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Effect of Temperature and Mole Ratio on the Synthesis Yield of Rhenium-Tetrofosmin

Widyastuti* and A.H. Gunawan

Center for Radioisotopes and Radiopharmaceuticals Technology, National Nuclear Energy Agency
Puspiptek Area Serpong, Tangerang 15310, Indonesia

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ABSTRACT

Technetium-99m (^{99m}Tc) tetrofosmin is widely used in nuclear medicine as a diagnostic agent for myocardial perfusion and as a tumor imaging agent. As a parenteral preparation it requires an evaluation of its pharmacokinetics and stability in-vivo. Since ^{99m}Tc has a short half-life and is only available in very low concentrations, it is impossible to characterize its chemical properties and presence in the body. Due to this reason, only technetium-99 ($T_{1/2} = 5 \times 10^5$ years), which is available in macro quantities, or natural rhenium can be used for this purpose. In this study rhenium-188 (^{188}Re) tetrofosmin will be synthesized and applied, because non-radioactive Re can be easily obtained. Synthesis and radiochemical purity analysis of carrier-added ^{188}Re-tetrofosmin were carried out as a model to study the in-vivo stability of technetium-99m tetrofosmin. Rhenium-188 was used as a tracer to identify the formation of rhenium tetrofosmin. Rhenium gluconate was synthesized first prior to the formation of rhenium tetrofosmin. The quality of labeling for both rhenium gluconate and rhenium tetrofosmin was analyzed using paper- and thin-layer chromatography, respectively. Rhenium gluconate can be synthesized with high labeling yield within 1 hour, whereas rhenium tetrofosmin was synthesized both in room temperature and in an elevated temperature with various tetrofosmin-to-rhenium mole ratios.The results showed that heating at 95°C led to a higher yield of more than 90% within 30 minutes. Rhenium tetrofosmin could be produced in high radiochemical purity using an excess of tetrofosmin with mole ratio of 2000. It is concluded that rhenium tetrofosmin could be synthesized through the formation of rhenium gluconate, and a higher yield could be obtained in a shorter time by heating process.

INTRODUCTION

Radiopharmaceuticals employed to detect myocardial perfusion, such as technetium-99m MIBI (^{99m}Tc-MIBI) and ^{99m}Tc-tetrofosmin, have been widely used in nuclear medicine in Indonesia,. MIBI or Sestamibi kits has been locally produced by PTRR-BATAN in colaboration with Kimia Farma pharmaceutical company and is initially marketed in Indonesia. ^{99m}Tc-tetrofosmin is widely used and known as a newer radiopharmaceutical for myocardial perfusion imaging which has some advantages compared to ^{99m}Tc-MIBI. Nowadays its application has expanded to tumor imaging, as with ^{99m}Tc-MIBI [1-16].

In hospitals (nuclear medicine department) sterile radiopharmaceutical kits or ”cold kits” are labeled with ^{99m}Tc to produce ^{99m}Tc-labeled compounds which will be administered to patients through intravenous injection. Afterwards, the exposure of gamma ray from the body will be detected by gamma camera to generate an image that will be evaluated by nuclear medicine specialists for diagnosis purposes [10,15,16].

Systemic administration of a radiopharmaceutical into the human body must be assured with regard to its safety aspects and benefits as parentheral preparation in general, so it is necessary to study its physico-chemical properties, mainly related to the stability and purity

*Corresponding author.
E-mail address: widyast@batan.go.id
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of the radiopharmaceutical that contribute to the safety and efficacy in the body. Prior to patient study the stability of the radiopharmaceutical in the body should have been studied through in vitro studies. Therefore it is necessary to study the chemical stability of ^{99m}Tc-tetrofosmin in vitro in the blood plasma as a simulated chemical stability in the body over a span of imaging with a gamma camera [17].

