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Study on Labeling of Tc-99m with L-Aspartic Acid†

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A L-aspartic acid was used as a chelating ligand for labeling Tc-99m. Labeling experiments of Tc-99m with L-aspartic acid were carried out at varying temperature, time, pH and concentration ratio of L-aspartic acid to $SnCl_2$. For the effects of temperature (25, 40, 60 and 80 ºC) and pH (2, 6-7, 10) on the labeling, the best results were obtained at 80 ºC and in the neutral pH range, respectively. For the effect of concentration ratio of L-aspartic acid to $SnCl_2$ (15, 30, 45, 80 and 100), the best one was obtained at 45. The labeling of Tc-99m was investigated by radio-ITLC using methyl ethyl ketone and 0.9 % saline solution as solvents. The method to determine percentages of Tc species formed at given reaction conditions was described. The best result for the labeling was obtained at 80 ºC in the neutral pH range with the concentration ratio of aspartic acid to $SnCl_2$ of 45. At those conditions, the extent of labeling reached 90 % for 1 h and 95 % for 90 min.

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

INTRODUCTION

Radiopharmaceuticals including a radionuclide are widely used in nuclear medicine, for both diagnostic and treatment purposes. Among radionuclides, Tc-99m is currently the most commonly used radionuclide to SPECT (single photon emission computed tomography), because of its ideal nuclear properties (half-life = 6 h, γ-particle energy = 140 keV), availability and relatively low cost[1]. 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. During the reduction by $SnCl_2$ commonly used, the ligand stabilizes the lower oxidation states, otherwise, colloidal TcO_2 is formed in aqueous media[2]. The formation of ^{99m}Tc-labeled complex depends on the stability of the complex itself[3]. It depends on the ligand in which oxidation state of technetium a complex will be stabilized. The coordination chemistry of technetium is attracting much attention due to the radionuclide-based application in radiopharmaceuticals. The structure of technetium complexes can be characterized by the coordination number (N), which can vary from 4 to 7, allowing geometries; tetrahedral (N = 4) for Tc(VII), tetragonal pyramidal (N = 5) for Tc(V), octahedral (N = 6) for Tc(I, III, IV, V), capped octahedral (N = 7) for Tc(III) or pentagonal bipyramidal (N = 7) for Tc(V)[4]. The pertechnetate anion, when reduced in the presence of ligand, usually does not release all the oxygen atoms, leading to complexes in which a $Tc{=}O^{3+}$ or a TcO_2^+ core is identified[5]. Complexes containing a $Tc{=}O^{3+}$ core show an octahedral six-coordinated or a square pyramidal five-coordinated spatial configuration; complexes containing a TcO_2^+ core form octahedral six-coordinated complexes. Chemically, the labeling of Tc-99m with ligands requires not only the presence of $Tc{=}O^{3+}$ or TcO_2^+ core through the reduction of $^{99m}TcO_4^-$ by stannous chloride, but also its chelation ability to bind with selected functional groups belonging to the ligand. Reduction and chelation should occur at the same time for the labeling, making it difficult to find reaction conditions for a high labeling yield. 5-Nitroimidazoles are a well-established group of antiprotozoal and antibacterial agents[6]. We are recently interested in the aminoethyl-2-methyl-5-nitroimidazole, which can interact with hypoxia tissue and hypoxia cellular target. Since this molecule is not easy to chelate with ^{99m}Tc species for itself, it needs to combine with some ligand to chelate with ^{99m}Tc species. L-aspartic acid, a kind of amino acids, will be such a good ligand to chelate with ^{99m}Tc species.

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The aim of this study is to investigate the labeling of ^{99m}Tc radioisotope with L-aspartic acid. The effects of temperature, reaction time, pH and the concentration ratio of L-aspartic acid to stannous chloride are investigated.

EXPERIMENTAL

L-Aspartic acid sodium salt monohydrate (ASP) was obtained from SIGMA; stannous chloride($SnCl_2 \cdot 2H_2O$) from SIGMA-ALDRICH; saline from JW pharmaceutical; methyl ethyl keton (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.

