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Article

Optimizing Labeling Conditions for Cysteine-Based Peptides with ^{99m}Tc

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Radiolabelled peptides have attracted a great deal of attention due to their wide applicability in the development of target-specific radiopharmaceuticals. They can easily be used in diagnostic imaging as carriers for the delivery of radionuclides to tumors as well as for therapy. Previous investigations revealed that technetium(V) could form stable complexes with peptide-based ligands of N_3S type such as Cys-Gly-Gly-Gly. Herein, a targeting HER-2 receptor peptide was labeled with technetium-99m (^{99m}Tc) with two different types of tetrapeptide-based ligands, Cys-Gly-Gly-Gly and Cys-Ser-Ser-Ser. The effect of experimental parameters in the labeling procedure such as type of buffer solutions, pH of media, and type of exchange ligands were optimized toward obtaining maximum labeling yield. The optimum labeling conditions were different for two peptides. Shelf life of both labeled peptides was determined by analytical reversed-phase high-performance liquid chromatography (RP-HPLC) and thin layer chromatography (TLC) that showed radiochemical yield up to 95% even after 4 h.

Keywords: optimizing labeling, cysteine based peptide, ^{99m}Tc

Introduction

Recent years have witnessed an increasing interest in radiolabelled peptides for nuclear medicine applications. This emergence is attributed to the overexpression of many receptors in numerous cancers compared to their relatively low density in normal organs.[1] One of the most widely used diagnostic radionuclides is technetium-99m (^{99m}Tc) due to short half-life and optimum energy gamma emission. On the other hand, the existence of technetium in many oxidation states from, −1 to +7, offers an advantage for doing the necessary chemistry to prepare the various radiopharmaceuticals.[2] Among these oxidation states, Tc^{5+} is widely used in radiopharmaceutical formulations because of the stability of the resulting complexes TcO^{3+} oxotechnecium(V) with quadridentate ligands such as N_3S and N_2S_2.[3,4]

These complexes have a distorted square pyramidal structure with the oxo group perpendicular to the plane of the peptide nitrogen and sulfur coordinating atoms. It is noteworthy to mention that the presence of large S-donor atoms helps occupying the space around the metal ion more effectively compared to smaller N or O donor atoms.[5,6]

One of the design strategies for ^{99m}Tc-labeled radiopharmaceuticals is bifunctional chelating agent (BFCA) approach in which BFCA is used for ^{99m}Tc chelating.[7] An important property of BFCAs is the ability to form complexes with high *in vivo* stability. The attachment of the BFCA-radiometal complex to peptide occurs through the following reactive groups: *N*-hydroxysuccinimide (NHS) esters, isothiocyanates or maleimide moieties.[8] A wide variety of BFCAs and prosthetic groups such as mercaptoacetyltri-glycine (MAG3), diaminedithiol (DADT), 2-hydrazinonicotinic acid (HYNIC) and peptide based BFCA have been developed in recent years, allowing rapid and convenient radiolabeling of peptides with ^{99m}Tc.[9] Among these various approaches, peptide labeling can be performed through peptide-based ligands via one-pot procedure and there is no need for a separate BFCA connection step. In other words, chelators consisting of amino acids may be added to small peptides during their solid phase synthesis. Targeting peptide containing tripeptide sequence Cys-Gly-Gly as N_3S and Cys-Gly-Cys as N_2S_2 groups can be chelated with ^{99m}Tc.[10]

These tripeptide chelating sequences usually form stable technetium complexes with the $[TcO]^{3+}$ core. Coordination of the radiometal to a linear peptide increases the receptor binding affinity to its intended receptor by creating a constrained macrocyclic metallopeptide with less conformational freedom.[11,12] The site-specific metal cyclization can also improve the *in vivo* stability of the

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radiolabeled peptide.[13,14] Moreover, peptide-based ligands can simplify the chemistry of adding the metal binding sites.

