Asian Journal of Chemistry; Vol. 26, No. 13 (2014), 4027-4030

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17705

Comparative Studies on Experimental Procedures for Labeling of Tc-99m with L-Aspartic Acid†

YEONG-JAE PARK[1,*], SUNG-A KANG[2], JEI-WON YEON[3] and KI-JIN KIM[4]

[1]Department of Biotechnology, Hoseo University, 79-20 Hoseo-ro, Baebang-eup, Asan-si, Chungcheongnam-do 336-795, Republic of Korea
[2]R & D Center, Envirokorea Co., Ltd, 167-10, Techno 2, Yuseong-gu, Daejeon 305-500, Republic of Korea
[3]Nuclear Chemistry Research Division, Korea Atomic Energy Research Institute, P.O. Box 105, Yuseong-gu, Daejeon 305-353, Republic of Korea
[4]Konyang University Hospital, 685 Gasuwondong Seo-gu, Metropolitan city, Daejeon, Seoul 302-718, Republic of Korea

*Corresponding author: Fax: +82 42 934-2343; E-mail: nyjpark@hoseo.edu

Published online: 23 June 2014; AJC-15400

For the labeling of Tc-99m radioisotope with L-aspartic acid (ASP), six different procedures were designed. The key points to the procedures are focused on the phase (solid or solution) used of major reactants (L-aspartic acid sodium salt monohydrate and $SnCl_2$) and the pH of reacting solution. Labeling experiments were carried out according to the procedures at conditions; the concentration ratio ([ASP]/[$SnCl_2$]) = 35-45, temperature = 80 ºC, radioactivity of ^{99m}Tc = 5 mCi, reaction time = 1 h. The labeling was investigated by radio-ITLC using methylethyl keton (MEK) and 0.9 % saline solution as solvents, respectively. The labeling yields were determined from the radioactivity distributions in the two solvents. Solid typed $SnCl_2$ gave better yields than solution typed $SnCl_2$. While, regardless of whether L-aspartic acid sodium salt monohydrate was used as solid or solution, there was found a negligible effect on ^{99m}Tc labeling. The pH dependence on the ^{99m}Tc labeling with L-aspartic acid sodium salt monohydrate was found. The labeling yields were found higher in the following order; weakly acidic and neutral (pH 5-7) > alkaline (pH 10) > acidic (pH 4) media. The best yield (*ca.* 90 %) was obtained from the procedure that both L-aspartic acid sodium salt monohydrate and $SnCl_2$ were used as solid without adjustment of pH, providing a good method for the preparation of pharmaceutical kits using L-aspartic acid sodium salt monohydrate.

Keywords: Labeling, Procedures, Tc-99m, L-Aspartic acid, Radio-ITLC.

INTRODUCTION

Radiopharmaceuticals including a radionuclide are widely used in nuclear medicine, for both diagnostic and treatment purposes. Tc-99m is currently the most commonly used radionuclide. Research on new molecules for labeling radioisotope Tc-99m has been growing steadily, stimulated by the demand for new medical application. 5-Nitroimidazole is reported to be a well-established group of antiprotozoal and antibacterial agents[1]. This molecule can interact with hypoxia tissue and hypoxia cellular target. We are now interested in its application to radiopharmaceuticals using 5-nitroimidazole derivatives. Since 5-nitroimidazole itself is not willing to chelate with ^{99m}Tc species, it needs a certain ligand to chelate with ^{99m}Tc species. Here, 5-nitroimidazole can be a driving engine for hypoxia cellular target and a ligand is a ^{99m}Tc-transporting car. Prior to the combination of a derivative of 5-nitroimidazole with a ligand, it is necessary to investigate ^{99m}Tc-transporting abilities of the ligand. L-aspartic acid, a kind of amino acids, will be such a good ligand to chelate with ^{99m}Tc species.

A pertechnetate anion (TcO_4^-) occupying the highest oxidation state (VII) does not bind directly to any ligand since its chemical reactivity is negligible. Thus, for the production of Tc-99m pharmaceuticals, reduction to lower oxidation states in the presence of a suitable ligand is a prerequisite for the synthesis of ^{99m}Tc-labeled molecules. For this reduction, $SnCl_2$ is commonly used as reducing agent.

