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Synthesis of highly concentrated, carrier free ^{188}Re-mercaptoacetyltriglycine

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Abstract Chelation of ^{188}Re to compounds such as MAG$_3$ will further reduce the radiation dose to the patient in case of balloon rupture through the rapid excretion from the body. In order to prepare highly concentrated, carrier free ^{188}Re-MAG$_3$, S-benzoyl mercaptoacetyltriglycine (S-Bz-MAG$_3$) was synthesized, labeled with carrier free ^{188}Re. The overall yield of S-Bz-MAG$_3$ is higher than those published in the literature. Dependence of the labeling yield of ^{188}Re-MAG$_3$ upon concentrations of reducing agent, pH, reaction time, etc. was examined and optimum conditions were confirmed. The concentration procedure was succeeded with Sep-Pak C18 cartridge to obtain highly concentrated ^{188}Re-MAG$_3$. In the case of optimun conditions, the labeling yield of ^{188}Re-MAG$_3$ was more than 98%. Radiochemical purity of ^{188}Re-MAG$_3$ was more than 92% after 24 hour storage at room temperature.

Keywords ^{188}Re-MAG$_3$, Synthesis, Labeling, Carrier free, RIT

CLC number O615.4 A

1 INTRODUCTION

The radioisotope of ^{188}Re, with a half-life of 17 h, may be an attractive isotope for radioimmunotherapy (RIT).[1] ^{188}Re decays by β emission with high energies (maximum 2.11 MeV) and it also emits a γ photon (155 keV, 15%) suitable for imaging. The greatest importance is the availability of carrier-free ^{188}Re-perrhenate at any time in the clinical setting by saline elution of a ^{188}W/^{188}Re generator system. Furthermore, the high-energy β-particles emitted by ^{188}Re have a longer average range (about 2.2 mm in tissues). The chemical property of rhenium is very active, like Tc, so that it can form many stable complexes.

Rhenium chemistry is dominated by redox reactions, and perrhenate is a negatively charged anion that is rapidly excreted from living systems, preventing the ultimate

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metabolite from accumulating in non-target tissues. Because ^{188}Re is very promising for RIT, we developed a series of ^{188}Re labeled radiopharmaceuticals.[2–5]

Mercaptoacetyltriglycine (MAG_3) is a compound that has been extensively used in nuclear medicine both as ^{99m}Tc radiopharmaceutical for kidney imaging and as a prosthetic group for labeling antibodies and biomolecules with ^{99m}Tc, ^{186}Re, ^{188}Re.[6–9]

Catheter balloon filled with ^{188}Re-labelled radiopharmaceuticals for intracoronary radiation therapy to reduce restenosis provides technically a simple, safe and inexpensive method to deliver a radiation field that conforms to the vessel shape in the optimal geometry. Chelation of ^{188}Re to compounds such as MAG_3 will further reduce the radiation dose to the patient in case of balloon rupture by the rapid excretion from the body. Highly concentrated ^{188}Re-labelled radiopharmaceuticals may shorten irradiation time for patients with prerequisite concentration of solutions. S-Bz-MAG_3 was synthesized and ^{188}Re-MAG_3 chelate was prepared in this study.

2 MATERIALS AND METHOD

2.1 Materials

^{188}Re was obtained from an alumina-based ^{188}W/^{188}Re generator. The sodium tungstate [^{188}W] solution was provided by the Oak Ridge National Laboratory (Oak Ridge, TN USA). Elution of the generator with 0.9% NaCl provided solutions of carrier-free ^{188}Re as perrhenate. The nuclide purity of ^{188}Re was greater than 99% analyzed by γ-spectroscopy with a high purity germanium (HPGe) detector (GEM-15190, EG&G Ortec, Oak Ridge, TN USA) and the radiochemical purity of $Na^{188}ReO_4$ was more than 95% by paper chromatography developed with 0.9% NaCl.[10] A solid scintillation counter with NaI(Tl) crystal was used for radioactivity measurements. The IR spectra were taken with a NICOLET 360FT-IR spectrophotometer (potassium bromide). Dicyclohexylcarbodiimide (DCC, 99%), Glycylglycylglycine (98.5%) were from Fluka and N-hydroxysuccinimide (NHS) (98%) was from Acros. All other chemicals were from Shanghai Chemical Co. and all were of guaranteed grade. Thin layer chromatography (TLC) was carried out on silica gel GF_{254}, developing agent: $CHCl_3$:CH_3OH:glacial acetic acid=60:40:1. Xinhua No.1 paper was used for paper chromatography.