The aim of this study was to use the peptide sequences of cysteine-triserine (CSSS) and cysteine-triglycine (CGGG) that would enable labeling of LTVSPWY peptide with ^{99m}Tc (Figure 1). Shadidi and Sioud[15] have identified a 7-mer peptide, referred to as LTVSPWY that exhibited preferential binding and internalization into breast cancer cell lines by HER-2 receptors.

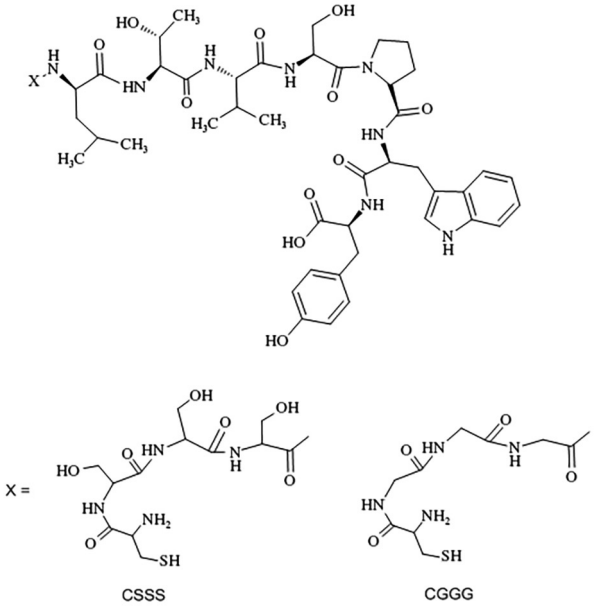

Figure 1. Structure of cysteine-triserine (CSSS) and cysteine-triglycine (CGGG)-LTVSPWY peptides.

Experimental

Materials and instruments

CGGGLTVSPWY and CSSSLTVSPWY peptides were synthesized and purchased from ProteoGenix (Schiltigheim, France). The $^{99m}TcO_4Na$ was eluted from a ^{99}Mo/^{99m}Tc radionuclide generator (Parsisotope, Tehran, Iran). Acetonitrile (HPLC grade), sodium tartrate, sodium bicarbonate, phosphate, ammonium acetate, and ethylenediaminetetraacetic acid (EDTA) were obtained from Merck company (Darmstadt, Germany); trifluoroacetic acid (TFA), tin(II)-chloride dehydrate, gluconic acid sodium salt and tricine were from Sigma-Aldrich company (St. Louis, MO, USA). Solutions were prepared by standard procedures and using high-quality water. The distribution of radioactivity on the instant thin layer chromatography (ITLC) strips was quantified using a Lablogic mini scan TLC scanner (Sheffield, UK) and analyzed with Lura image analysis software. Radioactivity in the samples was measured using a gamma counter with a NaI (Tl) detector gamma detector (Delshid, Tehran, Iran). Analytical reversed-phase high-performance liquid chromatography (RP-HPLC) was performed on a Knauer HPLC system (Berlin, Germany). The HPLC analyses of radiolabelled peptides were performed on Lablogic radioactivity gamma detector (Sheffield, UK). Column: Eurospher 100-5 C18, 4.6 × 250 mm (Knauer, Berlin, Germany) with pre-column; the mobile phase consisted of (A) 0.1% TFA in acetonitrile and (B) H_2O. RP-HPLC elution was performed with a solvent system consisting of: 0.1% TFA in acetonitrile (solvent A) and 0.1% TFA in water (solvent B). A gradient with solvents A and B was run as follows: 0 min, 10% A; 0-10 min, 10-30% A; 10-20 min, 30-80% A; 20-25 min, 80-10% A for a total time of 25 min. Flow rate: 1.0 mL min^{-1}; all solvents were filtered and degassed earlier entering the column.

