

Mechanism of Carbonylation Reactions of Technetium-99m

G. E. Kodina,^{*,a} A. O. Malysheva,^a O. E. Klement'eva,^a A. A. Inkin,^a N. I. Gorshkov,^b
A. A. Lumpov,^b and D. N. Suglobov^b

^aISSC Institute of Biophysics, 123082, Moscow, Russia, Zhivopisnaya, 46

^bRPA Khlopin Radium Institute, 194021, St.-Petersburg, Russia, 2nd Murinskii, 28

Received: July 31, 2005; In Final Form: September 30, 2005

The aim of this work was to study products produced in basic and acidic media upon atmospheric pressure and high-pressure carbonylation and to propose probable mechanism of reaction. It was found that tricarbonyltechnetium(I) fragment forms strong complexes with charged ligands. The polydentate anions (like citrate anion), which occupies three coordination places in octahedral technetium arrangement forms strongest complexes. So $\text{Tc}(\text{CO})_3$ moiety in aqueous solutions exhibits properties of a typical d-element and a "soft" acid.

1. Introduction

Within 40 years technetium-99m occupies the leading position among the radionuclides used in diagnostic nuclear medicine.

One of perspective methods of synthesis of bioconjugates with technetium is the embedding of $\text{Tc}(\text{CO})_3^+$ cation in a peptide chain. Therefore the study of tricarbonyltechnetium (TC-Tc) chemistry and its complexes, and also their biological behavior is extremely actual task for the development of new technetium-99m radiopharmaceuticals (RPh). The similar works with radionuclides of rhenium-186 and 188 are guided on the development of RPh for radionuclide therapy.

Practically all foreign publications of the last years describe the method firstly offered by Alberto et al.^{1,2} which based on reduction of sodium pertechnetate, $^{99\text{m}}\text{Tc}$ in an alkaline medium in the presence of tartrate-ions. It is supposed that the tartrate is the stabilizer of an intermediate pentavalent state of technetium. The commercial lyophilized kit "IsoLink"TM (Mallinckrodt Medical) has been developed. $\text{TC-}^{99\text{m}}\text{Tc}$ was prepared by simple introduction of sodium pertechnetate solution in a kit vial with the subsequent heating of the vial in a boiling water bath during 20 minutes.

Alternative method for high-pressure (160–200 atm) carbonylation of sodium pertechnetate under elevated temperature (150–180 °C) in acidic media was developed in Khlopin Radium Institute³ (Russia). The main advantage of this procedure is absence of organic impurities in preparation, however it requires special microautoclave unit.

The goal of this work was to study products obtained in basic and acidic media upon atmospheric pressure and high-pressure carbonylation and to propose probable mechanism of reaction.

2. Material and Methods

The commercially produced generators of Tc-99m (Obninsk, Russia) were used in this work.

Synthesis of the $\text{TC-}^{99\text{m}}\text{Tc}$ in acid medium was carried out by autoclave carbonylation according.³ After cooling of the reaction mixture it was neutralized by sodium carbonate or citrate.

$\text{TC-}^{99\text{m}}\text{Tc}$ in an alkaline medium was prepared using commercial kit "IsoLink" or potassium boranocarbonate.

Reaction mixtures and obtained radiopharmaceuticals were analyzed by the following methods:

1. TLC on silica gel (Merck 5554) in methanol/HCl conc. 99/1 and Whatman 3 MM in acetone.

2. HPLC was carried out on a fluid chromatograph "Analist-7800" (LDC, USA); terms of separation:

Column: Ultrasphere-ODS (Altex, USA), (250 × 4.6 mm, 5 μ)

Mobile phase: 55% of acetonitrile, 45% of 0.1% solution of phosphoric acid. Flow rate: 1 mL/min.

The retention time (R_f) for KTcO_4 was 1.9–2.1 min and for $\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3\text{Cl}$ was 3.6–3.9 min.

Chloride of pentacarbonyltechnetium-99 (PC-Tc) and solution of $\text{TC-}^{99}\text{Tc}$, prepared from the PC-Tc with the subsequent NMR identification, were used as the standards. $\text{K}^{99}\text{TcO}_4$ was also used for the preparation of the artificial mixtures. A position of components on chromatograms was determined by an autoradiography. The position of ^{99}Tc -components was also identified in UV light (F_{254}).

