Concentration of ^{188}Re-Perrhenate for Therapeutic Radiopharmaceuticals

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(Received on 29th February 2012, accepted in revised form 2nd August 2012)

Summary: Rhenium-188 ($T_{1/2}$=16.9h) has great potential for a variety of therapeutic applications, including radionuclide synovectomy, oncology and bone pain palliation. The radioactive concentration of ^{188}Re is dependent upon the specific activity of ^{188}W, which dictates the bed size of the alumina/gel column. Due to the high content of inactive tungsten in neutron irradiated WO_3, large columns containing aluminum oxide or gel are needed to prepare to double neutron capture based ^{188}W/^{188}Re generators that results in large elution volumes containing relatively high ^{188}W contents and low concentrations of $^{188}ReO_4^-$. This decrease in specific volume of $^{188}ReO_4^-$ places a limitation because a high radioactive concentration of $^{188}ReO_4^-$ is always needed for filling angioplasty balloons or other therapeutic radiopharmaceuticals like ^{188}Re -EHDP ^{188}Re -EDTMP, ^{188}Re –MAG_3 and ^{188}Re -DTPA. We report post elution concentration of $^{188}ReO_4^-$ using in- house prepared lead cation exchange and alumina columns. Using these columns high bolus volume (10 mL saline) of $^{188}ReO_4^-$ can conveniently be concentrated in 1 mL of physiological saline for therapeutic use.

Keywords*:* Isotonic saline as eluent; ^{188}W/^{188}Re generator; Concentration of ^{188}Reperrhenate, Lead (Pb) cation exchange column; Tiny alumina column.

Introduction

High specific activity of the radiotracer is an important requirement for most of its applications in biology and medicine. Rhenium-188 has emerged as a nuclide for therapy due to its availability in the form of ^{188}W/^{188}Re generator and attractive properties including the emission of β^- particles with an average energy of 764 keV and the emission of a 155 keV γ-ray (15 %) suitable for gamma camera imaging. Most of the work with ^{188}W/^{188}Re generators has focused on an alumina-based column chromatography system, in which tungsten-188 ($T_{1/2}$ = 69 d) is adsorbed on alumina as hydrated tungsten oxide and ^{188}Re is eluted from the column with 0.9% physiological saline solution [1, 2] Currently low specific activity ^{188}W is produced by double neutron capture by ^{186}W, in a nuclear reactor. Various gel generators of ^{188}W/^{188}Re have also been investigated [3]. High radioactive concentration of ^{188}Re is necessary for several clinical applications, including the use of ^{188}Re liquid-filled balloons for coronary restenosis therapy, and for the preparation of ^{188}Re-DEDC and ^{188}Re-HDD radiopharmaceuticals that are used for liver cancer therapy [4, 5]. Many techniques have been described for concentration of $^{188}ReO_4^-$ in the literature. The prime method is based on commercially available silver and anion exchange cartridges that have been reported by many groups [6, 7]. A system was developed for concentration and purification of pertechnetate solutions involving reduction, adsorption, reoxidation and elution steps which improves the critical quality parameters of the pertechnetate solution in such a way that they meet the highest requirements [8]. Chattopadhyay *et al* [9] describe the use of inexpensive, commercially available Dowex-1 x 8 resin and in-house prepared silver chloride columns for the concentration of ^{99m}Tc-pertechnetate from an 80 g alumina column generator loaded with ^{99}Mo produced via (n, γ) reaction. Acetone was used an eluent for $^{99m}TcO_4^-$/ $^{188}ReO_4^-$ and evaporated by gentle heating for concentration of pertechnetate/perrhenate [10]. In another method a mixture of 0.7 M acetic acid and 0.0225 M sodium chloride was used and concentration of $^{99m}TcO_4^-$/ $^{188}ReO_4^-$ was achieved by

commercially available QMA Sep-Pak® anion exchange cartridge [11]. In house preparation of silver cation column and its use for concentration of $^{99m}TcO_4^-$/ $^{188}ReO_4^-$ was reported [5, 12].The extraction of medically interesting ^{188}Re-perrhenate in methyl ethyl ketone was also reported [13].