Radiolabeling methods for two peptides

Two different approaches were used for radiolabeling of these peptides. The ligand exchange method, that was used gluconate, tartarate, tricine and methylene diphosphonate (MDP) ligands and different types of buffer solutions such as bicarbonate, ammonium acetate, phosphate and phosphate-citrate. In direct labeling method, different buffers such as phosphate buffer saline (PBS, pH 7.4), NH_4OAc (pH 5.5) and NaOH (pH 12) were used at different temperatures (60, 90 ºC and room temperature, r.t.) and amounts of $SnCl_2$ (20, 40 and 100 µg). Various labeling conditions were used for obtaining high radiochemical yield and shelf life for ^{99m}Tc-labeled peptide (Table 1).

Radiolabeling of [$^{99m}Tc^{(V)}O$] Cys-Gly-Gly-Gly-LTVSPWY

For this peptide, the optimum labeling yield was accomplished from $^{99m}Tc^{(V)}O$ gluconate precursor. For this purpose, 5 mg of sodium gluconate ($C_6H_{11}NaO_7$) and 10 mg of sodium bicarbonate ($NaHCO_3$) were dissolved in 100 µL of deionized water separately. To this mixture, 40 µL $SnCl_2$ (2 mg mL^{-1}, dissolved in 0.1 mol L^{-1} HCl) was added and followed by a sodium pertechnetate ($Na^{99m}TcO_4$) containing 300-400 MBq of ^{99m}Tc. The final volume of mixture was reached to 1 mL by normal saline. The reaction was accomplished at room temperature for 10 min. An aliquot of 200 µL of the above solution (2-3 mCi) was added to 120 µL of an aqueous solution (pH 1-2) containing 10 µg peptide. The radiolabeled mixture was incubated at 37 ºC for 30 min. Then the radiochemical yield was determined by ITLC in different mobile phases such as PBS and mixture of pyridine/acetic acid/water and RP-HPLC. In ITLC, when PBS was used as mobile phase, free $^{99m}TcO_4^-$ and

Table 1. Summarized methods and conditions for labeling of two peptides cysteine-triserine (CSSS) and cysteine-triglycine (CGGG)-LTVSPWY

Method for labeling	Buffer	$SnCl_2$ / μg	Temperature / °C	Ligand exchange
Direct	PBS NH_4OAc NaOH $NaHCO_3$	20 to 100	r.t. to 90	–
Indirect	PBS NH_4OAc NaOH $NaHCO_3$ NaH_2PO_4 saline NaH_2PO_4-citrate	1.3 to 175	r.t. to 100	gluconate MDP tartrate tricine

MDP: Methylene diphosphonate; NH_4OAc: ammonium acetate; PBS: phosphate buffer saline; r.t.: room temperature.

^{99m}Tc-gluconate migrate with the solvent front (Rf = 1.0), while peptide-bound ^{99m}Tc and colloid remain at the application point (Rf = 0.0). However, for pyridine/acetic acid/H_2O system the colloids (Rf = 0.0-0.3) remain at the application point (Rf = 1.0) and peptide-bound ^{99m}Tc, free $^{99m}TcO_4^-$ and ^{99m}Tc-gluconate migrate move with the solvent front. In HPLC analysis, retention times of free $^{99m}TcO_4^-$ and ^{99m}Tc-gluconate were less than 5 min while for ^{99m}Tc-labeled peptide was between 18-20 min.

Radiolabelling of [$^{99m}Tc^{(V)}O$] Cys-Ser-Ser-Ser-LTVSPWY

For CSSSLTVSPWY, the optimum labeling reaction conditions was the same as CGGGLTVSPWY except for the amount of sodium bicarbonate, which was 25 mg and the activity of sodium pertechnetate was 100-200 MBq. Also, the peptide was dissolved in 100 μL ammonium acetate and the final volume of the mixture did not reach 1 mL.

Results

In direct labeling method, buffer solutions including PBS (pH 7.4), NH_4OAc (pH 5.5) and NaOH (pH 12) were used. The amounts of $SnCl_2$ used were 20 to 40 μg. The reactions were accomplished at 90 °C and r.t. for 30 and 60 min. The obtained radiochemical yield in NH_4OAc buffer was better than other buffers. In order to confirm the good shelf life (efficacy of labeled compounds in over a period of time) of radiolabeled complexes with no considerable release of $^{99m}TcO_4^-$, we checked shelf life up to 2 h by incubation of reaction mixture at r.t. However, the shelf life after 2 h was unacceptable; radiochemical yield was less than 86% (Supplementary Information, Table S1).