Preparation of samples: Three sets of samples were prepared: Five different concentration ratios of L-aspartic acid sodium salt monohydrate to stannous chloride (15:1, 30:1, 45:1, 80:1, 100:1) at constant conditions of 80 ºC and pH 6-7. Three different pHs (2, 6-7, 10) at constant conditions of 80 ºC and [ASP]: [stannous chloride] = 45:1 Four different reaction temperatures (25, 40, 60, 80 ºC) at constant conditions of pH 6-7 and [ASP] : [stannous chloride] = 45:1.

^{99m}Tc- labeling experiments: First, reduction behaviours of $^{99m}TcO_4^-$ alone were investigated without and with stannous chloride($>10^5$ than [^{99m}Tc]), varying reaction temperature and time. ITLC-SG (Merck) strips/plates for the reduction of $^{99m}TcO_4^-$ were obtained using methyl ethyl ketone and 0.9 % NaCl solution as mobile phase and the distributions of radioactivity on the developed and dried chromatographic plates were analyzed using linear gamma Radio-TLC Scanner (BioScan). For the labeling of $^{99m}TcO_4^-$ with L-aspartic acid, appropriate amounts of L-aspartic acid (tens mg) and stannous chloride (1-2 mg), required for a given concentration ratio, were put in a vial. 5 mCi of $^{99m}TcO_4^-$ solution was added to each vial containing L-aspartic acid and stannous chloride. Total volume of 1.2 mL was maintained by adding 0.9 % saline solution if necessary. When pH adjustments were needed, a small amount of NaOH or HCl was added. Given reaction temperatures were kept to be constant using a water bath. At every given reaction time, ITLC-SG plates for reaction samples were obtained and then their distributions of radioactivity were analyzed. In the analysis of distributions of radioactivity from ^{99m}Tc labeling experiments, it is generally accepted that in methyl ethyl ketone, reduced, hydrolyzed ^{99m}Tc species (colloidal Tc) 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 remained at the origin[5].

RESULTS AND DISCUSSION

Reduction of $^{99m}TcO_4^-$ by $SnCl_2$ in the absence of ligand: By stannous chloride, $^{99m}TcO_4^-$ is reduced to Tc(V) and further to colloidal $Tc^{IV}O_2$ in the absence of ligand. In this study, in the absence of $SnCl_2$, free $^{99m}TcO_4^-$ migrated with SF in both methyl ethyl ketone and saline (Fig. 1(b)), whereas in the presence of $SnCl_2$, colloidal Tc remained at the origin in the both solvents (Fig. 1(a)). For the reduction of $^{99m}TcO_4^-$ at 80 ºC, colloidal Tc species was rapidly increased with time, reaching 90 % in 10 min and the reduction was completed in 1 h. The

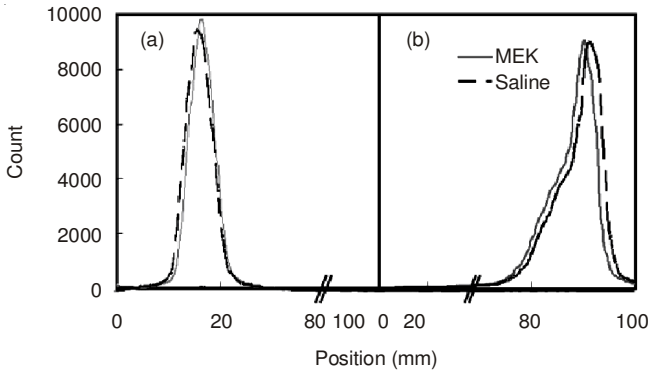

Fig. 1. ITLC of (a) colloidal Tc with $SnCl_2$ (b) $^{99m}TcO_4^-$ without $SnCl_2$, using two different solvents, methyl ethyl ketone (MEK) and saline. Conditions: 0.5 h; 80 ºC

effect of temperature on the reduction was not significant, resulting from $[SnCl_2]/[^{99m}Tc] > 10^5$.

Determination of the extent of ^{99m}Tc labeling: The extent of ^{99m}Tc-complex was determined from the distribution of radioactivity analyzed by Radio-TLC scanner using methyl ethyl ketone and saline solution, respectively. As a typical example for Tc-complex (Fig. 2), in methyl ethyl ketone, both colloidal Tc species and Tc-complex remained at the origin; a little bit of free $^{99m}TcO_4^-$ migrated with SF (R_f = 0.8-1.0). On the other hand, in saline, colloidal Tc species remained at the origin; Tc-complex and free $^{99m}TcO_4^-$ ions migrated with SF (R_f = 0.8-1.0). From the activity distributions (% area) obtained using two solvents, percentages of Tc-complexes, colloidal Tc and free $^{99m}TcO_4^-$ ions were determined.

