SOLID PHASE REDUCTION OF $^{99m}TcO_4^-$ WITH ZINC : A METHOD FOR THE PREPARATION OF DIFFICULT ^{99m}Tc COMPLEXES

C. Kremer*^, A. León*, D. Gambino^, S. Cartesio^, C. Ures*,

E. Savio*, E. Campos*, E. Kremer^.

*Cátedra de Radioquímica

^Cátedra de Química Inorgánica

Facultad de Química, Gral. Flores 2124

Universidad de la República, Montevideo, Uruguay.

SUMMARY

Two groups of compounds: aminoacids (glutamic acid, glutamine, glycine, lysine) and amines (ethylenediamine, diethylenetriamine, o-phenantroline, o-phenylenediamine) have been labelled with ^{99m}Tc by means of a solid phase reduction using fine powdered zinc as reducing agent. In all cases radiochemical purity in excess of 90% was obtained, with no evidence of colloid formation. Labelling yields were variable depending on the ligand used. The results show that the method presented allows to label with ^{99m}Tc some molecules for which the use of conventional techniques is not feasible.

Key words: ^{99m}Tc-aminoacids, ^{99m}Tc-amines, solid phase reduction, zinc as reducing agent.

INTRODUCTION

The preparation of ^{99m}Tc-labelled radiopharmaceuticals generally requires the initial reduction of pertechnetate anion followed by reaction with a complexing agent. Choice of the

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reducing agent is mainly limited to non-toxic, water-soluble species which do not result in appreciable amounts of ^{99m}Tc-colloid formation (1,2).

The majority of the radiopharmaceutical compounds are labelled with ^{99m}Tc using stannous chloride as reducing agent. However, its use presents two main problems: oxidation and hydrolysis. It has been demonstrated (3) that the presence of oxygen in the mixture of reaction interferes with the reduction of $^{99m}TcO_4^-$, oxidating the Sn(II) to Sn(IV). Also, the Sn(II) could hydrolyse in aqueous solution even at pH values lower than those commonly used for labelling (4).

In addition to these problems TcO_2 formation acts in a competitive way to the production of the complex, particularly in the case of weak chelating agents. It was reported that at pH 4 or higher, the Sn(II) ion and the reduced ^{99m}Tc precipitate, producing a co-colloid (5). This results in products of low radiochemical purity. Moreover, formation of side products may occur, thus affecting the biological distribution of the labelled product.

The problems described above have been found in the labelling with Sn(II) of diamines (6) and glutamic acid (7), ligands of low chelating power. It is concluded that for these ligands a new reducing agent should be found, with lower solubility in the reaction media. This would decrease the concentration of the ions responsible for the production of colloidal species.

Beyond the Radiopharmacy area, zinc has been used by some investigators to obtain reduced forms of Re and Tc (8,9). In this way, zinc was used for the deposition of microquantities of reduced Tc from TcO_4^- as starting material, either in acid or basic media (10) as well as for the TcO_2 synthesis (11).

With this in mind, a solid phase labelling technique has

been developed, where a mixture of $^{99m}TcO_4^-$ and ligand is exposed to zinc powder.

In order to verify the feasibility of this technique and to establish optimum labelling conditions , two different series of ligands were chosen. Both of them included complexing agents of very different ligand capability.

EXPERIMENTAL

A preliminary study was done, in order to determine which variables had to be taken into account to obtain the optimal labelling conditions for both types of complexes studied, ^{99m}Tc-aminoacids and ^{99m}Tc-amines. A fractional factorial design (13) showed that they are: number of moles of chelating agent (n), reaction volume (V), initial pH (pH_o), reaction time (t_r), amount of zinc and ^{99m}Tc activity.

General labelling technique

To a mixture of an aqueous solution of the chelating agent (n moles in V ml at pH_o) and carrier free ^{99m}Tc generator eluate (370 to 540 MBq (10 to 20 mCi)in a maximum volume of 1 ml), 200 mg of zinc powder were added.