As a result, we switched to ligand exchange method in which different ligands were used such as sodium gluconate, tartrate, tricine, and MDP. In the case of gluconate ligand, different amounts of $SnCl_2$ (40 to 150 μg) was added to constant quantity of gluconate (5 mg) and was dissolved in PBS, ammonium acetate and normal saline (Table 2, No. 1-12). Since stable $^{99m}Tc^{(V)}O$-gluconate complexes form in alkaline pH,[16] we decided to change the buffer solutions from PBS to alkaline buffers such as phosphonates or bicarbonate. All of the reactions were performed in one step, which means that the conditions of labeling for gluconate and peptide with ^{99m}Tc were the same (Table 2, No. 13-18). It was observed a short-term radiochemical shelf life. Although the radiochemical yield of ^{99m}Tc-peptide was more than 90% even after 1 h, ^{99m}Tc-peptide complex was unstable up to 4 h. Thus other exchange ligands such as tartrate, tricine and MDP were used .

The competing reaction for tricine and tartrate with peptide in labeling with ^{99m}Tc started at the beginning of the reaction while for gluconate displayed after 1 or 2 h. This result indicates that at the same conditions, tricine and tartrate desire to react with ^{99m}Tc in the presence of complex ^{99m}Tc-peptide more than gluconate. Moreover, changing the amounts of $SnCl_2$ and $Na^{99m}TcO_4^-$ were ineffective in the formation of ^{99m}Tc-tartarate, ^{99m}Tc-tricine and TcO_4^- (Table 2, No. 19-24). By using MDP as a weak competing ligand,[17] radiochemical purity was achieved at low yield (Table 2, No. 25-28).

In the next strategy, sodium gluconate was dissolved it in bicarbonate buffer was used as ligand exchange. Labeling of gluconate in the presence of $SnCl_2$ with ^{99m}Tc was performed in 10 min. After performing various set of reactions, we figured out that to obtain an effective radiolabeling it is essential to change the pH of the peptide media and to create acidic conditions (pH = 1-2). Hence, the combination of two mixtures results the pH of the final solution to be around 6-7, which prevented relabeling of gluconate with ^{99m}Tc in the presence of peptide (Table 2, No. 29-32).

Table 2. Conditions for optimizing labeling of peptides cysteine-triglycine (CGGG)-LTVSPWY via ligand exchange

Number of formulation	Buffer	Ligand exchange[a] / mg	$SnCl_2$ / µg	EDTA / µg	Temperature[b] / °C	time[c] / min	Labeling yield / %	Labeling yield up to 2 h[d] / %
1	saline	gluconate (5)	50	–	60	20	100	60
2	saline	gluconate (5)	75	100	60	20	74	–
3	saline	gluconate (5)	40	–	95	20	100	40
4	saline	gluconate (5)	75	100	95	20	30	–
5	saline	gluconate (5)	40	–	98	20	20	–
6	saline	gluconate (5)	40	–	98	15	50	–
7	NH_4OAc	gluconate (5)	40	–	98	20	19	–
8	NH_4OAc	gluconate (5)	100	–	98	15	40	–
9	NH_4OAc	gluconate (5)	40	–	98	15	60	–
10	PBS	gluconate (5)	40	–	98	15	90	–
11	PBS	gluconate (5)	175	100	60	20	47	–
12	PBS	gluconate (5)	125	100	60	20	70	–
13	KH_2PO_4	gluconate (5)	40	–	60	30	57	–
14	$NaHCO_3$	gluconate (5)	40	–	r.t.	30	83	–
15	$NaHCO_3$	gluconate (5)	40	–	r.t.	30	100	50
16	$NaHCO_3$	gluconate (5)	40	–	60	30	70	–
17	$NaHCO_3$	gluconate (5)	100	100	60	20	80	–
18	$NaHCO_3$	gluconate (5)	50	–	60	20	70	–
19	saline	tricine (1)	40	–	95	20	47	–
20	saline	tricine (10)	40	–	98	20	20	–
21	saline	tricine (5)	40	–	100	15	50	–
22	NH_4OAc	tricine (5)	40	–	100	15	10	–
23	Na_2HPO_4	tartarate (2)	50	–	60	20	86	–
24	$NaHCO_3$	tartarate (5)	40	–	60	30	50	–
25	saline	MDP (8.3 µg)	1.3	–	r.t.	30	80	–
26	saline	MDP (33.3 µg)	5.3	–	37	30	83	–
27	saline	MDP (8.3 µg)	1.3	–	r.t.	30	70	–
28	saline	MDP (33.3 µg)	5.3	–	37	30	75	–
29	$NaHCO_3$	gluconate (5)	80	–	37	30	100	97
30	$NaHCO_3$	gluconate (5)	80	–	45	30	70	–
31	$NaHCO_3$	gluconate (5)	80	–	95	15	42	–
32	$NaHCO_3$	gluconate (5)	80	–	r.t.	30	100	95