Technetium can only be obtained in the form of radioactive ^{99m}Tc so its physico-chemical properties can only be studied through nuclear techniques. Since the rhenium can be obtained in non-radioactive form, its physico-chemical properties such as elucidation of the structure and characteristics of complex compounds can be studied. Because the physico-chemical properties of rhenium and technetium are similar, especially in terms of the ability to form complex with ligands, rhenium can be used as a model to study the characteristics or properties of technetium radiopharmaceutical [18-20]. The position of Re in the molecule is believed to be the same as the position of Tc in Tc-tetrofosmin, as can be seen in Fig. 1 [17].

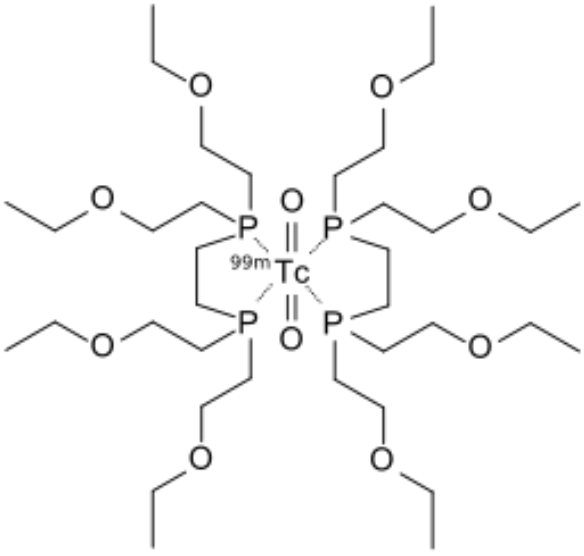

Fig. 1. Chemical structure of ^{99m}Tc-tetrofosmin

As with technetium complexes, to synthesize rhenium-ligand complexes requires a compound which serves as a reducing agent to lower the valence of rhenium from 7 so it can bind to the ligand to form a stable complex compounds, and the reducing agent commonly used is the tin(II) ion. Rhenium in reduced state is relatively unstable or more easily reoxidized (to Re(VII)) than technetium; therefore, it needs tin(II) chloride in a larger amount, and usually addition of antioxidant such as ascorbic acid is required to stabilize the tin. It indicates that rhenium is more difficult to reduce than technetium because its oxidation potential is higher [21-24].

Synthesis of rhenium tetrofosmin using the same method as for technetium-99m tetrofosmin has been carried out but the results were not promising. Efforts to improve the results have also been made through several modifications such as increasing the amount of reducing agent, changing the type of reducing agent, and changing the pH, but they did not give as good results as expected. Increasing the quantity of $SnCl_2$ as reducing agent by a factor of 50 did not make any difference, and the reaction solution became turbid because of the amphoteric Sn–(II) which turned into tin hydroxide compounds which is poorly soluble in water [17].

Synthesis of Re(V) with various ligands can be done through the synthesis of Re-oxo-gluconate as precursor or intermediate complex, such as for tartrate, dextran, and proteins. The complex formation requires 1-2 hours at pH 5 using stannous chloride or stannous tartrate as reducing agent under nitrogen or argon flow to prevent oxidation of stannous ions and reoxidation of Re(V) to Re(VII) [22-35].

The study and preparation of carrier-free ^{188}Re-tetrofosmin was done with radiochemical purity of over 90% and stable over 6 hours at room temperature [17,23]. The term carrier means an isotope (usually non-radioactive) that is added to the radioactive atoms to facilitate the reaction, for example non-radioactive ^{185}Re that is added to ^{188}Re in the labeling process or synthesis of radiolabeled compound.

Based on literature search, neither the research regarding carrier-added $^{186/188}$Re-tetrofosmin synthesis nor the study of physico-chemical characteristics of non-radioactive rhenium tetrofosmin has been done anywhere, so the approach which was followed to synthesize non-radioactive Re-tetrofosmin in micro scale was through the route of Re-oxo-gluconate synthesis.