This mixture was allowed to react for t_r minutes, stirring on a Vortex mixer for a period not less than 20 minutes and filtered through a membrane filter (0.22 µm pore size). The conditions n, V, pH_o, and t_r were adjusted for each ligand using one of the following methods: fractional factorial design or independent analysis of the influence of each variable.

Conditions were changed until radiochemical purity exceeded 90%, in a reaction time not more than 60 minutes. These were considered optimal conditions.

Radiochemical purity was tested by chromatographic methods. For the detection of $^{99m}TcO_4^-$ and $^{99m}TcO_2$, ascending paper chromatography was performed on Whatman N°1 paper using acetone and 0.9% sodium chloride as developing solvents.

The R_f values obtained with o-phenylenediamine and o-phenantroline complexes led to the search for new chromatographic systems which would allow us to differentiate those complexes from $^{99m}TcO_4^-$ and $^{99m}TcO_2$ respectively.

Table I. Chromatographic data.

Support medium	Solvent	R_f values		
		Tc-complex	TcO_4^-	TcO_2
paper	acetone	0.0	0.9-1.0	0.0
paper	saline	0.8-1.0	0.7-0.8	0.0
paper	BuOH:AcOH:H_2O (2:1:1)	0.9-1.0	0.5-0.6	0.0
TLC	EtOH:toluene (1:1)	0.0	0.5-0.6	0.0

In the case of ^{99m}Tc-o-phenylendiamine, radiochemical purity was checked by thin layer radiochromatography on silica gel Merck 60F 254 (solvent: ethanol:toluene (1:1)). For ^{99m}Tc-o-phenantroline, the choice was ascending paper chromatography (Whatman N°1) with butanol:acetic acid:water (2:1:1) as developing solvent (see Table I).

RESULTS AND DISCUSSION

Previous studies show that the mechanism involved in the process of obtaining the complex implies a first reduction step from TcO_4^- to TcO_2, which would further react with the ligand (14). The O=Tc=O structure is especially suitable to coordinate

with bidentated ligands, forming the final compound with coordination number 6, having two oxo groups in trans position. For this reason bidentated aminoacids and amines were chosen to study the possibilities offered by this method.

Preliminary experiences show that it is convenient to reach the optimal conditions of reaction in two stages. During the first one, an adjustement of the n, t_r, and pH_o variables is done, in order to reach a radiochemical purity not lower than 90%, using reaction times shorter than 60 minutes. During the second stage, the reaction volume is changed, keeping the ligand concentration constant and the maximum value of labelling yield (percentage of activity eluted through the filter) is determined. Table II shows for some particular cases, the influence of the different variables that affect the value of radiochemical purity obtained.

Table II. Influence of the variables on the radiochemical purity.

Compound	n(moles) x 10^5	V(ml)	pH_o (*)	t_r(min)	radiochem. purity(%) (**)
ethylenediamine	9	3.0	10.0	30	62
ethylenediamine	9	3.0	10.0	40	77
ethylenediamine	45	3.0	10.0	30	98
diethylenetriamine	15	2.5	10.0	15	27
diethylenetriamine	15	2.0	10.0	30	56
diethylenetriamine	140	3.0	4.5	30	93
diethylenetriamine	140	3.0	1.5	30	83
glycine	36	6.0	5.2	48	90
glycine	36	6.0	7.8	48	82
glycine	36	6.0	2.5	48	82

Although the amount of zinc is considered as a variable to be taken into account to determine the optimal labelling conditions, its mass is mantained at 200 mg in all cases after being adjusted for the labelling of glutamic acid (12). Under

*) pH does not remain constant during the reaction.

**) Mean values of at least five equivalent experiences.

similar conditions, smaller amounts of zinc decrease radiochemical purity.

In order to obtain appropiate values for further quality control by biodistribution studies, ^{99m}Tc activity is maintained between 370 MBq (10 mCi) and 540 MBq (20 mCi) in no more than 1 ml.

An increase in number of moles of ligand favors the formation of the complex (see ethylenediamine, Table II). This variable is especially important in the case of weak chelating agents. However, maximum ligand concentration is limited by its toxic dose.

With regard to the influence of initial pH in the system, it is important to point out that a pH increase favors formation of zinc hydroxide, lowering metallic zinc effective surface.