[a]Type of ligand exchange added to labeling reaction to obtain a ^{99m}Tc-labeled peptide; [b]temperature used for labeling peptide with ^{99m}Tc; [c]time needed for labeling; [d]radiochemical yield ^{99m}Tc-labeled peptide assessed up to 2 h. EDTA: Ethylenediaminetetraacetic; NH_4OAc: ammonium acetate; PBS: phosphate buffer saline; r.t.: room temperature.

Scanning of ITLC in order to detect radiolabeled peptide with a TLC scanner showed no $^{99m}TCO_4^-$, complexes ^{99m}Tc-gluconate (Supplementary Information, Figure S1a) and no reduced hydrolyzed technetium colloids (RHT; Supplementary Information, Figure S1b). This result indicates the radiochemical yield of 100%.

After 1, 2 and 4 h, radiochemical yield were assessed by ITLC to be 97, 96 and 95%, respectively (Supplementary Information, Figure S2). In these conditions, RHT was not observed. Moreover, the yield was also assayed by RP-HPLC at 1 and 4 h. The retention time of peptide was from 18 to 20 min and for mixture of $^{99m}TCO_4^-$ and ^{99m}Tc-gluconate complexes was 2-4 min. The obtained radiochemical yield at 1 and 4 h was 97 and 95%, respectively (Figure 2). Resulting acceptable radiochemical yield due to single peak at 18-20 min retention time, showed a high specific activity (2-3 mCi *per* 10 µg or 8.42-12.64 GBq *per* µmol).