The labeling of ^{99m}Tc with L-aspartic acid involves two reactions, reduction of $^{99m}TcO_4^-$ by $SnCl_2$ and chelation of the reduced ^{99m}Tc(V) species ($^{99m}Tc{=}O^{3+}$) with amino- and carboxyl- groups in the aspartic acid. These reactions occur at the same time during the labeling, making it very difficult to find reaction conditions for better labeling yields. The coordination chemistry of technetium is important to investigate the chelation behaviour with ligand. The structure of technetium complexes, depending on ligand, can be characterized by the coordination number (N), which can vary from 4-7, allowing tetrahedral (N = 4), tetragonal pyramidal (N = 5), octahedral (N = 6), capped octahedral (N = 7) or pentagonal bipyramidal (N = 7) geometry[2,3]. Pertechnetate anion (N = 4) is the most

†Presented at 5th International Symposium on Application of Chemical and Analytical Technologies in Nuclear Industries (Nu-ACT 2013), Daejeon, Korea

stable form of technetium in aqueous media. When pertechnetate ions are reduced in the presence of ligand, they usually do not release all the oxygen atoms, leading to complexes in which a $Tc{=}O^{3+}$ or a TcO_2^+ core is identified. Complexes containing a $Tc{=}O^{3+}$ core show an octahedral six-coordinated or a square pyramidal five-coordinated spatial configuration. Chemically, the labeling of Tc-99m with ligands requires the presence of metal-containing chemical cores that are stable enough to exist in aqueous solutions and versatile enough to bind with selected functional groups belonging to the ligand.

In the earlier study, ^{99m}Tc labeling experiments with L-aspartic acid were conducted with varying concentration ratios ([ASP]/[$SnCl_2$]), temperature, reaction time and pH. From these experiments, the best labeling yield (95 % at 90 min) was obtained at the conditions; 80 ºC, [ASP]/[$SnCl_2$] = 45 and pH 6. At that time, both ASP and $SnCl_2$ were used in forms of solid and pH was not adjusted. This method will be applied to procedure #1 in this study and the reproducibility will be examined. Considering the preparation of ^{99m}Tc-labeled pharmaceutical kits, the effects of phase used of reactants such as L-aspartic acid sodium salt monohydrate and $SnCl_2$ and pH adjustment on the Tc-99m labeling are investigated at a fixed reaction time.

The aim of this study is to investigate the chelation behaviour of L-aspartic acid with ^{99m}Tc(V) species using six different procedures and to compare the labeling yields at the reaction time of 1 h and finally to find the best method for the labeling. This study may contribute to the preparation of pharmaceutical kits.

EXPERIMENTAL

L-Aspartic acid sodium salt monohydrate (ASP) was obtained from SIGMA; stannous chloride($SnCl_2{\cdot}2H_2O$) from SIGMA-ALDRICH; 0.9 % saline from JW pharmaceutical; methyl ethyl ketone (MEK) from MERCH. These reagents were used without further purification. $^{99m}TcO_4^-$ was taken from a commercial ^{99}Mo/^{99m}Tc generator (Mallinckrodt) and 5 mCi of ^{99m}Tc was used for each experiment.

^{99m}Tc-labeling experiments: Six different procedures for ^{99m}Tc labeling with ASP were examined at the conditions following as: the concentration ratio of aspartic acid to stannous chloride = 35-45:1; reaction temperature = 80 ºC; pH was adjusted if necessary. Total volume of solution was almost 1.1 mL.

Prior to the ^{99m}Tc labeling with L-aspartic acid, the reduction behaviours of $^{99m}TcO_4^-$ in the absence of ASP were investigated at 80 ºC without and with $SnCl_2$ ($>10^5$ than [^{99m}Tc]). ITLC-SG (Merck) plates for the reduction of $^{99m}TcO_4^-$ were obtained using MEK and 0.9 % NaCl solution as mobile phase, respectively and then the distributions of radioactivity on the developed and dried chromatographic plates were analyzed using linear gamma Radio-TLC Scanner (BioScan).