2.2 Synthesis of S-Bz-MAG_3 [11,12]

2.2.1 S-benzoylthioglycolic acid ("1")

8.8 g (0.22 mol) of sodium hydroxide and 9.2 g (0.1 mol) of thioglycolic acid were dissolved in a mixture of 75 mL of toluene and 75 mL of water and cooled in an ice bath at about -5–0°C. 14.05 g (0.1 mol) of benzoic acid chloride was added within 30 min while stirring, and stirring for another 30 min at -5–0°C and additional 30 min at room temperature. The organic layer was separated, washed four times with water, and the combined aqueous phases were acidified to pH 1.5 by the addition of concentrated hydrochloric acid. The precipitated product was filtered and dried. Recrystallization from ethyl acetate gave 16.1 g (83%) of product as colorless crystals with a melting point of 99–101°C (Ref. [11]: 102–103°C).

2.2.2 Succinimidyl-S-benzoylthioglycolate ("2")

9.8 g (0.05 mol) of S-benzoylthioglycolic acid ("1") and 5.75 g (0.05 mol) of N-hydroxysuccinimide were dissolved in 60 mL of absolute tetrahydrofuran and then cooled to -5°C. Then 12.38 g (0.06 mol) of dicyclohexylcarbodiimide, dissolved in 20 mL of tetrahydrofuran, were added within 20 min while stirring. Subsequently the reaction mixture was stirred for 2 h at -5°C, then at room temperature for 20 h. After the addition of 0.2 mL of glacial acetic acid and stirring for an additional hour, the product was filtered from the N,N'-dicyclohexylurea and the residue was extracted twice with boiling tetrahydrofuran. The combined filtrates were evaporated to dryness and the colorless residue was recrystallized from ethyl acetate to obtain 12.65 g (86.4%) of colorless needles with a melting point of 131–134°C (Ref. [11]: 135–137°C); R_f=1.0.

2.2.3 S-benzoyl mercaptoacetylglycylglycylglycine (S-Bz-MAG_3, "3")

1.42 g (7.5 mmol) of glycylglycylglycine was dissolved in 1 mol/L of NaOH (7.5 mL) solution. Then this solution was added in one portion to a warm solution (60°C) of 2.93 g (10 mmol) of "2" in 25 mL of acetonitrile. The mixture was stirred for 4 h at the temperature, and then stirred at room temperature for 20 h. The acidity of the mixture was adjusted to pH 2 by addition of 1 mol/L of hydrochloric acid, and the resulting precipitate was filtered off, washed with water and recrystallized from isopropanol. The solid was dried in silica gel desiccator, resulted in 2.0 g (73%) of "3"; R_f=0.45; m.p. 193–195°C (dec.) (Ref.[6]:195–196°C); IR, cm^{-1} (KBr): 3290(ν–NH), 1708 (ν–COOH), 1665(ν–S–C=O), 1649 (ν–N-C=O).

2.3 Synthesis and purification of ^{188}Re-MAG_3 compound

To a buffer solution of tartrate, 4 mg of $SnCl_2·2H_2O$, 4 mg of ascorbic acid, 1 mg of S-Bz-MAG_3 solution, and 0.5 mL of ^{188}Re solution were added. The reaction mixture was allowed to react at boiling point of water for 1 h. The labeling yields and radiochemical purity of ^{188}Re-MAG_3 were determined by two-strip paper chromatography. One PC strip was developed with tetrahydrofuran:chloroform:acetone=2:1:1 for detection of ^{188}Re-MAG_3 and hydrolyzed *rhenium*($^{188}ReO_2$); *and the other* PC *with normal saline* for detection of hydrolyzed rhenium($^{188}ReO_2$).[13]

^{188}Re-MAG_3 solution was obtained under the optimum conditions. The reaction mixture was loaded onto Sep-Pak C18 Cartridge, which had been primed with 10 mL of ethanol and 10 mL of 1% acetic acid solution. A further 5 mL of 1% acetic acid solution was eluted through the cartridge, then the residual solution was withdrawn by air. The cartridge was then eluted with 1 mL of ethanol/water solution, and the eluted solution was collected in a glass tube. After complete evaporation of the azeotropic ethanol/water solution, the residue was reconstituted with 1 mL of 0.9% saline.