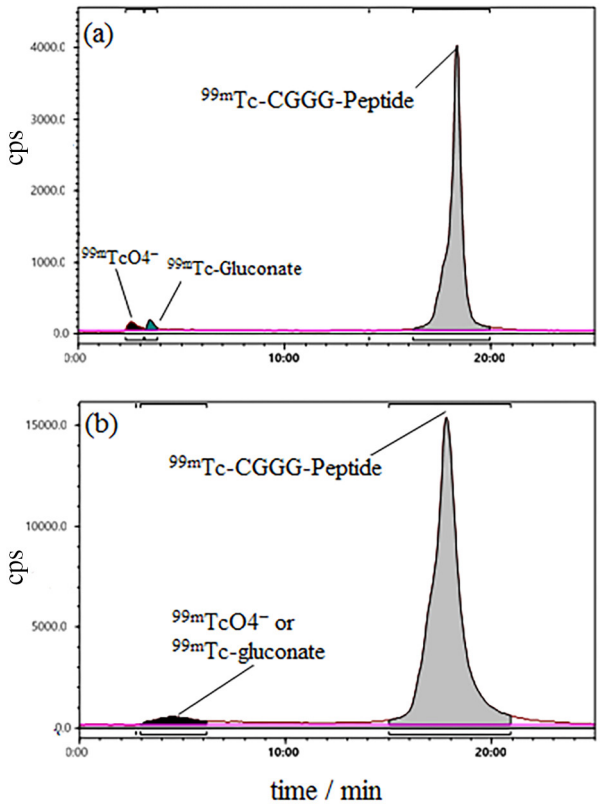

Figure 2. Radio high-performance liquid chromatography (HPLC) analysis of ^{99m}Tc- cysteine-triglycine (CGGG)-LTVSPWY peptide shelf life for 1 h (a); and 4 h (b) after labeling. The retention time of peptide was from 18 to 20 min and for mixture of $^{99m}TCO_4^-$ and complexes ^{99m}Tc-gluconate was 2-4 min.

Same optimized labeling conditions for CGGGLTVSPWY were repeated for CSSSLTVSPWY but the radiochemical yield was unsatisfactory. In order to optimize the labeling condition, dominant experimental parameters such as the quantity of $SnCl_2$ and the applied temperatures were changed as shown in Table 3. However, an effective conjugation was not achieved (Table 3, No. 1-3). Since CSSS amino acids sequence is structurally different from CGGGLTVSPWY due to the presence of serine groups in its structure, we decided to eliminate acidic media of peptide (pH = 1-2) due to prevent from protonating OH groups to obtain better radiolabeling. For this purpose, this peptide was dissolved in ammonium acetate and acetonitrile (Table 3, No. 4-7). Also, ligand exchange was changed from gluconate to tartrate, and buffer was phosphate and phosphate-citrate (Table 3, No. 8-41). But unfortunately, it was unsuccessful for obtaining a long-term stability for radiolabeled peptide

Finally, for optimizing labeling condition, gluconate was used as ligand exchange while the amount of bicarbonate buffer was increased up to two times with the peptide dissolved in ammonium acetate (Table 3, No. 42-43). The radiochemical yield was checked by ITLC and RP-HPLC like CGGG-peptide. Radiochemical yield was 99% at 30 min and 1 h, while it was reduced to 98 and 97% at 2 and 4 h after reaction, respectively. To prove the stable shelf life, results of RP-HPLC was also reported at 1 and 4 h. Radiochemical yield were 97 and 95% at 1 and 4 h, respectively (Figure 3). Resulting acceptable radiochemical yield due to single peak at 18-20 min retention time showed a high specific activity (3-4 mCi *per* 10 μg or 13.62-18.18 GBq *per* μmol).

Discussion

Biologically, active small peptides are favorable tools for diagnosis of various diseases because they offer many distinct advantages over proteins and monoclonal antibodies. Recently, a number of (^{99m}Tc)-labeled bioactive peptides have discovered to be useful diagnostic imaging agents. N_3S type ligand systems, such as Gly-Gly-Gly-Cys, form stable complexes with $^{185/187}Re^{(V)}/^{99m}Tc^{(V)}$ due to labeling peptide[18] in high yield and specific activity through the gluconate precursors.[4,19]

In the recent years, a series of studies involving labeling of affibody molecules with ^{99m}Tc using peptide-based N_3S chelators has been reported. Substitution of the amino acids forming the chelator for binding ^{99m}Tc effected on the biodistribution of affibody molecules.[20-24]

Our efforts were focused on radiolabeling of LTVSPWY peptide with ^{99m}Tc by two cycteine-based ligands of Cys-Gly-Gly-Gly and Cys-Ser-Ser-Ser. ^{99m}Tc radiolabeling of two peptides were accomplished through direct reduction and ligand exchange methods. In direct method, the use of reducing agents such as Sn (Π) can produce ^{99m}Tc intermediates that form unstable complexes with peptides.[25] In ligand exchange method, ^{99m}Tc-chelate intermediate allowed to react to peptides under mild conditions to form final acceptable radiolabeled peptides.[26] For optimizing labeling, in direct method, the influence of pH (changing buffers from acidic to alkaline), concentration of $SnCl_2$ and temperature was investigated on radiochemical yield. Although, in acidic conditions (using buffer NH_4OAc), maximum radiochemical yield was accepted but it was not stable for 2 h after labeling.