Since the synthesis result of carrier-free ^{188}Re-tetrofosmin (without non-radioactive rhenium) is assumed to be different from that using non-radioactive rhenium or carrier-added ^{186}Re/^{188}Re in micro scale, in this research, the reaction will be optimised by varying the mole ratio of tetrofosmin to perrhenate and heating the reaction mixture to increase the yield of complex compound as high as that of no-carrier added ^{188}Re-tetrofosmin (more than 90%).

The objective of this research is to obtain a protocol of rhenium-tetrofosmin synthesis in micro or semi-micro scale so as to be able to analyze its structure and physico-chemical characteristics using chemical instruments instead of radiochemical ones.

EXPERIMENTAL METHODS

Materials and equipment

The materials used are tetrofosmin (ABX), sodium D-gluconate (Sigma-Aldrich), stannous chloride dihydrate (Sigma-Aldrich), glacial acetic acid (Merck), sodium acetate (Merck), nitrogen gas (high purity, medical grade), ammonium perrhenate (Merck), ^{188}Re perrhenate solution from ^{188}W/^{188}Re generator (PTRR-BATAN), basic chemicals such as acetone, ethyl acetate, physiological NaCl solution (saline), and other materials.

The radiochemical purity of synthesis product was analysed using paper- and thin-layer chromatography, using Whatman-3 paper strips (Merck), TLC-SG strips (Merck), gamma counter or gamma management system (DPC), TLC scanner, glasswares and other equipment.

Methods

The method of synthesis was referred to the method of rhenium gluconate synthesis which acts as a precursor for the formation of complex compounds based on rhenium (V)-oxo [22-24].The synthesis of rhenium tetrofosmin was performed by first synthesizing Re-gluconate, followed by substituting gluconate with tetrofosmin (transchelation process). The scope of work includes the preparation of materials and equipment, synthesis of rhenium gluconate, synthesis of rhenium tetrofosmin, and analysis of the radiochemical purity of rhenium gluconate and rhenium tetrofosmin using paper chromatography and thin-layer chromatography. Prior to the synthesis of Re-tetrofosmin, the formation of Re-gluconate as an intermediate product was confirmed by analyzing it using paper chromatography. In the next reaction, Re-gluconate was replaced by tetrofosmin, and the Re-tetrofosmin formed was identified using thin-layer chromatography.

The scheme of Re-tetrofosmin synthesis can be seen in Fig. 2.

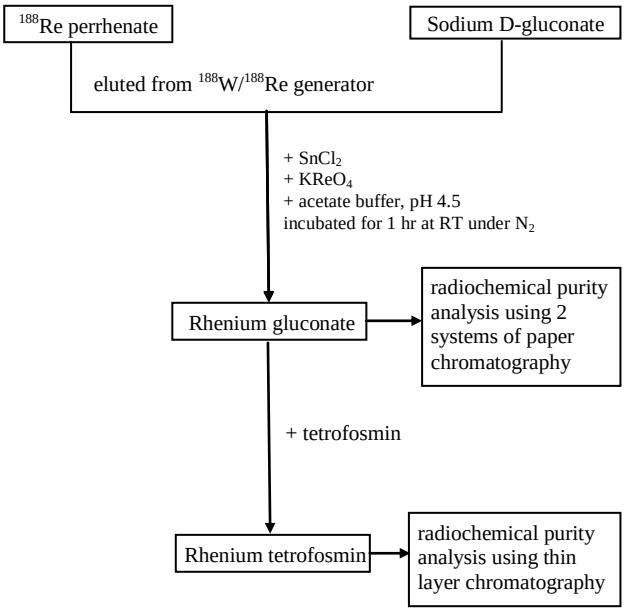

Fig. 2. Synthesis scheme and characterization of rhenium tetrofosmin.