Physiological (0.9%) saline is the most widely accepted eluent for alumina and gel chromatographic generators of $^{188}W/^{188}Re$. In this work we investigated the preparation of high specific volume solution of $^{188}ReO_4^-$ by using lead cation exchange and mini alumina columns. A safe concentrator system is also described.

Results and discussion

Many imaging and therapeutic applications are dependent on the use of concentrated solution of radionuclides. The principle of the concentration method developed consists of trapping of anionic perrhenate on a tiny alumina column (300mg). This can be only achieved in the absence of chloride ions. The chloride ions are removed from the eluate by reaction with lead ions loaded onto a cation exchange column, to precipitate lead chloride, which is filtered out by the column packing. Commercially available sulfonic acid resin AG 50W-X12, 200-400 mesh size, was loaded with 1.65g lead (Pb) which was found suitable for up to 60ml saline eluate. Any Pb ions present in the eluate from the laboratory prepared Pb ions loaded onto a cation exchange column may be detected by precipitation with sodium chloride solution since precipitation of lead chloride in the presence of chloride ions is an extremely sensitive test for lead ions. The absence of a precipitate in the final eluate is sufficient evidence that this product is free of lead. Samples were also analyzed by atomic absorption technique. None of the eluates showed detectable Pb. The detection limit by the atomic absorption technique was 20ppb for Pb.

Table-1 summarizes the results of concentration of $Na^{188}ReO_4$ eluted from a $^{188}W/^{188}Re$ column chromatographic generator. The eluate from the generator was sucked through lead cation exchange and alumina column with the help of evacuated vials. The eluate was desalined by lead cation exchange column while perrhenate, free from macroscopic quantity of eluent anions, was quantitatively adsorbed on the tiny alumina column. Finally 1.0 mL saline (~ 1mL/min) was used to elute perehenate from the alumina column. The alumina column also served as a purification column by adsorbing the ^{188}W, if present in the initial eluate. 10 mL of distilled water was passed through the lead column to remove any trapped $Na^{188}ReO_4$. Desorption of perrhenate was carried out with 1 mL 0.9% saline. In all cases the eluate was clear. The pH of eluates was 5-7. None of the eluates showed detectable Al or ^{188}W breakthrough. FigS. 1 and 2 show the radiochemical purity of ^{188}Re-EHDP assessed by ascending paper chromatography using ITLC-SG strip as stationary phase. In acetone (solvent system) the ^{188}Re-EHDP and reduced/hydrolyzed ^{188}Re remain at origin, while free perrhenate moves with the solvent front. When 0.9% sodium chloride (solvent system) is used the ^{188}Re-EHDP and free perrhenate move with the solvent front, while reduced/hydrolyzed ^{188}Re is retained at the origin.

The concentration factor was calculated by the following formula:

Concentration Factor=

$$\frac{\text{Activity of radionuclide}}{\text{Final eluate volume}} \times \frac{\text{Initial eluate volume}}{\text{Activity of radionuclide}}$$

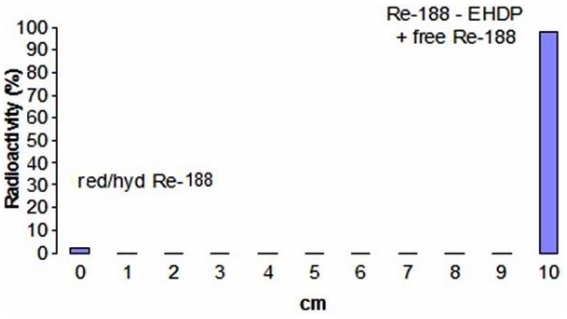

Fig. 1: Radiochromatogram showing positions of red/hyd ^{188}Re, ^{188}Re -EHDP complex and free ^{188}Re (Stationary phase = ITLC-SG, Solvent system = 0.9% NaCl).