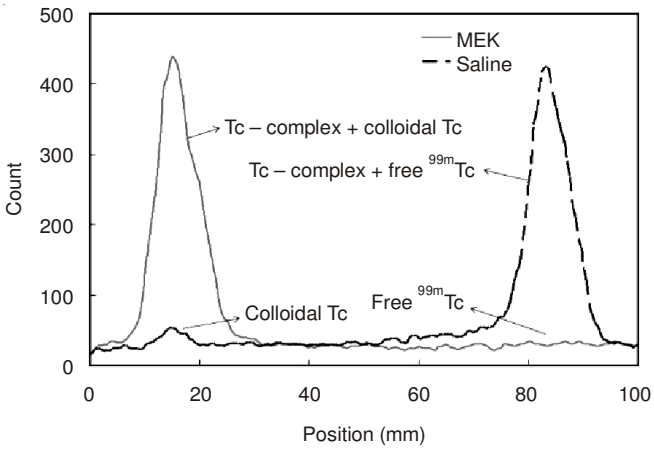

Fig. 2. ITLC of ^{99m}Tc species obtained from labeling experiment using methyl ethyl ketone (MEK) and saline. Conditions: 90 min; 80 ºC; pH 6-7; $[ASP]/[SnCl_2]$ = 45

Effect of concentration ratio ($[ASP]/[SnCl_2]$) on the labeling of ^{99m}Tc: On labeling ^{99m}Tc with L-aspartic acid sodium salt monohydrate, it is very important that the Tc(V) reduced by $SnCl_2$ should not be further reduced to lower oxidation states of technetium. L-Aspartic acid sodium salt monohydrate plays a key role in stabilizing Tc(V) by complexation, not being further reduced. For this reason, the concentration ratio of L-aspartic acid sodium salt monohydrate to $SnCl_2$ is expected to be a very important factor to obtain a higher yield of Tc-complex. The results from experiments carried out with the varied concentration ratios at 80 ºC and at

pH 6-7 show that the best yield of ^{99m}Tc-labeling was obtained at the ratio (45:1) (Fig. 3). This gives satisfactory information for preparing a radiopharmaceutical kit when L-aspartic acid is used as a ligand for labeling ^{99m}Tc.

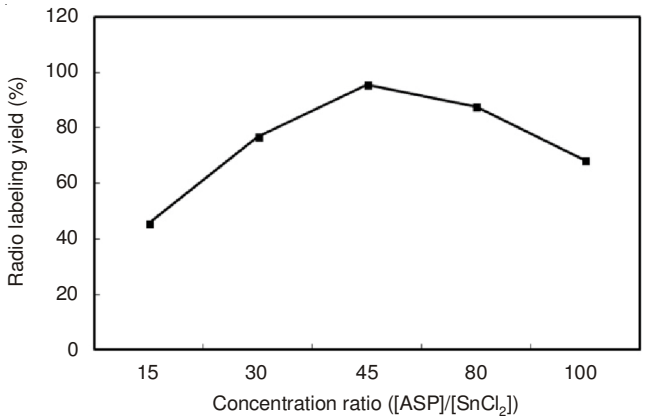

Fig. 3. Percentage of 99mTc-complex *vs.* concentration ratio([ASP]/[$SnCl_2$]). Conditions: 5 mCi; pH 6-7; 90 min; 80 ºC

The extent of ^{99m}Tc labeling at the ratio ([ASP]/[$SnCl_2$]) of 45 was also obtained with a varied reaction time using the conditions; 80 ºC, pH 6-7 (Table-1).