3. Results and Discussion

According to the literature data $\text{TC-}^{99\text{m}}\text{Tc}$ obtained in an alkaline media has R_f 0.4 (silica gel in methanol/HCl conc. 99/1), whereas the pertechnetate-ions move with solvent front. On our data R_f for $\text{TC-}^{99\text{m}}\text{Tc}$ (obtained with or without of technetium-99, and also for artificial mixtures) was from 0.3 up to 0.6 in dependence on the grade of silica gel and conditions of experiment. R_f for TcO_4^- was 0.8–1.0.

Practically in all experiments on autoclave carbonylation of technetium we observed a component (which originally considered as free TcO_4^-) with R_f close, but not identical, with R_f for TcO_4^- -ions.

In Figure 1 the chromatograms of reaction mixtures and solution of $\text{Na}^{99\text{m}}\text{TcO}_4$ (eluate of the generator) are submitted. Two components with R_f 0.5 and 0.8 are observed in initial solution of a reaction mixture (pH 1). It is possible to find up to 10% of activity on start of a chromatogram. After dilution and neutralization up to pH 6 in solution there is mainly one component $\text{TC-}^{99\text{m}}\text{Tc}$.

Two components with R_f 0.0–0.1 and 0.4 is also possible to observe on chromatogram of initial solution of reaction mixture (pH 11) after carbonylation of technetium in alkaline media. There is one component in solution after neutralization (0.1 M HCl) up to pH 6–7. In both cases the addition of sodium citrate (5×10^{-3} mole/L) to the neutral solution of $\text{TC-}^{99\text{m}}\text{Tc}$ results in formation of component with R_f 0.8–1.0. However the real con-

*Corresponding author. E-mail: vkorsuns@rcibph.dol.ru. FAX: +7-495-190-3590.

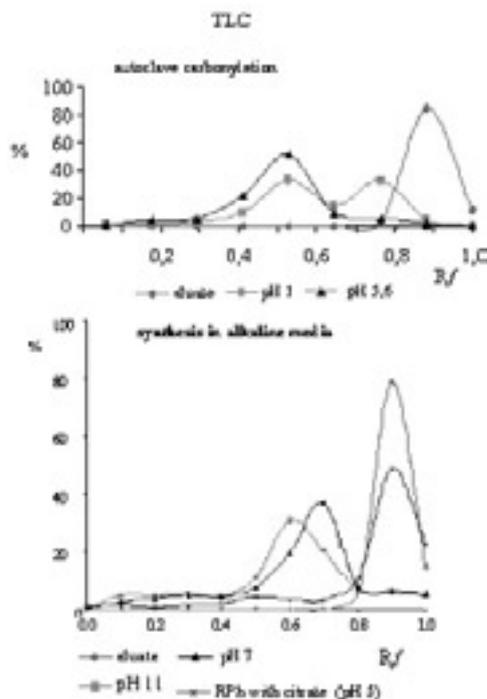


Figure 1. Chromatograms of reaction mixtures and solution of $\text{Na}^{99\text{m}}\text{TcO}_4$

tent of TcO_4^- -ions does not exceed 10% according to the chromatography of citrate solution on Whatman 3 MM in acetone.

The HPLC results of artificial mixture of $\text{K}^{99\text{m}}\text{TcO}_4$ and $\text{TC}^{99\text{m}}\text{Tc}$ are submitted in Figure 2a. At analysis of reaction mixtures, obtained upon autoclave carbonylation at the carrier presence we observed the appearance of the third component, the retention time of which is 4.6–5.0 min (Figure 2b). The solution of PC-Tc chloride was prepared for identification of this component. The retention times for TC-Tc, $[\text{Tc}(\text{CO})_4(\text{H}_2\text{O})_2]^+$ and PC-Tc were 3.7 min, 4.0 min and 4.9 min correspondingly (Figure 2c). Such interpretation was assumed on the data.³ We did not observe the presence of a component with retention time 4.0 min in reaction mixtures. At dilution and heating of a reaction mixture the signal of PC-Tc disappeared. Besides during TLC of neutralized reaction mixtures we always observed some of activity on start of chromatograms, we have assumed, that these impurities (probably hydrolyzed reduced technetium) are retained in a column during HPLC. As a result of column treatment with 0.1 M HCl after elution of TcO_4^- -ions and products of carbonylation we obtained one more fraction containing Tc in an amount ~10% from total concentration in initial sample. Thus it is possible to conclude, that upon autoclave carbonylation of technetium in amounts $(1-5) \times 10^{-3}$ mole/L we obtained mixture of TcO_4^- , $[\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, $[\text{Tc}(\text{CO})_5]^+$ and partially reduced Tc (IV) or Tc (V).