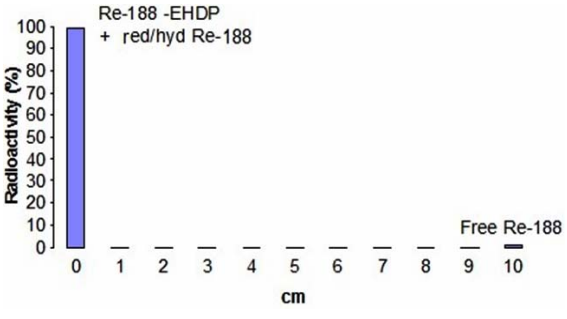

Fig. 2: Radiochromatogram showing positions of red/hyd ^{188}Re, ^{188}Re -EHDP complex and free ^{188}Re (Stationary phase = ITLC-SG, Solvent system = Acetone).

Table-1: Concentration of ^{188}Re-perrhenate eluted from ^{188}W/^{188}Re generator using lead cation and alumina columns.

10mL Saline MBq	Loss of ^{188}Re-perrhnate during concentration			Recovered in 1mL Saline MBq	% Recovered In 1mL Saline MBq	Concentration Factor
	Not adsorbed on Al_2O_3 column MBq	Retained on Lead Column MBq	Retained on Al_2O_3 Column MBq			
848	**46**	**14**	**8**	**775**	**91.4**	**9.1**
804	**34**	**12**	**7**	**737**	**91.6**	**9.1**
772	**32**	**11**	**7**	**716**	**92.7**	**9.2**
737	**30**	**11**	**6**	**677**	**91.8**	**9.1**
710	**28**	**9**	**6**	**656**	**92.4**	**9.2**
684	**26**	**8**	**6**	**628**	**91.8**	**9.1**

The concentration factor obtained by the described system was ~9 folds for double neutron capture based ^{188}W/^{188}Re column chromatographic generator. The radiochemical purity of $Na^{188}ReO_4$ in the concentrated eluates was >99% and the compatibility for formulation of ^{188}Re radiopharm-aceutical included satisfactory labeling of EHDP. Thus we have demonstrated a method for high specific volume solution of ^{188}Re-perrhenate by using laboratory produced lead cation exchange and mini alumina columns. Desalination of initial eluate (10 mL) is carried out by lead cation column while alumina adsorbs ^{188}Re-perrhenate quantitatively. The weight of the lead cation exchange resin dictates the volume of eluate which can be used for concentration purposes while the tiny alumina column anion exchanger was found sufficient to adsorb >Ci amounts of $^{188}ReO_4^-$ which can be subsequently eluted with 1.0 mL 0.9% saline.

Experimental

Materials and methods

All chemicals were purchased from commercial sources, E. Merck (Germany) and were mostly of GR/AR grade. Al_2O_3 (90 active acidic I for column chromatography, 70-230 mesh ASTM) was used as an adsorbent. Sulfonic acid resin AG 50W-X12, 200-400 mesh size, was obtained from Bio-Rad. No purification of chemicals was performed.

^{188}W/^{188}Re Generator

Sodium perrhenate (^{188}Re) was eluted with physiological saline from an alumina based ^{188}W/^{188}Re generator which was prepared by Oak Ridge National Laboratory (ORNL) USA [1, 2]. The ^{188}W/^{188}Re generator was received through International Atomic Energy Agency (IAEA) for a clinical trial involving the use of Rhenium-188-Lipiodol for therapy of hepatocellular carcinoma at NORI, Nuclear Medical Center, Islamabad.

Radionuclide Analysis

Radionuclide measurements were made using a calibrated HpGe detector coupled to a Canberra series 85 multichannel analyzer. Samples of constant geometry were counted with low (<5%) dead time. Nuclear data were taken from Lederer and Shirley [14] and the radioisotope levels were determined by the quantification of the following photo peaks: ^{188}Re, 155 keV (15%); ^{192}Ir, 316 keV (82.8%); ^{191}Os, 129 keV (25.9%). The amounts of ^{188}W were quantified by measurements of the155 keV γ-ray from decay of the^{188}Re daughter in the^{188}W/^{188}Re equilibrium mixture. Routine generator yields were determined using a calibrated ionization chamber Capintec (USA).