The activities were carried out as follows:

a. Synthesis of Re-gluconate as a precursor of the formation of Re-tetrofosmin

Re-gluconate synthesis was performed by reacting sodium gluconate 0.5 M (in 0.2 M sodium acetate solution) with 0.04 mg of potassium perrhenate (1 mg/ml solution in water) to which was previously added a minimum of 30 µCi ^{188}Re as a tracer. To the reaction mixture was then added 0.1 ml of $SnCl_2$ dihydrate solution (20 mg/ml in 10% acetic acid) was then added; the mixture was then incubated for 1-2 hours (Du *et al.*, 2000). The pH of the reaction should be 4-5, and it was obtained by using acetate buffer as the medium. Reactions were performed in a sealed container with a nitrogen gas flow for 15 minutes, and then the atmosphere containing N_2 gas was maintained by covering the container with a balloon containing N_2 gas.

Analysis of Re-gluconate was carried out using paper chromatography with Whatman-3 paper as a stationary phase and acetone as mobile phase (system 1) and the same method using physiological NaCl solution or saline as the mobile phase (system 2) [22-24]. In system 1, Re-gluconate and rhenium oxide (colloid form) stayed at the bottom (Rf = 0) while perrhenate ion was eluted up to the front edge or solvent line (Rf = 1). In system 2, Re-gluconate and perrhenate were eluted nearly to the front edge (Rf = 0.8) while ReO_2 colloid retained at the bottom (Rf = 0).

The chromatograms was measured for radioactivity using both Gamma Management System (in which the strips were cut into 1 cm-long pieces and put into holes at which the gamma detector counted the radioactivity of the strip cuts) and radiochromatography scanner (at which the detector ran along the whole strips to measure the radioactivity). The stability of Re-gluconate was also studied over elevated temperature.

b. Synthesis of Re-tetrofosmin from Re-gluconate

The process was conducted by mixing an amount of Re-gluconate with 0.5 ml of an aliquot containing 1 mg of tetrofosmin, incubated at room temperature for 1-2 hours with and without heating in the water bath.

The identification of Re-tetrofosmin was carried out by thin-layer chromatography with TLC-SG as solid phase and a mixture of acetone-dichloromethane (35: 65) as mobile phase, in which Re colloids was retained at the bottom (Rf = 0), Re perrhenate was eluted to the solvent line (Rf = 1), and Re-tetrofosmin was eluted to the middle (Rf = 0.5) [36,37]. Synthesis optimization was performed by varying the amount of tetrofosmin, temperature, and reaction time.

RESULTS AND DISCUSSION

The reaction of sodium D-gluconate with perrhenate using no-carrier-added ^{188}Re resulted in the formation of ^{188}Re-gluconate with a 92.4% yield, where the impurity was non-reduced ^{188}Re or free perrhenate. In this reaction, the formation of rhenium oxide was not detected, indicating that the reaction pH was correct so the Re reduced from valence 7 to 5 was mostly reacted with gluconate.

In the chromatography system with acetone as eluant, ^{188}Re-gluconate and $^{188}ReO_2$ (Re colloid) did not migrate while $^{188}ReO_4^-$ (^{188}Re perrhenate) did, whereas in the system with saline ^{188}Re-gluconate migrated along with $^{188}ReO_4^-$ up to the solvent line (Rf = 1). It indicates that acetone is less polar than saline so it can elute Re-gluconate but not perrhenate. On the other hand, saline can elute both Re-gluconate and perrhenate at Rf = 0.8 while rhenium colloid remained at the bottom (Fig. 3).

