction time 30 min, $NaBH_4$ 5.5 mg and Na/K tartrate 20 mg. The other parameters remained the same as in the published labeling. **Conclusion**: Optimization of the preparation of $^{99m}$Tc-carbonyl was successfully done, so that the precursor could be easily prepared and good radiochemical yield could be achieved. Further studies for potential use in the labeling of other biomolecules is suggested.

## 1. INTRODUCTION

The use of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ as a radiopharmaceutical precursor opens new routes in the labeling of biomolecules. During many years, the low oxidation state of organometallic Tc-Re complexes received little attention because few practical applications had been devised for them. Trop et al. 1980 [1] discovered a very stable water-soluble hexakis (isonitrile) complex $[^{99m}Tc(MIBI)_6]^+$, which was successful and is used as a myocardial perfusion agent. Some years afterwards Alberto et al. 1998 [2], developed a fully aqueous-based kit preparation of the organometallic technetium precursor under mild reaction conditions in the presence of gaseous carbon monoxide and sodium borohydrate, which is here investigated.

The $^{99m}$Tc-tricarbonyl core allows the labeling of even the smallest biomolecules with high specific activity and without damaging the biological activity and specificity. This precursor is not only readily water soluble but reveals good stability in aqueous solutions over a broad pH range, for several hours. The three water molecules coordinated to the highly inert $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core are readily substituted by a variety of functional groups such as amines, thioethers, thiols and phosphines.

Technetium-99m is a gamma emitter radioisotope, important in diagnostic nuclear medicine due to its nuclear characteristics, and is readily available from $^{99}Mo/^{99m}Tc$ generator for a convenient cost.

Although the preparation of Tc-carbonyl described above is suitable for research purposes, it still relies on toxic gaseous carbon monoxide. So the group of Alberto optimized this preparation in terms of kit-stability and commercial production by means of substitution of both gaseous CO and $NaBH_4$ by $K_2[H_3BCO_2]$ [3]. This formulation named ISOLINK kit will soon be commercially available by Mallinckrodt Medical B.V. (Netherlands). Until then we have to use the primary formulation, so it was our aim the study of the optimization of this preparation using the CO gas.

## 2. MATERIAL

Technetium-99m was obtained from an alumina-based $^{99}Mo/^{99m}Tc$ generator locally supplied by Radiopharmacy Center of Institute of Energetic and Nuclear Research (IPEN/CNEN) - São Paulo, Brazil.

Reagents used in the study were purchased from Merck and Sigma-Aldrich Brazil Ltda. The CO gas was purchased from White Martins Gases Industriais SA, São Paulo, Brazil.

## 3. METHODS

### 3.1. Synthesis of $^{99m}$Tc-carbonyl

The organometallic precursor was prepared according to a published procedure [2]. Briefly, 4.4 mg $Na_2CO_3$, 15 mg Na/K-tartrate and 5.5 mg $NaBH_4$ were purged for 40 min. with monoxide carbon gas. Then, 1 mL of $Na^{99m}TcO_4$ (185 MBq) eluted from a generator was added. The vial was heated at 75°C for 30 min. The reaction was stopped in ice bath. Then pH was adjusted to 7 using 0.2 mL of 1M HCl / 1M phosphate buffer solution (2:1) previously nitrogenated. Optimization of this preparation was done by varying parameters such as radioactivity of the isotope (185-3700 MBq), time to flushing with CO gas (30-60 min), time of reaction (20-60 min), $NaBH_4$ mass (4.0-5.5 mg) and Na/K-tartrate (15-20 mg).

### 3.2. Radiochemical evaluation

Radiochemical evaluation was done using paper chromatography (Whatman 1) and thin layer chromatography (TLC-Aluminium). The mobile phase was the mixture of MeOH/HCl 6M (99.5:0.5). This evaluation was confirmed by HPLC using as solvents $H_2O$ containing 0.1% trifluoracetic acid (Solvent A) and acetonitrile containing 0.1% trifluoracetic acid (Solvent B).

## 4. RESULTS AND DISCUSSION

The developments in $^{99m}$Tc chemistry are considered of great importance for investigating new $^{99m}$Tc labeled molecules with potential applications. In this sense the tricarbonyl technology which is currently being exploited is remarkable [4].

The synthesis of the precursor $^{99m}$Tc-carbonyl is a convenient method producing three tighly bound CO radicals and three labile water ligands. The metal centre ($^{99m}$Tc) is in the low oxidation state +1 and is therefore chemically inert [4, 5].

After optimization of labeling conditions, the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ percusor was produced in high yields (97.1 ± 1.2%). The improvement of the preparation was done by varying the parameters. The best results obtained in each assay were adopted, and the next parameter was studied in sequence, until good overall conditions were achieved.

