



Automation of the synthesis of highly concentrated $^{188}\text{Re-MAG}_3$ for intracoronary radiation therapy[☆]

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Abstract

We have developed an efficient method and an automated synthetic system for the preparation of highly concentrated $^{188}\text{Re-MAG}_3$. Routine production of $^{188}\text{Re-MAG}_3$ for use in intracoronary radiation therapy was performed by compressed air driven semi-automated shielded system. $^{188}\text{Re-MAG}_3$ was prepared with a commercial kit and reducing agents, purified and concentrated by C_{18} Sep-Pak cartridges to desired radioactivity and volume. Using this automated system, reproducible radiolabeling yields of 80–85% were obtained. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

Intracoronary radiation therapy using a balloon catheter filled with liquid ^{188}Re -labeled radiopharmaceuticals has recently been used successfully in prevention of restenosis after percutaneous transluminal coronary angioplasty (Giedd et al., 1997; Eigler et al.,

1998; Eigler, 1999; Weinberger, 1998, 1999). Intracoronary radiation using ^{188}Re solution-filled balloons have advantages of uniform dose distribution to the vessel wall and versatile application in case of larger vessel or angulation of artery (Knapp et al., 1999; Amols et al., 1998; Fox, 1997). ^{188}Re also has suitable physicochemical properties as a therapeutic radioisotope (Knapp et al., 1997, 1999). However, there are concerns in the use of the liquid balloon approach with ^{188}Re , since in case of balloon rupture, large amounts of ^{188}Re radiopharmaceutical could be released into circulation, and radiation toxicity may occur. Other drawbacks are radiation exposure during preparation of ^{188}Re radiopharmaceuticals and the

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reaction mixture was changed from 1 to 7 with 0.1 N HCl and 1 N NaHCO₃ solution. After addition of 370 MBq/ml of ¹⁸⁸Re-perrhenate solution from the ¹⁸⁸W/¹⁸⁸Re generator without concentration of ¹⁸⁸Re-perrhenate, the reaction mixture was stirred at 100°C for 10–70 min. The reaction mixture was loaded onto C₁₈ Sep-Pak cartridges, which had been primed with 10 ml of ethanol and 10 ml of 0.001 N HCl. A further 5 ml of distilled water was eluted through the cartridge, and the eluted solution was collected in test tubes. The cartridge was then eluted with 1 ml of 7:3 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 1–2 ml of 0.9% saline. The solution was filtered through a 0.22 μm membrane filter and collected in a sterile vial. Radiolabeling yield and radiochemical purity of ¹⁸⁸Re-MAG₃ were checked by TLC. The TLC strip was developed with THF:chloroform:acetone = 2:1:1 for detection of ¹⁸⁸Re-perrhenate, and with ethanol:10% ammonium acetate = 1:1 for detection of reduced hydrolyzed rhenium (¹⁸⁸ReO₂).

2.2.1.1. Stability studies of ¹⁸⁸Re-MAG₃. After synthesis, the radiochemical purity of ¹⁸⁸Re-MAG₃ was determined at 0.5 to 6 h in saline, blood plasma, and mixed solution with 20% (v/v) contrast agent. Radiochemical purity was checked by TLC.

2.2.2. Composition and construction of an automation system for ¹⁸⁸Re-MAG₃ synthesis

The scheme for our new system is shown in Fig. 1. All parts are housed in a U-shaped panel, which consists of three aluminum plates. Each plate is 30 × 30 cm in size and the components consist of three syringe pumps, with each syringe pump consisting of a pneumatic air cylinder, disposable syringe, connection between syringe plunger and pneumatic cylinder axis, and speed controller for control of movement. The system is actuated by compressed air and three-way toggle valves, which are also actuated by compressed air, guide the solution flow direction. The solution is moved in 1.56 mm OD, 0.76 mm ID teflon tubing or PEEK tubing. Reaction vessel is in the shape of a cylinder with 60 × 18 mm size, and it has a 20 μm frit at the bottom to remove insoluble materials from the reaction mixture. Flexible heaters are used to heat the reaction vessel and evaporation set, and are fixed to the vessel wall by silicon rubber tape. The temperatures are set to 90°C for the reaction vessel and 65°C for the evaporation set by a variable transformer.