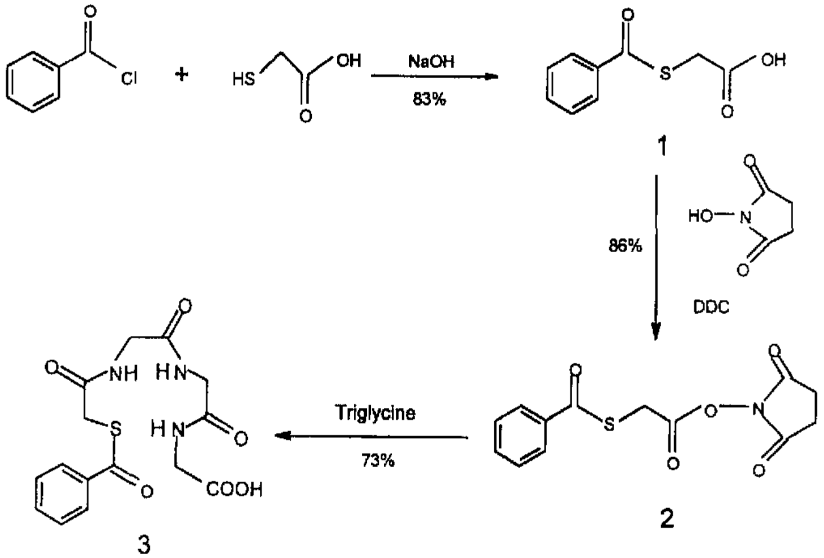
2.4 Stability of ^{188}Re-MAG$_3$ compound

After synthesis and purification, ^{188}Re-MAG$_3$ was allowed to stand at room temperature for 24 h and the radiochemical purity was checked at intervals of 0 h, 3 h, 6 h and 24 h by two-strip paper chromatography.

3 RESULTS AND DISCUSSION

3.1 Synthesis of S-Bz-MAG$_3$

The benzoyl protected precursor of mercaptoacetylglycylglycylglycine was synthesized following the synthetic route outlined in Scheme 1. The yield of "1" and "2" as well as the overall yield at -5–0°C are higher than those by Brandau.[10] By using acetonitrile as solvent, the preparation of "3" avoided complicated extraction course.



Scheme.1 Reaction scheme for the synthesis of S-benzoyl mercaptoacetylglycylglycylglycine (S-Bz-MAG$_3$)

3.2 Synthesis of ^{188}Re-MAG$_3$ compound

3.2.1 Effect of pH

The influence of pH on the labeling yields was shown in Fig.1. The influence was found to be negligible. The labeling yields were over 95% for the pH from 1 to 6, and reached maximum at pH 5.

3.2.2 Effect of stannous chloride concentration

As a reducing agent, stannous chloride is very important in labeling process. The dependence of the labeling yields of ^{188}Re-MAG$_3$ on the concentration of stannous chloride was shown in Fig.2. When the concentration of stannous chloride was less than 3 mg, the yield of ^{188}Re-MAG$_3$ is under 90%; and the labeling yield reached 98% when the concentration of stannous chloride was 4 mg.

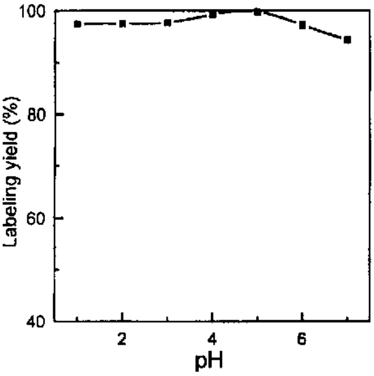


Fig.1 Effect of pH of reaction mixture in the ^{188}Re-MAG$_3$ synthesis

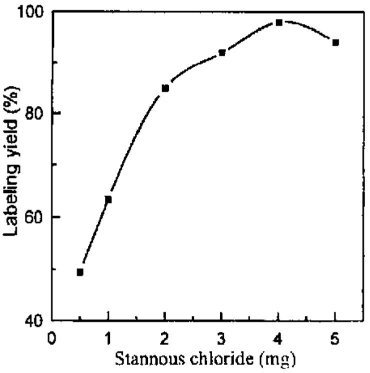


Fig.2 Effect of the concentration of stannous chloride

3.2.3 Effect of ascorbic acid concentration

Ascorbic acid can stabilize low-valence rhenium. Fig.3 shows that the labeling yield of ^{188}Re-MAG$_3$ is over 95% with ascorbic acid from 1 mg to 4 mg. 4 mg ascorbic acid was selected.