Having in mind the mechanism proposed for the reaction, it is not convenient to work at very low pH values, wich can prevent the formation of TcO_2. For the studied compounds, an increase in pH produces better conditions for the reaction, because it diminishes the concentration of protonated forms of the ligand. This evidence leads to the conclusion that the best pH_o for labelling in each case results from a compromise between these factors. The values found for glycine are a clear example of this fact.

As far as the reaction time is concerned, its increase is followed by a higher radiochemical purity. Table II shows this influence for ethylenediamine and diethylenetriamine.

Chromatographic controls show that even in those experiences in which low radiochemical purities are obtained, there are no appreciable amounts of Tc detected in colloidal form. This is also verified by electrophoresis (14, 15).

Once the radiochemical purity and reaction time are set within acceptable values, a second stage is performed changing

reaction volume in order to optimize labelling yield. For instance, increasing the volume by a factor of three, a fivefold increase of labelling yield is obtained.

The summary of optimal conditions for the studied ligands, presented in Table III, allows an evaluation of the possibilities offered by this labelling method.

Table III. Optimal conditions of the ^{99m}Tc-labelling of different substrates.

Compound	n(moles) x10^4	V(ml)	pH_o	t_r (min)	final pH
ethylenediamine	4.5	3.0	10.0	30	10.0
diethylenetriamine	14.0	3.0	4.5	30	7.0
o-phenantroline	3.7	3.0	4.5	30	7.0
o-phenyldiamine	2.2	3.0	1.5	45	5.8
L(-) glutamic acid	4.5	6.0	3.5	45	6.0
L(-) glutamine	4.1	3.5	3.0	60	3.5
L(+) lysine	4.5	5.5	5.9	50	8.0
glycine	4.5	6.5	5.1	45	7.2

In every case, radiochemical purities are high, being irrelevant the capacities as ligand of the amine or the aminoacid (see Table IV.).

Table IV. Total radiochemical yield and purity of the ^{99m}Tc-labelling of different substrates in optimal conditions.

Compound	radiochem. purity(%)	labelling yield(%)
ethylenediamine	98	39
diethylenetriamine	93	13
o-phenantroline	96	20
o-phenylenediamine	98	12
L(-) glutamic acid	90	23
L(-) glutamine	95	12
L(+) lysine	90	11
glycine	90	16

Elution yields are usually low, because an important fraction of total activity remains adsorbed on zinc powder surface. Best results are obtained with ethylenediamine among amines and with glutamic acid within the aminoacids. It is worthy

to note that the former, because of its size and molecular shape, and the latter, because of its anionic character at optimal labelling conditions, form Tc complexes of high thermodynamic stability. However, it is not possible to relate in a simple way the elution yields and capacity of different compounds to act as ligands. There are several variables that also have influence on the final result, including ligand concentration and pH of labelling.

Having in mind to develop future studies on the effects of ligand size, lipophilicity and pKa of the ligands correlated with the biodistribution of the ^{99m}Tc-labelled amines, preliminary studies with the simplest member of the group (^{99m}Tc-ethylenediamine) were made. This complex is cleared from the blood mainly by renal accumulation and excretion. It is also observed hepatic uptake and biliary excretion, in a less percentage. The diagnostic potential of this group of ^{99m}Tc complexes will be investigated in a future.

The biodistribution profile in animals and the possibilities of in vivo applications have been tested for ^{99m}Tc labelled aminoacids. Some of them have been assayed in normal mice and wistar rats. Different aminoacids show similar biodistribution profiles. It is discussed in detail for glutamic acid in a previous paper (14), together with stability and toxicity tests as well as clinical studies in humans.

CONCLUSIONS

For the two groups of molecules studied, both capable of acting as bidentate ligands, the solid phase labelling technique using zinc as reducing agent results in ^{99m}Tc complexes with radiochemical purity in excess of 90%. These compounds, difficult to label by conventional techniques, are formed in this way free of colloidal components. The method used is useful as an

alternative labelling technique, mainly to synthetise complexes for which the use of conventional techniques is not feasible.

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