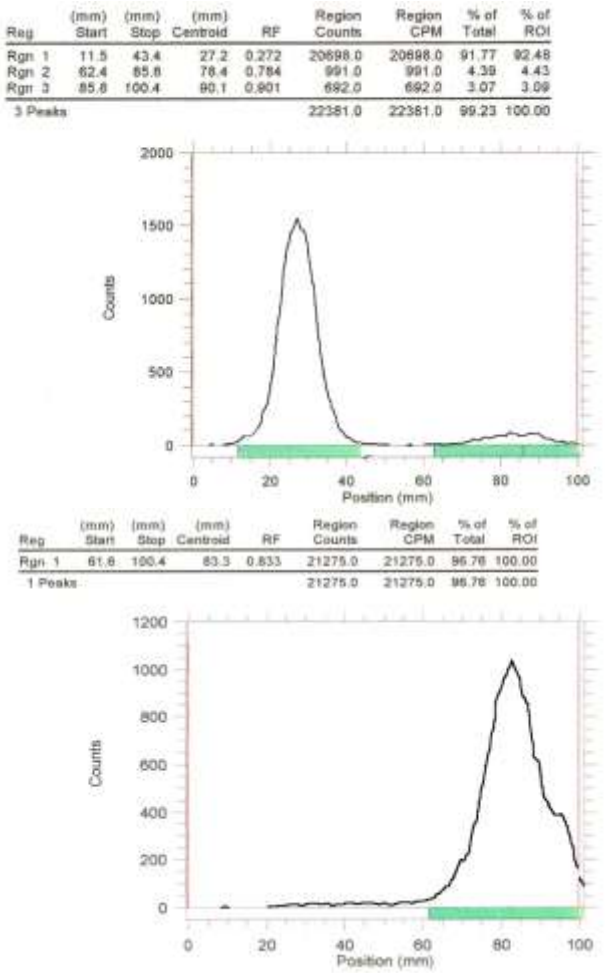

Fig. 3. The peak of ^{188}Re-gluconate in the chromatography system using acetone (top) and saline as mobile phase (bottom).

Heating Re-gluconate at the boiling point of water (~95°C) for 15 minutes decreased the radiochemical purity from 89.26% to 59.5% (Fig. 4). It indicates that the stability became lower due to reoxidation of Re(V) to Re(VII).

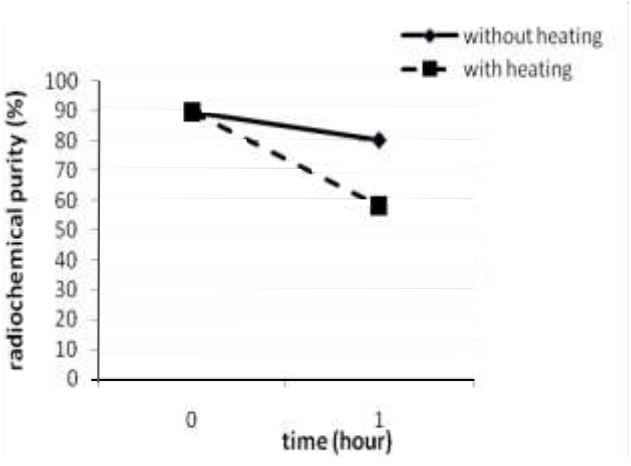

Fig. 4. Effect of heating on the radiochemical stability of Re-gluconate.

The chemical structure of Tc-tetrofosmin is Tc(V)-dioxo-tetrofosmin is assumed to be the same as that of Re-tetrofosmin since Re and Tc have similar physico-chemical properties.

In the synthesis of Re-tetrofosmin from Re-gluconate, radiochemical purity could not be analyzed with TLC scanner instrument since the low activity of the complex was below the limit of detection. The radioactivity of the radiochromatograms was measured using Gamma Management System (GMS) which is more sensitive.

Radiochemical purity analysis of Re-tetrofosmin using TLC with mixture of acetone and dichloromethane (35:65) as eluent should give peak of Re-tetrofosmin at Rf = 0.5 and perrhenate at Rf = 1.0, but in practice, the peaks of Re-perrhenate and Re-tetrofosmin were at the same spot, at Rf = 1, so this method cannot distinguish between perrhenate and Re-tetrofosmin. From the observation of the presence of Re-gluconate in the paper chromatogram, it was assumed that Re-gluconate was entirely replaced by tetrofosmin.