The labeling of ^{99m}Tc with ASP for each procedure was investigated. ITLC-SG (Merck) plates corresponding to each procedure were obtained and analyzed by Radio-TLC scanner using methyl ethyl ketone and saline solution, respectively. In methyl ethyl ketone, colloidal Tc species and Tc complex remained at the origin (R_f = 0.0-0.2); free $^{99m}TcO_4^-$ migrated with the solvent front(SF) (R_f = 0.8-1.0), while in saline, free $^{99m}TcO_4^-$ and Tc complex migrated with SF; colloidal Tc species remained at the origin.

From the activity distributions (% area) obtained using two solvents, percentages of Tc-complex, colloidal Tc species and free $^{99m}TcO_4^-$ at the reaction time of 1h were determined.

Procedure #1: A powdered 1.5 mg of $SnCl_2{\cdot}2H_2O$ and a powdered 40.7 mg of ASP were put into a vial, 5 mCi of $^{99m}TcO_4^-$ solution (0.1 mL) added and finally doubly deionized water was added until total volume was 1.1 mL. At that time, the pH of solution was measured. This sample solution was left to react at 80 ºC for 1 h. The pHs of sample after 1 h and 17 h were measured.

Procedure #2 : A solution of ASP was prepared from dissolving a powdered 48.3 mg of ASP in 1 mL of doubly deionized water. A powdered 1.8 mg of $SnCl_2{\cdot}2H_2O$ was put into a vial, followed by an addition of 1 mL of ASP solution prepared in advance and then 5 mCi of $^{99m}TcO_4^-$ solution (0.1 mL) was added. The total volume was almost 1.1 mL. At that time, the pH of solution was measured. This sample solution was left to react at 80 ºC for 1 h. The pHs of sample after 1 and 17 h were measured.

Procedure #3: A solution of ASP was prepared from dissolving a powdered 53.8 mg of ASP in 1 mL of doubly deionized water and the pH of this solution was adjusted to pH 10 by adding NaOH solution drop by drop. 5 mCi of $^{99m}TcO_4^-$ solution (0.1 mL) was added. And then, a powdered 2.0 mg of $SnCl_2{\cdot}2H_2O$ was put into the solution, followed by measurement of pH. This sample solution was left to react at 80 ºC for 1 h. The pHs of sample after 1 h and 17 h were measured.

Procedure #4: A solution of ASP was prepared from dissolving a powdered 42.8 mg of ASP in 1 mL of doubly deionized water and the pH of this solution was adjusted to pH 10 by adding NaOH solution drop by drop. 5 mCi of $^{99m}TcO_4^-$ solution (0.1 mL) was added. And then, a solution of $SnCl_2$ containing 1.3 mg of $SnCl_2{\cdot}2H_2O$, dissolved in HCl solution, was added drop by drop to the solution, followed by measurement of pH. This sample solution was left to react at 80 ºC for 1 h. The pHs of sample after 1 h and 17 h were measured.

Procedure #5: A solution of ASP was prepared from dissolving a powdered 48.3 mg of ASP in 1 mL of doubly deionized water and the pH of this solution was adjusted to pH 11 by adding NaOH solution drop by drop. 5 mCi of $^{99m}TcO_4^-$ solution (0.1 mL) was added. And then, a powdered 1.6 mg of $SnCl_2{\cdot}2H_2O$ was added to the solution, followed by measurement of pH. This sample solution was left to react at 80 ºC for 1 h. The pHs of sample after 1 h and 17 h were measured.

Procedure #6: A solution of ASP was prepared from dissolving a powdered 39.4 mg of ASP in 1 mL of doubly deionized water and the pH of this solution was adjusted to pH 10 by adding NaOH solution drop by drop. 5 mCi of $^{99m}TcO_4^-$ solution (0.1 mL) was added. And then, a solution of $SnCl_2$ containing 1.2 mg of $SnCl_2{\cdot}2H_2O$, dissolved in doubly deionized water, was added drop by drop and the pH of final solution was measured and adjusted to pH 10. And then, the sample solution was left to react at 80 ºC for 1 h. The pHs of sample after 1 h and 17 h were measured.