TABLE-1
EXTENT OF ^{99m}Tc LABELING OBTAINED WITH A VARIED REACTION TIME

Reaction time (min)	Colloidal Tc (%)	Free $^{99m}TcO_4^-$ (%)	Tc-Complex (%)
30	43.2	0	56.8
60	9.4	0	90.6
90	4.4	0	95.6

Effect of temperature on the labeling of ^{99m}Tc with L-aspartic acid: Labeling experiments were carried out varying temperature at [ASP]/[$SnCl_2$] = 45:1 in the neutral pH range. At a given temperature, the extent of labeling was investigated at reaction times of 30 and 90 min. The results show that the higher temperature is in the temperature range investigated (25-80 ºC), the more Tc-complex is formed. The extent of ^{99m}Tc labeling obtained at each temperature is displayed (Table-2).

Effect of pH on the labeling of ^{99m}Tc with L-aspartic acid: The effect of pH on ^{99m}Tc labeling was investigated at pH 2, 6 and 10 using the conditions of 80 ºC, [ASP]/[$SnCl_2$] = 45:1 and reaction time = 90 min. The labeling yields were obtained 31 % at pH 2, 54 % at pH 10 and 95 % in the neutral pH range (pH 6-7), respectively (Table-3). 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[7]. It is expected that deprotonation of L-aspartic acid sodium salt monohydrate is essential for chelation with Tc(V) ions with a $Tc{=}O^{3+}$ or a TcO_2^+ core. In the neutral pH range, two carboxylic groups are almost unprotonated and negatively charged, being able to chelate with Tc(V) ions. At pH 2, the carboxylic groups in the side chain are not unprotonated, the carboxyl groups attached to α-carbon half-unprotonated and amino groups protonated ($-NH_3^+$). This makes it difficult for L-aspartic acid sodium salt monohydrate to chelate with Tc(V) ions. At pH 10, two carboxylic groups are fully unprotonated and the amino group exists as a mixture of $-NH_2$ and $-NH_3^+$. The circumstance at pH 10 for chelation is expectedly favoured over that at neutral pH; at pH 10, non-bonding electrons of nitrogen atom in the $-NH_2$ group as well as two unprotonated carboxylic groups can coordinate to Tc(V) ions. However, the labeling yield (54 %) at pH 10 was much lower than that (95 %) at neutral pH. The reason is thought to be due to a decrease in reduction of free $^{99m}TcO_4^-$ by hydrolysis of $SnCl_2$ at high pH. This can be well explained by the experimental data that free $^{99m}TcO_4^-$ remains by a portion of 39 % yet at pH 10 (Table-3).

TABLE-3
EXTENT OF ^{99m}Tc LABELING OBTAINED WITH A VARIED pH

pH	Colloidal Tc (%)	Free $^{99m}TcO_4^-$ (%)	Tc-Complex (%)
2	28.5	40.5	31.0
6	4.4	0	95.6
10	7.6	38.5	53.9

Structures of ^{99m}Tc-labeled complexes with L-aspartic acid: The chelation of Tc(V) ions with L-aspartic acid sodium salt monohydrate is related to the coordination number (N = 5). In this study, four structures of L-aspartic acid sodium salt monohydrate-complexes with a $^{99m}Tc{=}O^{3+}$ core are suggested (Fig. 4). The structures of L-aspartic acid sodium salt monohydrate-complexes with a $^{99m}TcO_2^+$ ($[O{=}Tc{=}O]^+$) core are expected substantially the same. There are seen two types of Tc-complex, 2:1 complex (ASP:Tc) [Fig. 4(a-c)] and 1:1 complex [Fig. 4(d)]. For 2:1 Tc-complex, there are N_2O_2 type (a) and (b) and O_4 type (c). The difference between complexes (a) and (b) is which carboxylate in L-aspartic acid sodium salt monohydrate participates in the coordination. In complex (a), the carboxylate attached to α-carbon coordinates to $Tc{=}O^{3+}$ ions together with amino group, forming 5-membered rings. In complex (b), the carboxylate in the side chain coordinates to $Tc{=}O^{3+}$ ions together with amino group, forming 6-membered rings. For both N_2O_2 typed complex, when two L-aspartic acid sodium salt monohydrate molecules coordinate to one $Tc{=}O^{3+}$ core, L-aspartic acid sodium salt monohydrate molecule should hold its L-configuration in the complex. For this reason, the complex formed at pH 2 might be complex (a) rather than complex (b), considering from the deprotonation of α-carboxyl group at pH 2. The reason for the low extent (*ca.* 31 %) at pH