In experiments using no-carrier-added ^{188}Re, Re-gluconate was formed with a radiochemical purity of 100%, then after substitution reaction gluconate was replaced by tetrofosmin within 2 hours producing Re-tetrofosmin with a radiochemical purity of 90.1%.

Transchelation process between Re-gluconate and tetrofosmin was performed by varying mole ratio between tetrofosmin and Re. The results show the mole ratio of tetrofosmin/Re of 2000 resulted in the highest yield of 60% whereas the mole ratio of 3700 resulted in a slightly lower yield (Fig. 5). Thus, the mole ratio between tetrofosmin to Re of 2000 is recommended for the formation of Re-tetrofosmin.

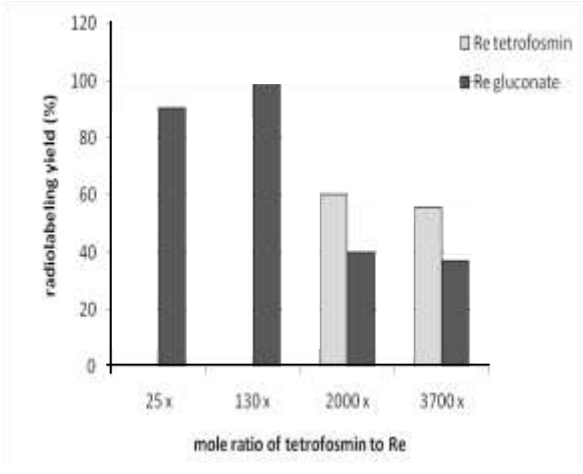

Fig. 5. Effect of tetrofosmin/Re mole ratio on the radiolabeling yield of Re-tetrofosmin, in which Re-tetrofosmin was formed when mole ratio was 2000 or higher.

Results of varying the reaction time from 30 minutes to two hours showed that the higest yield of Re-tetrofosmin was obtained after one hour, while afterwards the yield decreased due to the instability of the complex, as shown in Fig. 6. In this figure, the presence of perrhenate as impurity is not displayed.

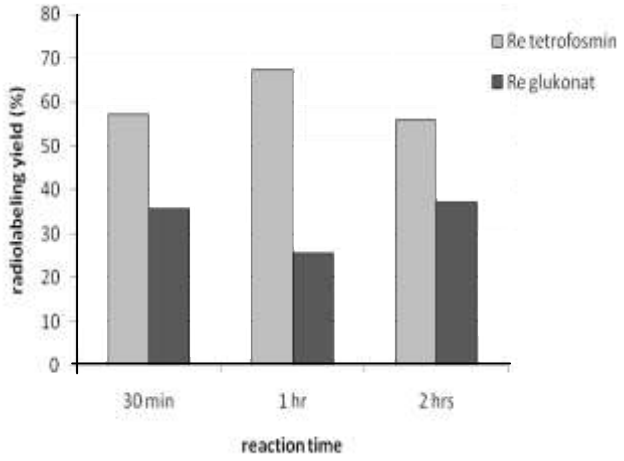

Fig. 6. Effect of reaction time on the radiolabeling yield of Re-tetrofosmin.

The substitution of gluconate by tetrofosmin in the reaction between Re-gluconate and tetrofosmin using no-carrier-added ^{188}Re with heating in the water bath (water boiling point) for 15 minutes resulted in a 90% yield of Re-tetrofosmin, but the one without heating requires a longer time (Fig. 7). It is considered that heating process may increase the enthalpy of molecules that increase the activation energy so the substitution reaction can take place more quickly. This result conformed with that described in the previous publication [23].