TABLE-1
SUMMARY OF SIX EXPERIMENTAL PROCEDURES TOGETHER WITH pH VALUES MEASURED

Name of procedure	1st reactant	2nd reactant	3rd reactant	Initial pH	pH adjusted	pH after 1 h	pH after 17 h
#1	ASP(s)	$SnCl_2$(s)	$^{99m}TcO_4^-$	5-6	Not	5-6	5
#2	$SnCl_2$(s)	ASP(aq) pH 4-5, not adjusted	$^{99m}TcO_4^-$	5	Not	6	5
#3	ASP(aq) pH 10, adjusted	$^{99m}TcO_4^-$	$SnCl_2$(s)	6-7	Not	7	7
#4	ASP(aq) pH 10, adjusted	$^{99m}TcO_4^-$	$SnCl_2$(aq)dissolved in acid	4	Not	4	4
#5	ASP(aq) pH 11, adjusted	$^{99m}TcO_4^-$	$SnCl_2$(s)	10	Not	10	10
#6	ASP(aq) pH 10, adjusted	$^{99m}TcO_4^-$	$SnCl_2$(aq) dissolved in acid	7	10	10	10

Note: (s) and (aq) represent reactants which are used as solid and aqueous solution, respectively.

RESULTS AND DISCUSSION

Reduction of $^{99m}TcO_4^-$ with and without stannous chloride: In the absence of stannous chloride, free $^{99m}TcO_4^-$ migrated with SF in both methyl ethyl ketone and saline. In the presence of stannous chloride, reduced colloidal Tc species remained at the origin in both methyl ethyl ketone and saline. In the earlier study, for the reduction of $^{99m}TcO_4^-$ at 80 ºC, colloidal Tc species was rapidly increased with increasing reaction time, reaching about 90 % in 10 min and the reduction was completed in 1 h.

^{99m}Tc labeling with L-aspartic acid according to six procedures: Six procedures for the labeling of ^{99m}Tc with L-aspartic acid are briefly described, together with pH values measured at given times (Table-1). The key points to the procedures are focused on the phase (solid or solution) used of reactants (ASP and $SnCl_2$) and the effect of pH and will be described later in details. It was found in all the experiments that conspicuous changes in pH of solutions before and after reactions were not observed, although each procedure gave quite a different yield of ^{99m}Tc labeling. The ^{99m}Tc labeling yields obtained from the execution of six procedures are displayed (Table-2). Procedure #1 provided the best yield (90 %), while procedure #4 did the worst one (53 %). A few procedures were executed prolonging reaction time up to 90 min, showing that the labeling yields were slowly increased (not shown here). Especially, Procedure #1 gave the same yield (95 %) as in the earlier study at the reaction time of 90 min, exhibiting the reproducibility.

TABLE-2
YIELDS OF ^{99m}Tc LABELING WITH L-ASPARTIC ACID AT REACTION TIME OF 1 h

Procedure	#1	#2	#3	#4	#5	#6
[ASP]/[$SnCl_2$]	35.4	35	35.3	44.4	40.0	43.0
Yield (%)	90.2	88.1	72.4	53.1	69.6	60.1