In indirect method, the effect of types of ligand exchanges (gluconate, tartarate, tricine and MDP) , buffers (PBS, phosphate, phosphste-citrate, ammunium acetate and bicarbonate), pH, amount of $SnCl_2$ and temperatures was studied for accepting maximum labeling yield. The high long-term radiochemical stability was accomplished through gluconate and bicarbonate buffer in pH range of 6-7. In addition to bicarbonate buffer, other buffers like

Table 3. Conditions for optimizing labeling of peptides cysteine-triserine (CSSS)-LTVSPWY via ligand exchange

Number of formulation	Buffer	Ligand exchange[a] / mg	$SnCl_2$ / µg	Temperature[b] / °C	time[c] / min	Labeling yield / %	Labeling yield up to 2 h[d] /%
1	$NaHCO_3$	gluconate (5)	80	37	30	80	–
2	$NaHCO_3$	gluconate (5)	80	45	10	70	–
3	$NaHCO_3$	gluconate (5)	80	r.t.	30	30	–
4	$NaHCO_3$	gluconate (5)	80	37	30	20	–
5	$NaHCO_3$	gluconate (5)	80	r.t.	30	77	–
6	$NaHCO_3$	gluconate (5)	80	45	30	82	–
7	$NaHCO_3$	gluconate (5)	80	95	30	20	–
8	$NaHCO_3$	tartarate (2.5)	7.5	r.t.	30	80	–
9	$NaHCO_3$	tartarate (2.5)	7.5	37	30	82	–
10	$NaHCO_3$	tartarate (2.5)	7.5	25	30	85	–
11	$NaHCO_3$	tartarate (2.5)	7.5	37	30	90	60
12	$NaHCO_3$	tartarate (2.5)	7.5	25	30	80	50
13	$NaHCO_3$	tartarate (2.5)	7.5	37	30	70	65
14	$NaHCO_3$	tartarate (5)	25	37	30	84	55
15	$NaHCO_3$	tartarate (5)	25	95	30	20	–
16	$NaHCO_3$	tartarate (5)	25	r.t.	30	100	60
17	phosphate-citrate	tartarate (10)	100	r.t.	30	67	–
18	phosphate-citrate	tartarate (10)	100	37	30	70	–
19	phosphate-citrate	tartarate (10)	100	90	10	20	–
20	phosphate-citrate/NH_4OAc	tartarate (10)	100	r.t.	60	100	60
21	phosphate-citrate/ NH_4OAc	tartarate (10)	100	37	60	67	–
22	$NaHCO_3$	tartarate (10)	100	r.t.	60	60	–
23	$NaHCO_3$	tartarate (10)	100	37	60	100	50
24	phosphate-citrate/HCl	tartarate (10)	100	r.t.	60	70	–
25	phosphate-citrate/HCl	tartarate (10)	100	37	60	100	60
26	phosphate-citrate/citric acid	tartarate (10)	100	r.t.	60	20	–
27	phosphate-citrate/citric acid	tartarate (10)	100	37	60	30	–
28	$NaHCO_3$	tartarate (10)	80	37	60	99	56
29	$NaHCO_3$	tartarate (10)	80	r.t.	60	60	–
30	phosphate	tartarate (1)	8	37	60	35	–
31	phosphate	tartarate (1)	8	r.t.	60	45	–
32	phosphate/NH_4OAc	tartarate (5)	20	37	60	90	45
33	phosphate/NH_4OAc	tartarate (5)	20	r.t.	60	40	–
34	phosphate/CH_3CN	tartarate (5)	20	37	60	40	–
35	phosphate/CH_3CN	tartarate (5)	20	r.t.	60	30	–
36	phosphate/NH_4OAc	tartarate (5)	20	r.t.	60	82	–
37	phosphate-citrate/NH_4OAc	tartarate (5)	20	r.t.	60	30	–
38	phosphate/NH_4OAc	tartarate (5)	5	r.t.	60	70	–
39	phosphate/NH_4OAc	tartarate (5)	80	r.t.	60	40	–
40	phosphate	gluconate (5)	80	r.t.	60	40	–
41	phosphate	gluconate (5)	80	37	60	40	–
42	$NaHCO_3$/NH_4OAc	gluconate (5)	80	r.t.	30	70	–
43	$NaHCO_3$/NH_4OAc	gluconate (5)	80	37	30	100	97