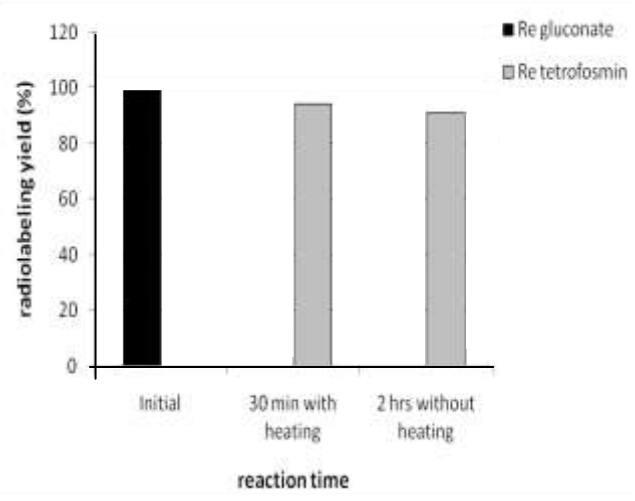

Fig. 7. Effect of heating on the yield of no-carrier-added Re-tetrofosmin (left peak is initial position in which Re-gluconate is predominant).

Transchelation process in experiments using carrier-added ^{188}Re in elevated temperature showed that the same higher yield of 90% can be achieved both in 30 minutes or 1 hour, whereas in 15 minutes with heating and in 1 hour without heating gave yield of 70% and 67% respectively (Fig. 8).

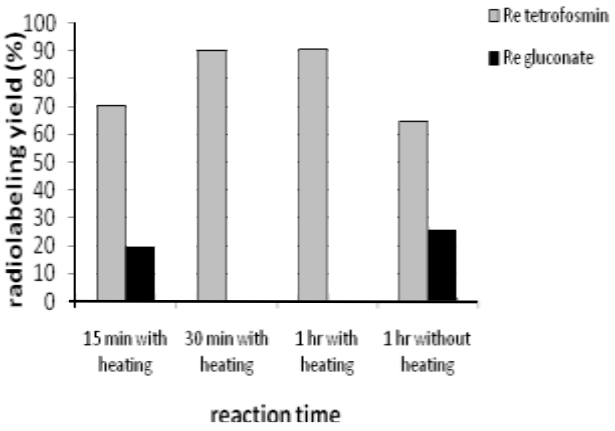

Fig. 8. Effect of heating and reaction time on the yield of carrier-added Re-tetrofosmin.

Re-dioxo-tetrofosmin complex was formed through substitution of gluconate by tetrofosmin in the transchelation reaction. Optimization has been performed by varying the mole ratio between tetrofosmin and Re to obtain high yield of Re-tetrofosmin and by elevating the temperature to accelerate the formation of the complex.

Rhenium tetrofosmin with a radiochemical purity of ~ 90% has been successfully synthesized using carrier-added ^{188}Re. Radiochemical purity or labeling yield was analysed using paper chromatography and thin-layer chromatography.

After ^{188}Re-tetrofosmin has been successfully labeled, the work will proceed with synthesis of non-radioactive rhenium tetrofosmin in amounts sufficiently high to enable characterization of rhenium tetrofosmin using chemical instruments.

The identification of Re-tetrofosmin in nano scale can be done by a method using radioactive ^{188}Re which is added as a tracer. Simulation of stability study of ^{99m}Tc-tetrofosmin in the body can be conducted only if Re-tetrofosmin can be synthesized in macro scale. It requires high amounts of tetrofosmin, and since tetrofosmin is expensive, such a study is unlikely unless the tetrofosmin can be easily provided or locally synthesised. To continue the stability study, it is necessary to use no-carrier-added radioactive rhenium in higher activities, so that it can still be detected after dilution process to test the stability of the blood plasma.

CONCLUSION

Re-gluconate as a precursor for the synthesis of Re-tetrofosmin was produced with high radiochemical purity but low stability at high temperature.

Re-tetrofosmin can be formed with high radiolabeling yield of ~ 90% through the synthesis route of Re-gluconate with an excess of tetrofosmin and under heating. The high yield of Re-tetrofosmin was reached within 30 minutes with heating. It is suggested to apply this optimum synthesis condition to a larger scale using non-radioactive rhenium so the resulted product could be analyzed using conventional instrumental methods such as FTIR or NMR.

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