First the reaction time from 20 to 60 min, was determined. The best yield obtained was at 30 min of reaction (94.8 ± 1.6%) (Fig. 1) showing that further increase in reaction time doesn't improve the labeling yield. Then we defined this parameter and changed the flushing time of CO (30, 40 and 60 min). The highest yield was obtained with 60 min of CO flushing (97.1 ± 1.2) and that can be observed in Figure 1. The second parameter was fixed as 60 min for CO time flushing. Tartrate mass was studied at 15, 17 and 20 mg, and the best result was reached using 20 mg, (Table 1).

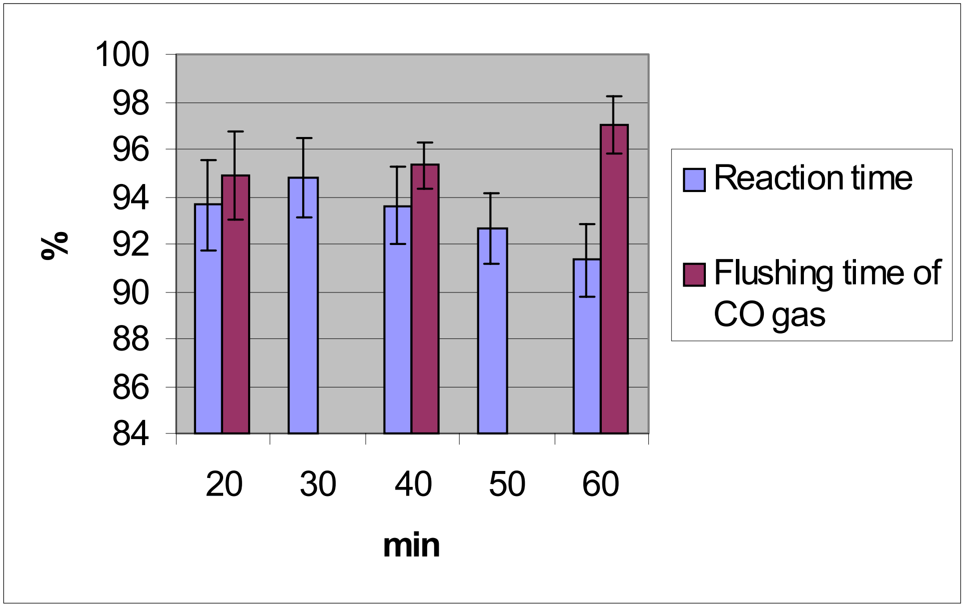

**Figure 1. Radiochemical yield with different labeling parameters.**

**Table 1. Radiochemical purity of $^{99m}Tc(CO)_3^+$ varying the mass of tartrate**

| | 15 mg | 17 mg | 20 mg |
|---|---|---|---|
| $^{99m}Tc(CO)_3^+$ | 94.0 ± 1.0 % | 95.0 ± 1.1 % | 97.1 ± 1.2 % |
| $^{99m}TcO_2$ | 3.1 ± 0.7 % | 2.8 ± 0.9 % | 1.3 ± 0.6 % |
| $^{99m}TcO_4^-$ | 2.9 ± 0.6 % | 2.2 ± 0.5% | 1.7 ± 0.7 % |

n=5

In Figure 2 we can observe that increasing $^{99m}Tc$ activity above 1100 MBq, a decrease in radiochemical purity was observed. That was due to increase in perthecnetate. Probably, for higher activity, a greater amount of the reducing agent borohydride is needed.

When a reduced amount of sodium borohydride was used (4 mg) an increase in the pertechnetate impurity was demonstraded (Table 2). Figure 3 shows the HPLC curve of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ with a retention time of 17.07 min, and a retention time for $^{99m}TcO_4^-$ of 5.33 min.