3.2.4 Effect of MAG$_3$ concentration

The labeling yield of ^{188}Re-MAG$_3$ is over 95% with MAG$_3$ from 0.2 mg to 1.2 mg (Fig.4). Obviously, MAG$_3$ concentration does not affect the labeling yield within this range.

3.2.5 Effect of reaction time

The labeling yield increase as a function of reaction time at 100°C between 10 to 50 min is shown in Fig.5. The maximum yield of over 95% was reached at about 50 min and apparently constant between 50 and 70 min.

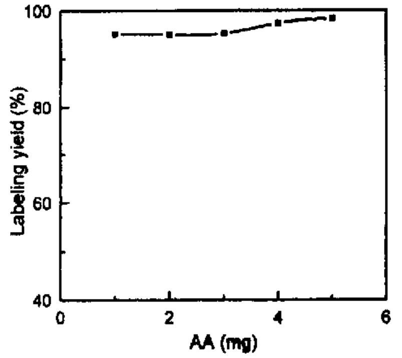


Fig.3 Effect of the concentration of AA

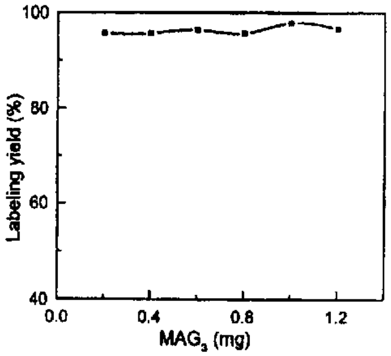


Fig.4 Effect of the concentration of MAG_3

3.2.6 Effect of volume of ^{188}Re added

The labeling yield of ^{188}Re-MAG_3 depended on the volume of ^{188}Re added. It decreased as a function of volume of ^{188}Re added (Fig.6). When the volume of $^{188}ReO_4^-$ was increased to 4 mL, the labeling yield of ^{188}Re-MAG_3 was reduced to 93%.

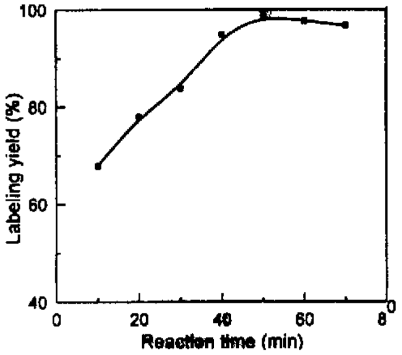


Fig.5 Effect of the reaction time

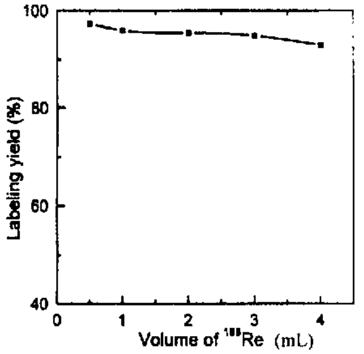


Fig.6 Effect of the volume of ^{188}Re

3.2.7 Effect of different transchelation reagents

Under the optimum conditions, citrate and gluconate were used as transchelation reagents, respectively. The results show that the transchelation reagents don't affect the labeling yield.

3.3 Stability of ^{188}Re-MAG_3

The results of stability of ^{188}Re-MAG_3 at pH 5 are shown in Table 1. Radiochemical purity of ^{188}Re-MAG$_3$ was still more than 92% after 24 h storage at room temperature.

Table 1 Radiochemical purity (%) of ^{188}Re-MAG$_3$

t (h)	0	1	3	6	24
Radiochemical purity (%)	98.8	96.8	96.4	95.4	92.6

In our studies, MAG$_3$ and ^{188}Re-MAG$_3$ were synthesized via two procedures including chelation of ^{188}Re to transchelation reagent, ligand exchange, and deprotection of the benzoyl group. The labeling procedure was at higher pH with stannous chloride as a reducing reagent. ^{188}Re-MAG$_3$ was purified and concentrated with Sep-Pak C$_{18}$ cartridge to obtain highly concentrated, carrier free ^{188}Re-Mercaptoacetyltriglycine. The separation efficiency of Sep-Pak C$_{18}$ cartridge is more than 96%. ^{188}Re-MAG$_3$ exhibits good stability.

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