Radiochemical Analysis

To determine the amount $^{188}ReO_4^-$, the sample was chromatographed on ITLS-SG (Gelman Sciences Inc, USA) using acetone as mobile phase. Unbound$^{188}ReO_4^-$ migrated with the solvent front, whereas reduced/hydrolyzed and labeled material remained at the origin. The amount of reduced/hydrolyzed species was determined using saline (0.9%) to develop the ITLC strip. In this system the reduced/hydrolyzed form was retained at the origin, whereas free$^{188}ReO_4^-$ and labeled material moved with the solvent front. The radioactivity was determined by cutting the strips into 1cm pieces and counting in a well type gamma counter. Sometimes a 2π Scanner (Berthold, Germany) was used for scanning the radioactivity on strips.

Determination of Al^{3+}, pH and labeling efficiency

The Al^{3+} content of the final solution was determined by a spot test, based on the reaction of Al^{3+} ions with alizarin S (sodium salt of the 1, 2-dioxy-anthraquinone sulfonic acid). The concentrated pertechnetate obtained was checked for clarity, pH, radioactive concentration and efficacy to label with EHDP.

Preparation of lead (Pb)-cation column for concentration of ^{188}Re-perrhenate

A slurry of 3g of cation exchange resin in 5 mL 0.3 M HNO_3 was filled into a glass column (60 x 8 mm) containing a sintered disc at the bottom. The other end of the column was packed with glass wool. The liquid was drained off with the help of vacuum

and the column was loaded with 0.5 M $Pb(NO_3)_2$ in 0.3 M HNO_3 at a flow rate of 1-2 mL/min using controlled vacuum. The effluent was checked for the absence/presence of Pb^{++} by addition of sodium chloride solution. The appearance of Pb in the effluent determined the end point. The amount of Pb sorbed on the column was calculated. The column was washed with 10 mL water. The chloride removal capacity of the Pb column was tested by passing the 0.9% saline through the column at a rate 1 mL/min. Effluents from the Pb column were tested for chloride by addition of lead nitrate solution.

Preparation of Al_2O_3 Column for Concentration of ^{188}Re-Perrhenate

Acidic alumina (350mg) was washed with distilled water to remove fine particles and transferred to a glass column (1.3 x 0.4 cm^2).The glass wool was used to pack both ends of the alumina column.

Concentrator System for ^{188}Re-Perrhenate

In order to minimize the radiation exposure to the worker, the system is used behind a lead glass. The tandem cation/anion concentration system consists of an in-house made lead cation exchange column (5 g AG 50W-X12, 200-400 mesh size, loaded with Pb, bed size; dia 1.0 cm and length 8 cm) attached to a three-way stopcock connected at the outlet to the mini alumina column. ^{188}Re- perrhenate generator eluate, followed by 10 mL sterile water, was sucked with the help of evacuated vials through the columns at a flow rate of ~6 mL /min. The column effluents were run to waste. The lead cation column was disconnected and the alumina column was eluted under slight pressure with 1.0 mL 0.9% sterile saline.

Labeling of HEDP Kit with Concentrated ^{188}Re-perrhenate

Concentrated perrhenate solution was injected into the vial containing HEDP kit. The mixture was mixed well. After a few minutes the sample from reconstituted kit was spotted on ITLC-SG. Two strips (2cm width and 15 cm length) were used. One strip was developed in acetone and the other one in 0.9% NaCl. After development of the chromatogram in respective solvents, they were dried cut into 1 cm pieces and counted in a gamma counter.

Conclusions

We have developed a simple and low cost concentrator method for medically interesting $^{188}ReO^{4-}$ which is based on lead cation column connected in tandem to a tiny Al_2O_3 column. Removal of chloride in initial eluate (10mL) is performed on lead cation exchange column enabling $^{188}ReO_4^-$ to be adsorbed on Al_2O_3 quantitatively. The latter column also served as a purification column. Finally 1mL saline was employed to elute ^{188}Re-perrhnetate from acidic alumina column under slight pressure which is useful for formation of therapeutic radiopharmaceuticals like^{188}Re –EHDP. The radiochemical purity of ^{188}Re-perrhenate in the concentrated eluates was >99% and the compatibility for formulation of ^{188}Re radiopharmaceutical included satisfactory labeling of EHDP (labeling efficiency >95%).

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