Mainly one peak of $\text{TC}^{99\text{m}}\text{Tc}$ (no carry added) is observed during HPLC of the reaction products, obtained in an alkaline media after neutralization to pH 7 (Figure 2c). The data of HPLC practically coincide with the data TLC. Quantitative yield of $\text{TC}^{99\text{m}}\text{Tc}$ is possible only in presence of tartrate-ion. Presumably it stabilizes intermediate five-valence state of technetium, which is reduced to univalent state under elevated temperature and excess of carbonylation agent (CO, boranocarbonate). The same effect was observed by us in the case of preparation of Tc(I)-isocyanide complexes at the presence of L-cysteine.⁴ Last one is also known as powerful tridentate ligand for linking of $\text{Tc}(\text{CO})_3^+$ species and corresponding complex was isolated and characterized earlier.⁵

For comparative experiments on Tc-99m carbonylation we chose sodium citrate and L-cysteine, which introduced into a reaction mixture instead of tartrate in equimolar amounts.

In Figure 3a,b the chromatograms of products, obtained dur-

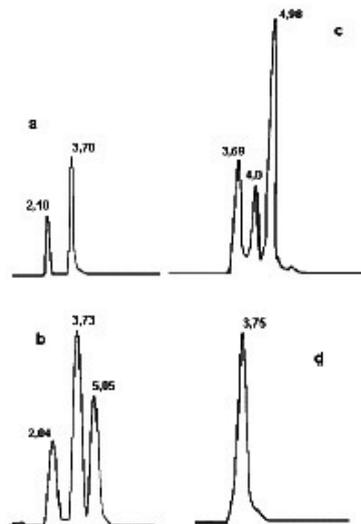


Figure 2. HPLC: 2a -artificial mixture of $\text{K}^{99\text{m}}\text{TcO}_4$ and $\text{TC}^{99\text{m}}\text{Tc}$; 2b - reaction mixtures, obtained upon autoclave carbonylation at the carrier presence; 2c -reaction mixture of TC-Tc, $[\text{Tc}(\text{CO})_4(\text{H}_2\text{O})_2]^+$ and PC-Tc; 2d -neutralized solution of $[\text{TC}^{99\text{m}}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, pH ~7

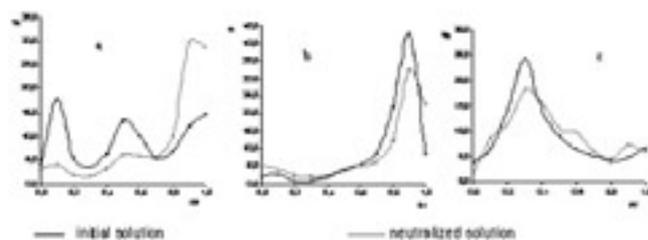
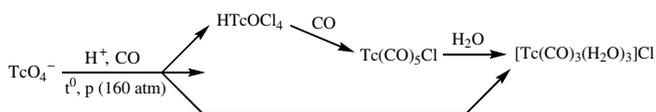


Figure 3. Chromatograms of products, obtained during carbonylation in the following conditions: 3a- at the presence of K, Na-tartrate with the subsequent neutralization of mixture by solution of sodium citrate; 3b- at the presence of sodium citrate with the subsequent neutralization by 0.1 M HCl; 3c- reaction mixture containing L-cysteine and sodium citrate

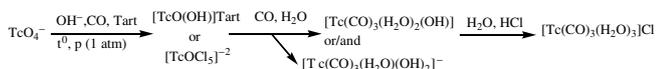
ing carbonylation in the following conditions, are submitted: 1) at the presence of K, Na-tartrate with the subsequent neutralization of mixture by solution of sodium citrate; 2) at the presence of sodium citrate with the subsequent neutralization by 0.1 M HCl. The behavior of radioactive components of the reaction mixture at pH 11 is completely different. We already mentioned, that citrate complex of $\text{TC}^{99\text{m}}\text{Tc}$ has R_f 0.8–1.0 on silica gel in methanol/HCl conc, (99/1). The chromatograms of both mixtures are identical after neutralization. Thus, it is possible to assume, that the citrate-ions substitute molecules of water or hydroxyl-ions in $\text{TC}^{99\text{m}}\text{Tc}$, synthesized at the presence of tartrate-ions. The functional groups of citrate at once are included into coordination core of technetium (I), when carbonylation is carried out in the presence of citrate-ions.