The first panel has one syringe pump for delivery of ¹⁸⁸Re-perrhenate from the vial to the reaction vessel, and three three-way toggle valves. The second panel consists of the reaction vessel, Sep-Pak cartridge for concentration and purification of synthesized ¹⁸⁸Re-MAG₃, and evaporation set with one syringe pump and three three-way toggle valves. The third panel is

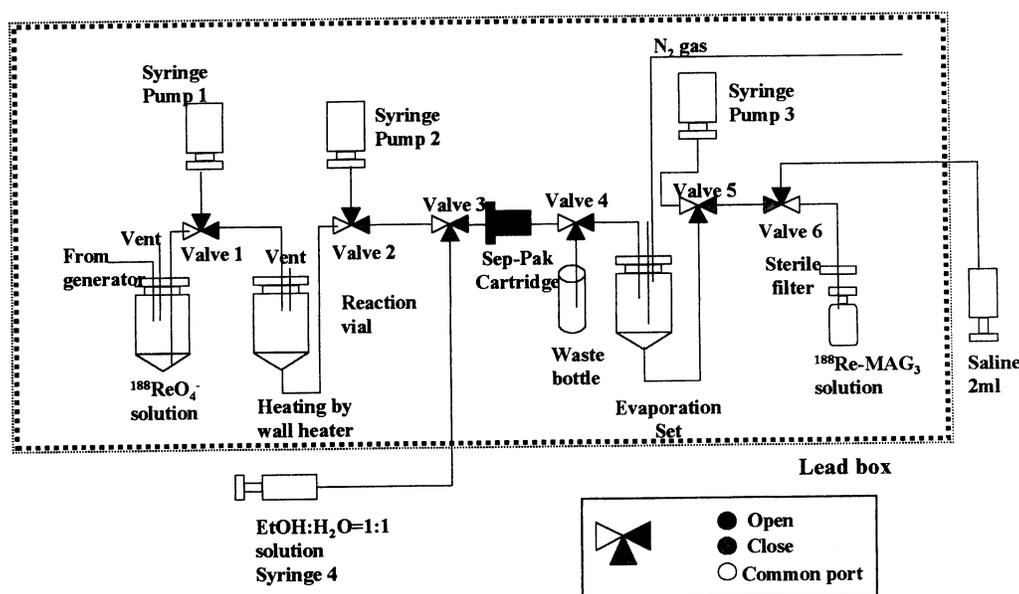


Fig. 1. Flow chart of the automated system for synthesis of ¹⁸⁸Re-MAG₃.

concentrated to the desired volume by C₁₈ Sep-Pak cartridge.

3.1.1. Effect of concentration of stannous chloride

Radiochemical yield increased with the concentration of stannous chloride (Fig. 2). The yield was improved from 37 to 95% by increasing the concentration of stannous chloride from 0.5–5 mg with 1 mg of MAG₃, although a white precipitate occurred with 5 mg of stannous chloride. When 2 mg of MAG₃ was used to prevent precipitation with 5 mg of stannous chloride, there was no increase of the radiolabeling yield. Therefore, the concentration of stannous chloride was fixed at 4 mg and more than 90% labeling yield was obtained without precipitation.

3.1.2. Effect of reaction time

As shown in Fig. 3, the radiolabeling yield increases as a function of reaction time at 100°C between 10 and 50 min, reaching a maximum yield of 95–99% at about 50 min. This maximum yield is apparently constant between 50 and 90 min reaction time.

3.1.3. Effect of concentration of ligand

The influence of MAG₃ concentration on the radiolabeling yield of the ¹⁸⁸Re–MAG₃ is shown in Fig. 4. No dramatic increase in the radiolabeling yield was observed at 0.2 mg (0.58 μmol) to 1 mg (2.9 μmol) MAG₃ concentration. In comparison with the results of other reported ¹⁸⁸Re-labeled radiopharmaceuticals (Hashimoto et al., 1996, 1998), the radiolabeling yield of ¹⁸⁸Re–MAG₃ did not depend on ligand concentration.

3.1.4. Effect of pH

The radiolabeling yield decreased slightly when the

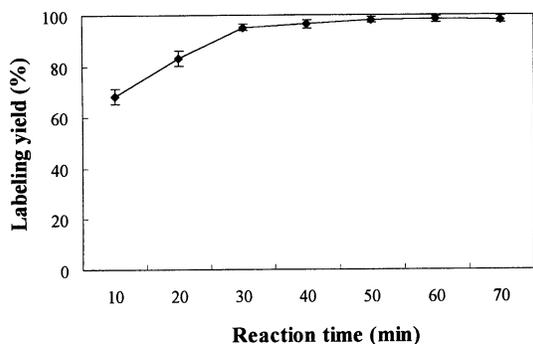


Fig. 3. Effect of the reaction time. Radiolabeling yield of ¹⁸⁸Re–MAG₃ increased by the reaction time at 100°C upto 95–99%. Then it stayed same after 30 min. Reaction was performed with 1 mg MAG₃ and 4 mg stannous chloride at 100°C. Radiolabeling yield was obtained after Sep-Pak purification and expressed as mean ± SD from three experiments.

