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A RADIOTHERAPEUTIC AGENT FOR BONE PAIN: THE CHROMATOGRAPHIC BEHAVIOR OF Re-188-HEDP IN DEXTRAN MATRIX FOR RADIOCHEMICAL QUALITY CONTROL

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ABSTRACT

The hydroxyethylidene diphosphonic acid (HEDP) is a bone seeking agent radiolabeled with Re-188, a β^- emitting radionuclide with favorable physical characteristics that demonstrated clinical potential in relieving intense bone pain resulting from metastases of the breast, prostate and lung cancer patients. After production of this radiopharmaceutical, the most common impurities in the complex are free Re as ReO_4^- and reduced Re. A different procedure of quality control for the determination of radiochemical purity of HEDP-Re-188 complex has been studied with chromatography dextran matrix – Sephadex G-25 (PD-10 by Amersham Pharmacia), and the gel chromatographic behavior of Re-188 and Re-188-HEDP was demonstrated by the separation of the species. The radiochemical purity of the complex also was measured by Whatman 1M paper using acetone for free perrhenate (Rf 0.9-1) and labeled product (Rf 0) and ITLC-SG using saline for reduced rhenium (Rf 0) and labeled product (Rf 0.9-1). The radiochemical purity yields determinations of Re-188-HEDP complex were carried out by gamma spectrometry with an HPGe detector calibrated for Re-188 (155 keV). The results of complex yields were compared. In these experiments, the results of complex yields showed that Sephadex G-25 column is a similar method of quality control when compared with the procedures of paper chromatography and thin layer chromatography on silica gel. It was also observed that chromatographic behavior in Sephadex was not affected by the pH adjustment in final preparation of the complex.

1. INTRODUCTION

Rhenium-188 (^{188}Re) is an important radioisotope in a variety of therapeutic applications. The use of targeted therapy with radionuclides has become increasingly popular in the field of oncology and one of the clinical interests for ^{188}Re is the palliative management of pain from bone metastases. The hydroxyethylidene diphosphonic acid (HEDP) is the most studied phosphonate in this therapeutical application and has been found to form a stable complex with $^{186/188}$Re. [1, 2, 3]

The W-188/Re-188 generator provides Re-188, a β^- emitting radionuclide with favorable physical characteristics with 16.9 hour-half-life, maximal energy of 2.12 MeV and gamma photon of 155 keV (15% abundance) suitable for imaging.

The quality control measurements before the administration of radiopharmaceuticals to humans are necessary to guarantee its effectiveness and safety. The desired chemical form of the radiopharmaceutical as an end product can be evaluated by several analytical methods using a fraction of total radioactivity and the result is expressed as the radiochemical purity

value (RCP), in percentage. The high yield of RCP is the goal in the development of radiopharmaceuticals and the value reflects their stability and the integrity of molecules.

The RCP measures the amount of impurities in the labeled products that can arise from several effects including the solvent, temperature, pH, oxidizing or reducing agents and decomposition which are related to the radiolabeling procedures and the chemical composition of the process.

The gel chromatography is a useful method for separating different components from a radiopharmaceutical preparation. The system is applied for the quality control of ^{99m}Tc-radiopharmaceuticals, where free, bound and unbound hydrolyzed ^{99m}Tc species can be separated and identified using Sephadex gel and saline as the elution solvent. In this case, the ^{99m}Tc-chelate is eluted first, $^{99m}TcO_4^-$ comes through next and the hydrolyzed ^{99m}Tc is retained by the column. In several ^{99m}Tc-labeled preparations the chelate binds to Sephadex gel, which causes problems in the separations of the impurities. Examples are ^{99m}Tc-gluconate and ^{99m}Tc-mannitol which are adsorbed on the Sephadex column. [4]

The aim of this work is to perform a comparative study of the ^{188}Re-HEDP behavior in gel chromatography with methods routinely used to determine the RCP in ^{188}Re-HEDP, such as paper and thin-layer chromatography, often described in the published literature. The reason for this study is that the RCP methods are not always complete or convenient, being sometimes difficult to select and perform an adequate RCP assay.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals and reagents were analytical grade. The bone seeking agent to be radiolabeled with ^{188}Re was in the form of a lyophilized kit which composition was 30 mg of HEDP, 3 mg of ascorbic acid and 7 mg of stannous chloride ($SnCl_2.2H_2O$). The ^{188}Re was obtained as sodium perrhenate ($Na^{188}ReO_{4)}$ from a 17 GBq alumina-based ^{188}W-^{188}Re generator system eluted with 0.9% saline, provided by Oak Ridge National Laboratory (ORNL, USA).