Use of solid or solution typed reactants: From all the experiments, it was found that solid-typed $SnCl_2$ (#1, #2, #3 and #5) gave better yields for the labeling than solution-typed $SnCl_2$ (#4 and #6). These results may be explained by means of hydrolysis of $SnCl_2$ which leads to a decrease of its ability to reduce TcO_4^- ions. When $SnCl_2$ is used as solid, it slowly dissolves by an amount corresponding to its solubility at surrounding pH and rightly reduces TcO_4^- before hydrolysis. On the other hand, in the case that $SnCl_2$ is used as solution, when Sn^{2+} ions are placed in alkaline media, they are apt to hydrolyze, leading to a decrease in their ability to reduce TcO_4^-. As such examples, there seem procedures #3 and #4. In the case of procedure #4 using solution-typed $SnCl_2$, Sn^{2+} ions are directly facing an alkaline solution of ASP (pH 10); the hydrolysis of Sn^{2+} ions occurs favourably over the reduction of TcO_4^-, leading to the worst labeling yield (53 %). While, procedure #3 using solid-typed $SnCl_2$ gave the 3rd good labeling yield (72 %) in spite of encountering an alkaline solution of ASP (pH 10). This can be explained by pH values measured after 1h in procedures #3 and #4. The hydrolysis of Sn^{2+} ions lead to an acidic media ($Sn^{2+} + H_2O = SnOH^+ + H^+$). The pH values after 1h were 7 for #3 and 4 for #4, respectively and their pH maintained constant even after 17 h. This indicates that Sn^{2+} ions, slowly dissolving from solid (for #3), mainly reduce TcO_4^-, while Sn^{2+} ions originating from solution (for #4) mainly hydrolyze.

It was investigated which typed ASP is more effective for the labeling. For procedures #1 and #2, the experimental conditions were almost the same except that ASP was used as solid for #1 and solution for #2, respectively. Those labeling yields were almost the same. Hence, it doesn't matter which type of ASP is used for the labeling.

Effect of pH: L-aspartic acid contains two carboxyl- and one amino- groups. The pK_a value is 2.10 for carboxylic acid attached to α-carbon, 3.86 for carboxylic acid in the side chain and 9.82 for α-amino, respectively[4]. ^{99m}Tc labeling with ASP is done through the chelation of $^{99m}Tc{=}O^{3+}$ ions with the functional groups above-mentioned. In this study, three typical pH ranges are targeted; acidic (pH 4), weakly acidic and neutral (pH 5-7) and alkaline (pH 10-11). At pH 4, the carboxylic acids attached to α-carbon are almost unprotonated, but the carboxylic acids in the side chain are half-unprotonated. This means that L-aspartic acids to chelate with $^{99m}Tc{=}O^{3+}$ ions are not enough in acidic media. Procedure #4 belongs to this case and gave the worst yield (53 %) (Table-2). In the neutral pH ranges, all the carboxylic acids in ASP are fully unprotonated and negatively charged, driving chelations. Procedures #1, #2 and #3 targeting weakly acidic and neutral pH ranges gave high labeling yields, compared to other procedures. At pH 10, all the carboxylic acids in ASP are fully unprotonated and negatively charged and the amino groups in ASP exist as a mixture of $-NH_2$ and $-NH_3^+$. The circumstance for chelation at pH 10 is expectedly favoured over that at neutral pH. That is, non-bonding electrons of nitrogen atom in the $-NH_2$ group as well as two unprotonated carboxylic groups can participate in coordinating to $^{99m}Tc{=}O^{3+}$ ions. Unexpectedly, the labeling yield (70 %) for procedure #5 at pH 10 was lower than that obtained in the neutral pH range. This is thought to be due to a decrease in reduction of free TcO_4^- ions by hydrolysis of

$SnCl_2$ at high pH. From comparison of procedures #5 and #6, it is interesting to note that ^{99m}Tc labeling yield is affected by the phase used of $SnCl_2$. Unlike procedure #5 using solid typed $SnCl_2$, procedure #6 (adjusted to pH 10) using solution typed $SnCl_2$ gave a worse yield (60 %) than procedure #5. This indicates the hydrolysis effect of $SnCl_2$ at high pH, additionally depending on the phase used of $SnCl_2$ even at the same pH.

Conclusion

^{99m}Tc labeling with L-aspartic acid was dependant on pH. The labeling yields were found higher in the following order; weakly acidic and neutral (pH 5-7) > alkaline (pH 10) > acidic (pH 4) media. Solid-typed $SnCl_2$ gave better labeling yields than solution-typed $SnCl_2$, whereas the phase (solid or solution) of ASP used had no effect on ^{99m}Tc labeling. The best labeling yield (90 % at 1h) was obtained from the procedure that both ASP and $SnCl_2$ were used as solids without adjustment of pH.

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