TABLE-2
EXTENT OF ^{99m}Tc LABELING OBTAINED WITH A VARIED TEMPERATURE

Temperature (ºC)	30 min			90 min		
	Colloidal Tc (%)	$^{99m}TcO_4^-$ (%)	Tc-Complex (%)	Colloidal Tc (%)	$^{99m}TcO_4^-$ (%)	Tc-Complex (%)
25	98.8	0	1.2	98.7	0	1.3
40	98.0	0	2.0	45.4	0	54.6
60	82.8	0	17.2	37.9	0	62.1
80	43.2	0	56.8	4.4	0	95.6

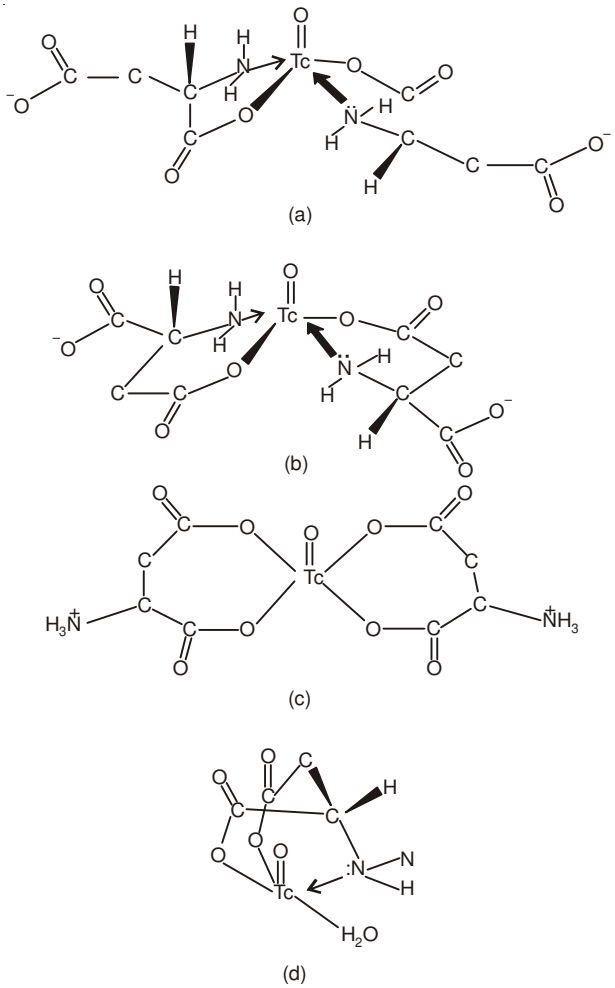

Fig. 4. Suggested-structures of ^{99m}Tc-complex with L-aspartic acid

2 can be explained by means of steric hindrances that α-amino and α-carboxylate groups in complex (a) should coordinate to a $Tc{=}O^{3+}$ core keeping their L-type configurations. On the other hand, in complex (b), the carboxylate in the side chain can coordinate free of L-configuration. Hence, in the neutral pH range, complex (b) is expected to be favoured over complex (a). In O_4 type-complex, two carboxylates in L-aspartic acid sodium salt monohydrate molecule participate in the coordination, forming 7-membered rings. Complex (c) is a possible species but Tc(V)-complex with 7-membered rings has been scarcely reported. In general, the concentration of organic ligand used for ^{99m}Tc labeling experiments is very higher than that of ^{99m}Tc, most of Tc(V)-complexes are expected to be 2:1 complexes. Tc-complex (d), 1: 1 complex, is suggested, but it needs one water molecule to satisfy the coordination number of 5. The 1:1 complex like this is thought to be very difficult to form.

Conclusion

The extent of ^{99m}Tc labeling with L-aspartic acid increased as reaction temperature is higher in the range from 25-80 ºC. From the labeling experiments carried out in acidic, neutral and alkaline media, the best result was obtained in the neutral pH. The concentration ratio of L-aspartic acid to stannous chloride is very important to obtain better ^{99m}Tc-labeling yield and the best one (95 %) was acquired at the conditions; [ASP]/[$SnCl_2$] = 45, temp. = 80 ºC, pH = 6-7. Several structures for ^{99m}Tc complex with L-aspartic acid are here suggested. Although the suggested structures are not verified, 2:1 complexes are expected to be major species, but not 1:1 complexes.

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