[a]Type of ligand exchange added to labeling reaction to obtain a ^{99m}Tc-labeled peptide; [b]temperature used for labeling peptide with ^{99m}Tc; [c]time needed for labeling; [d]radiochemical yield ^{99m}Tc-labeled peptide assessed up to 2 h. EDTA: Ethylenediaminetetraacetic; NH_4OAc: ammonium acetate; PBS: phosphate buffer saline; r.t.: room temperature.

as normal saline, phosphate, phosphate-citrate, PBS for accepting pH rang 6-7 was tested but radiochemical yield was unsuccessfull (less than 95%). *In vitro* stabilities of two peptides were assessed by ITLC and RP-HPLC. Results obtained during the shelf life studies demonstrated that two ligands stabilized as to chemical decomposition. Both complexes of peptides with ^{99m}Tc were easily formed in high radiochemical yield (> 95%) even after 4 h.

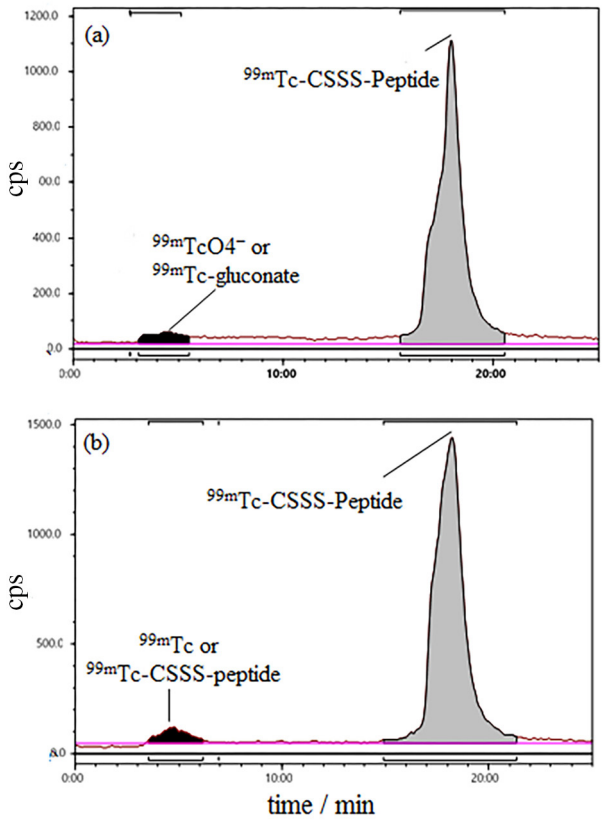

Figure 3. Radio high-performance liquid chromatography (HPLC) analysis of ^{99m}Tc- cysteine-triserine (CSSS)-LTVSPWY peptide shelf life for 1 h (a); and 4 h (b) after labeling. The retention time of peptide was from 18 to 20 min and for mixture of $^{99m}TCO_4^-$ and complexes ^{99m}Tc-gluconate was 2-4 min.

Conclusions

Radiolabeling of LTVSPWY peptide with ^{99m}Tc using peptide-based chelators and gluconate as a ligand exchange was performed through ligand exchange and direct reduction method. Optimized labeling for peptides was obtained through ligand exchange method. The obtained results during the shelf life studies demonstrated that two ligands stabilized ^{99m}Tc using two tetra peptides chelators of CGGG and CSSS. The radiolabeled peptide showed reasonable shelf life and high radiochemical yield (> 95%) in solution.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

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