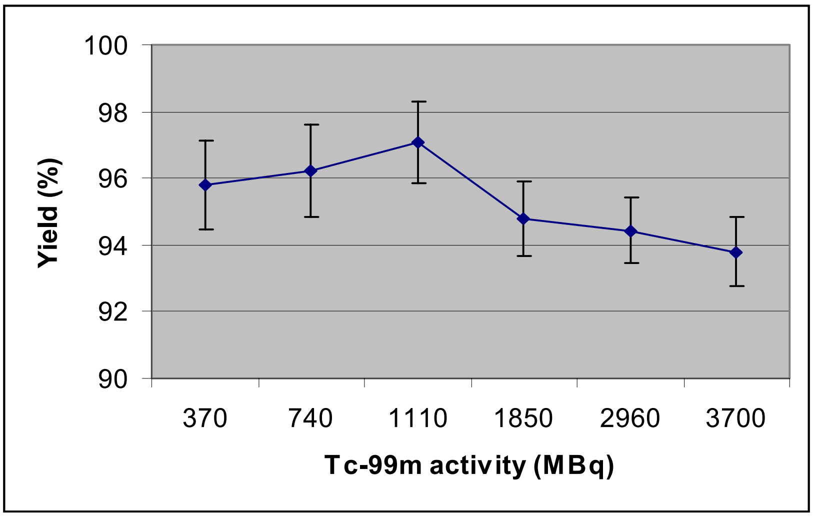

**Figure 2. Radiochemical purity of $^{99m}Tc(CO)_3^+$ with different activities of pertechnetate.**

**Table 2. Radiochemical purity of $^{99m}Tc(CO)_3^+$ varying the mass of sodium borohydrate**

| | 4 mg | 5.5 mg |
|---|---|---|
| $^{99m}Tc(CO)_3^+$ | 94.1 ± 1.6 % | 97.1 ± 1.2 % |
| $^{99m}TcO_2$ | 2.7 ± 0.8 % | 1.3 ± 0.6 % |
| $^{99m}TcO_4^-$ | 3.2 ± 0.9 % | 1.7 ± 0.7 % |

n=5

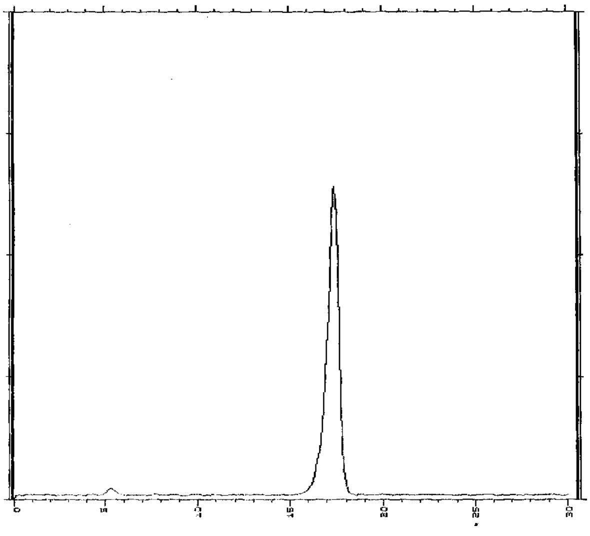

**Figure 3. Radiochromatogram of $[^{99m}Tc(CO)_3(H_2O)_3]^+$**

## 5. CONCLUSIONS

The parameters for best radiochemical purity of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ were: time for flushing the CO gas 60 min, reaction time 30 min, 20 mg of tartrate mass, activity of 1110 MBq and 5.5 mg of borohydride. Optimization of the preparation of $^{99m}$Tc-carbonyl was successfully done, so that the precursor could be easily prepared and good radiochemical yield could be achieved. Further studies of this standardization for potential use in the labeling of other biomolecules is suggested.

## REFERENCES

1. H. S. Trop, A. J. Davison, M. A. Davis, D. J. Szalda, S. J. Lippard, “Synthesis and physical properties of hexakis(isothiocyanato)technetate(III) and (IV) complexes, structure of the $[Tc(NCS)_6]^{3-}$ ion”, *Inorg. Chem*, **19**, pp. 1105-10 (1980).
2. R. Alberto R, R. Schibli, A. Egli, A. P. Schubiger, “A novel organometallic aqua complex of technetium for the labeling of biomolecules: Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in aqueous solution and its reaction with a bifunctional ligand”, *J. Am. Chem. Soc.,* **120**, pp. 7987-7988 (1998).
3. R. Alberto, K. Ortner, N. Wheatley, R. Schibli, A. P. Schubiger, “Synthesis and properties of boranocarbonate: A convenient in situ CO Source for the aqueous preparation of $[^{99m}Tc(OH_2)_3(CO)_3]^+$”, *J. Am. Chem. Soc.*, **123**, pp. 3135-3136 (2001).
4. R. Schibli, P. A. Schubiger, “Current use and future potential of organometallic radiopharmaceuticals”, *Eur. J. Nucl. Med.*, **29**, pp. 1529-1542 (2002).
5. S. R. Banerjee, K. P. Maresca, L. Francesconi, J. Valliant J, J. W. Babich, J. Zubieta, “New directions in the coordination chemistry of $^{99m}$Tc: a refletion on technetium core structures and a strategy for new chelate design”, *Nucl. Med. Biol.*, **32**, pp. 1-20 (2005).