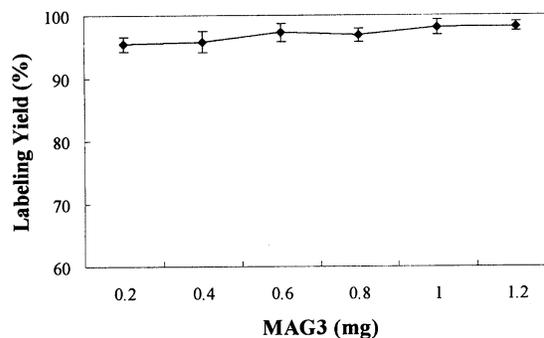


Fig. 4. Effect of the concentration of MAG₃. Concentration of MAG₃ did not affect radiolabeling yield. In comparison of other ¹⁸⁸Re-labeled radiopharmaceuticals, similar radiolabeling yield was observed with 0.2–1 mg MAG₃. Reaction was performed with 1 mg MAG₃ at 100°C for 1 h. Radiolabeling yield was obtained after Sep-Pak purification and expressed as mean ± SD from three experiments.

pH (Fig. 5) of the reaction solution increased from 1 to 7. There was a 5.8% decrease of radiochemical yield at neutral pH in comparison with that at pH 1. Although the ability of reducing agent appears to be slightly decreased at neutral pH, ¹⁸⁸Re–MAG₃ can be still synthesized under neutral condition in the above 90% radiochemical yield because ¹⁸⁸Re–MAG₃ is formed by ligand exchange reaction while other ¹⁸⁸Re-labeled radiopharmaceuticals are usually synthesized under strong acidic conditions.

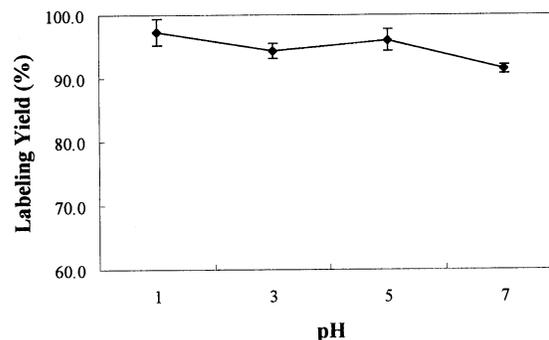


Fig. 5. Effect of pH of reaction mixture in the ¹⁸⁸Re–MAG₃ synthesis. The radiochemical yield of ¹⁸⁸Re–MAG₃ was slightly decreased by 5.8% by increasing the pH of reaction mixture. However, ¹⁸⁸Re–MAG₃ could be obtained in neutral pH in above 90% radiochemical yield without any additional procedure to adjust pH of the final product. Reaction conditions were 1 mg MAG₃ and 4 mg stannous chloride, 100°C, 1 h. Radiolabeling yield was obtained after Sep-Pak purification and expressed as mean ± SD from three experiments.

on reaction volume in the automated synthesis and is shown in Fig. 6. Lower radiolabeling yields were obtained with reaction volumes more than 12 ml. For synthesis of $^{188}\text{Re-MAG}_3$, reaction volume of 6–8 ml was preferable without $^{188}\text{Re-perrhenate}$ concentration.

4. Discussion

In these studies, $^{188}\text{Re-MAG}_3$ was synthesized, purified and concentrated with C_{18} Sep-Pak cartridge. $^{188}\text{Re-MAG}_3$ was concentrated to desired radioactivity per unit volume with C_{18} Sep-Pak cartridge. Using our new semi-automated synthesis system, reproducible radiolabeling yields of 80–85% were obtained. Highly concentrated $^{188}\text{Re-MAG}_3$ would reduce the irradiation time and myocardial ischemia during intracoronary brachytherapy would be minimized. In addition, automated synthesis will decrease radiation exposure to personnel who is involved in the routine preparation of $^{188}\text{Re-MAG}_3$ for intracoronary radiation therapy.