The results of chromatography of reaction mixture containing L-cysteine and sodium citrate are submitted in Figure 3c. At use of L-cysteine as the stabilizing agent in a reaction mixture, on the data of TLC, the complex of $\text{TC}^{99\text{m}}\text{Tc}$ with cysteine is mainly formed, which at the subsequent adding of sodium citrate does not variate the chromatographic behavior. Hydrolyzed forms of technetium-99m are not formed practically both in alkaline, and in neutral mediums. It is possible to conclude, that in the reactions of carbonylation cysteine behaves similarly to citrate, however the chromatographic mobility of complex $\text{TC}^{99\text{m}}\text{Tc}$ -cysteine (R_f 0.3) differs from mobility of complex $\text{TC}^{99\text{m}}\text{Tc}$ -citrate (R_f 0.8–1.0). The results of biological tests of these radiopharmaceuticals confirm this suppose. Both ligands are more “strong” complex agents in relation to tricarbonyltechnetium, as against of tartrate-ions, which capability to supersede water from the coordination core of Tc (I) is doubtfully.

It is possible to assume the various mechanism for carbonylation reactions in acidic and alkaline mediums on the basis of the obtained data. The comparison of TLC and HPLC results for reaction products and artificial mixtures, containing Tc-99, specifies probability of formation PC-^{99m}Tc as an intermediate product upon autoclave carbonylation. PC-^{99m}Tc- chloride turns into TC-^{99m}Tc at dilution (neutralization) of a reaction mixture. The probability of step reduction of technetium in acid medium basically exists (by analogy to the reaction carried out in an alkaline media). So the following sequence of reactions during autoclave carbonylation of technetium in acid medium can be proposed:



In an alkaline media at the presence of tartrate-ions the following reactions probably take place:



If the citrate is introduced into a reaction mixture instead of tartrate, schematically the reaction of carbonylation can be presented as follows:



Three carbonyl groups of citric acid or two carbonyl groups and oxy-group can participate in formation of pentavalent technetium complex with citrate in alkaline media according to.⁵ Therefore it is possible to consider the submitted above formulas symbolic.

We isolated and characterized complex with overall structure $[\text{Tc}(\text{CO})_3(\text{Cit})]$ with tridentate coordination of ligand which have the same R_f in TLC. In the ⁹⁹Tc NMR we observed singlet at -906 ppm and in PMR spectrum we observed complex multiplet, centered at 2.85 ppm, which assigned to

unequivocal methylene groups. This fact along with the data of IR spectroscopy, which show coordination of two COO groups of the ligand and absence of OH stretch, allows us to consider the tridentate coordination of citrate-anion. Thus we can conclude that $^{99m}\text{Tc}(\text{CO})_3^+$ converted to $[\text{Citrato-}^{99m}\text{Tc}(\text{CO})_3]$ at concentration of citrate $5 \times 10^{-3}\text{M}$. Proposed structure is shown below.

Spectral characteristics of $\text{Tc}(\text{CO})_3(\text{Cit})$ complex

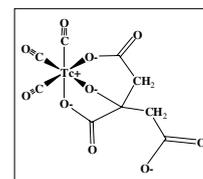
Tc NMP (ppm) -906

¹H NMR (ppm, D₂O) 2.85 (dd, dd (unequiv. CH₂))

IR (cm⁻¹, nujol) 2030, 1905 (ν_{CO}-fac coord.),

1710, 1605 (ν_{COO} coord. stretch),

1550 (ν_{COO} free stretch), ν_{OHval} is absent



Proposed structure

In conclusion we can say, that tricarbonyltechnetium(I) fragment forms strong complexes with charged ligands. The polydentate anions (like citrate anion), which occupies three coordination places in octahedral technetium arrangement forms strongest complexes. So $\text{Tc}(\text{CO})_3$ moiety in aqueous solutions exhibits properties of a typical d-element and a "soft" acid.

Acknowledgements. This work was financially supported by ISTC (Project N 1723). We acknowledge, *TYKO Healthcare (Mallinckrodt)* for the "IsoLink" kits.

References

- (1) R. Alberto and R. Schibli, Patent WO 98/48848, A61K 51/04.
- (2) S. Liu and D. S. Edwards, Chem. Rev. **99**, 2235 (1999).
- (3) N. I. Gorshkov, A. A. Lumpov, and A. E. Miroslavov et al., Radiochemistry, **42**, 213 (2000) (in Russian).
- (4) G. E. Kodina, A. O. Malysheva, and T. A. Babushkina et al., Coordination Chem. **23**, 682 (1996) (in Russian).
- (5) B. Johansson, H. Spice *Technetiumverbindungen Chemische und radiopharmakologische unter-suchungen unter besonderer Berucksichtigung von Technetium-Thiol-Komplexen*, (Rosendorf, 1981), p 213.