$^{188}\text{Re-MAG}_3$ was synthesized from two step procedures similar to that of $^{99\text{m}}\text{Tc-MAG}_3$. These procedures include chelation of ^{188}Re to sodium tartrate, ligand exchange, and subsequent deprotection of the benzoyl group. Other ^{188}Re -labeled radiopharmaceuticals used in clinical trials such as $^{188}\text{Re-DTPA}$ or HEDP require very harsh reaction conditions including strong acidic medium (Hashimoto et al., 1996, 1998). However, this synthetic method allowed synthesizing $^{188}\text{Re-MAG}_3$ at higher pH from a commercial kit with addition of extra reducing agent only. Purification of $^{188}\text{Re-MAG}_3$ by C_{18} Sep-Pak cartridge is performed to eliminate excessive reducing agent and other chemical impurities. $^{188}\text{Re-MAG}_3$ exhibits good stability

without ascorbic acid, while most ^{188}Re -labeled radiopharmaceuticals had to be mixed with ascorbic acid to prevent radiolysis. In this study, excessive reducing agent and other chemical impurities were eliminated by purification of $^{188}\text{Re-MAG}_3$ using C_{18} Sep-Pak cartridge.

Radiolabeling yield of $^{188}\text{Re-MAG}_3$ using the semi-automated synthesis system depended on the reaction volumes. While a radiolabeling yield of more than 80–85% was obtained in reaction volume of 6–10 ml, it was reduced to 70–80% with 10–12 ml of reaction volumes. There was no further increase of the radiolabeling yield with the additional ligand and reducing agent. According to the information on the expected performance characteristics of the 1.85 GBq size of $^{188}\text{W}/^{188}\text{Re}$ generator, 15–20 ml of elution volume is required to obtain maximum radioactivity of ^{188}Re . If less than 10 ml of eluted volume is used for the synthesis of $^{188}\text{Re-MAG}_3$, the obtained radioactivity of ^{188}Re will not be enough for intracoronary radiation therapy after 3–6 months. As indicated by package insert for $^{99\text{m}}\text{Tc-MAG}_3$, 10 ml is the upper limit that can be used in the synthesis of $^{188}\text{Re-MAG}_3$. Further optimization of the concentration of ligand and reducing agent is needed for longer utilization of the $^{188}\text{W}/^{188}\text{Re}$ generator.

Although 10 ml of ethanol/water solution was recommended for the extraction of $^{188}\text{Re-MAG}_3$ from Sep-Pak cartridge, 1 ml was adequate for manual separation in this study. However, 3–5 ml of ethanol/water solution extracted $^{188}\text{Re-MAG}_3$ completely from the dead volumes of teflon tube in the semi-automated synthesis device. Smaller volumes of ethanol/water solution have an advantage to reduce evaporation time within 5 min.

In our new semi-automated system, the reaction vessel is heated with a fitted flexible heater. Heat is transferred to reaction mixture directly, and this thermal capability is better than any other available heating methods, although sensitive control of heating is required. Although $^{99\text{m}}\text{Tc-MAG}_3$ is prepared in 100°C water bath, heating temperature higher than 90°C reduced labeling yield in the synthesis of $^{188}\text{Re-MAG}_3$ with wall type heater employed. This may be caused by different reaction temperature with different types of heating, and by decomposition of MAG_3 of the temperature higher than 100°C.

There was slightly high residual radioactivity on the evaporation set than any other part of the automated system. In an automated system for the production of PET radiopharmaceuticals, the final synthetic radiopharmaceutical products are usually reconstituted with 5–10 ml volumes, and radioactivity on evaporation device is mostly recovered. For radiopharmaceuticals utilized in intracoronary radiation therapy, there is a limit on the reconstituted volume, which led to a large

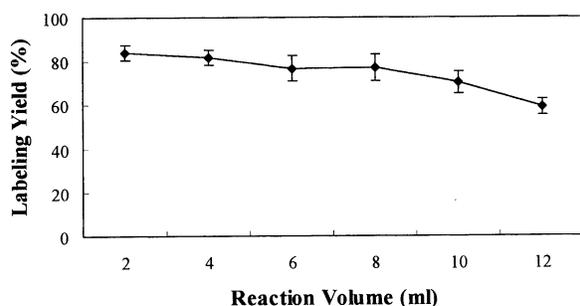


Fig. 6. Effect of the reaction volume in the automated synthesis of $^{188}\text{Re-MAG}_3$. In the automated synthesis, radiochemical yield of $^{188}\text{Re-MAG}_3$ depended on the reaction volume. High radiochemical yield obtained in the reaction was between 6 and 8 ml. Radiolabeling yield was mean \pm SD from three experiments.

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