2.2 Radiolabeling of ^{188}Re-HEDP

The labeling was carried out with 1 mL of perrhenate (^{188}Re) and 20 μL of rhenium carrier and the solution heated up to 100°C for 30 minutes. The carrier solution was prepared as follows: 20 mg of rhenium oxide (VII) into 200 mL of saline purged with N_2. After a 60 minutes cooling time, the final pH was adjusted to 5 with 2 mL of 0.3 M sodium acetate trihydrate buffer. The activity concentration was between 185 and 370 MBq/mL.

2.3 Radiochemical Purity (RCP)

The radiochemical purity of ^{188}Re-HEDP was determined by:

1- Instant thin layer chromatography (TLC) using in the ITLC plates (ITLC/SG, Gelman) as support and saline solution as mobile phase, where reduced rhenium had Rf=0 and the labeled product Rf=0.9-1,

2- Paper chromatography (PC) using in Whatman 1M paper support and acetone as mobile phase, where the free-perrhenate unreacted had Rf=0.9-1 and labeled product Rf=0.

The strips for both methods 1 and 2 were cut 5 cm from the spot line and the activities were measured in a HPGe detector. The labeling efficiency (E%) was calculated as follows:

$$\textit{Labeling efficiency} = E\,(\%) = 100 - (\%)\ \text{impurities} - (\%)\ ReO_4^- \quad (1)$$

3- Gel chromatography, where a sample of the labeled product was loaded on the top of a column of Sephadex G-25 PD-10 (medium) conditioned with 0.9% NaCl solution. Sequential fractions of the eluate were collected and the radioactivity was measured in each fraction with a HPGe detector and a dose calibrator from Capintec.

The labeling efficiency measured by the gel chromatography technique was calculated in two different ways, depending on the detector employed:

$$Yield = Y(\%) = [(A_1 - A_2) / A_1] \times 100, \text{ where:}$$
A_1 is the load activity at Sephadex, measured in the dose calibrator;
A_2 is the residual activity at Sephadex measured in the dose calibrator (2)

$$R_{area}(\%)\ or\ Counts\ (\%) = \{100 - [(A_t - A_o) / At\ ] \times 100\}, \text{ where:}$$
A_t is the total area or counts at the peak of 155 keV in HPGe;
A_o area or counts at the peak of 155 keV in HPGe of the first fraction eluted (10 mL) (3)

3. RESULTS AND DISCUSSION

Gel filtration is a simple chromatography technique and separates molecules on the basis of differences in their size. The technique can be applied to separations of components in a sample based on the molecular weight [5] or cation exchange properties.

For this reason, the first experiments were performed to analyze the behavior of the radiolabeled compound ^{188}Re-HEDP and $Na^{188}ReO_4$ in the Sephadex column. The separation can be seen in figure 1. The results with ^{188}Re-HEDP at pH 1.5, shows that 94.8% of total activity was eluted in the first 10 mL of the elution with saline.

Figure 1 also shows that the total activity of $Na^{188}ReO_4$ moved further into the matrix and the rate of recovering was 99.8%. The elution was between the 11 and 18 mL of the eluted solvent. This is in agreement to the theoretical separation process that predicts that larger molecules are eluted first.

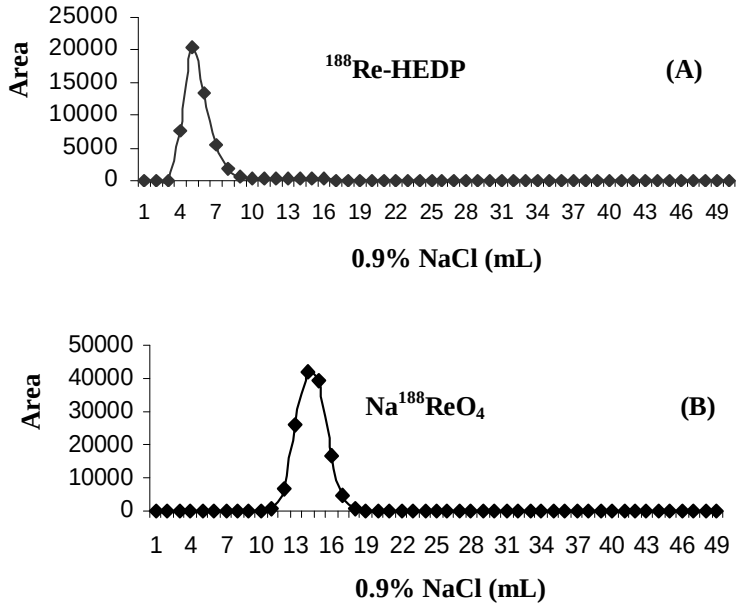

Figure 1. Elution profile of ^{188}Re-HEDP at pH 1.5 (A) and $Na^{188}ReO_4$ (B).

In the labeling procedure of ^{188}Re-HEDP, it is necessary to adjust the pH to 5 after the cooling time. So it was important to analyze the elution profile of ^{188}Re-HEDP at pH 5. Figure 2 shows that the behavior did not change with the addition of the buffer.

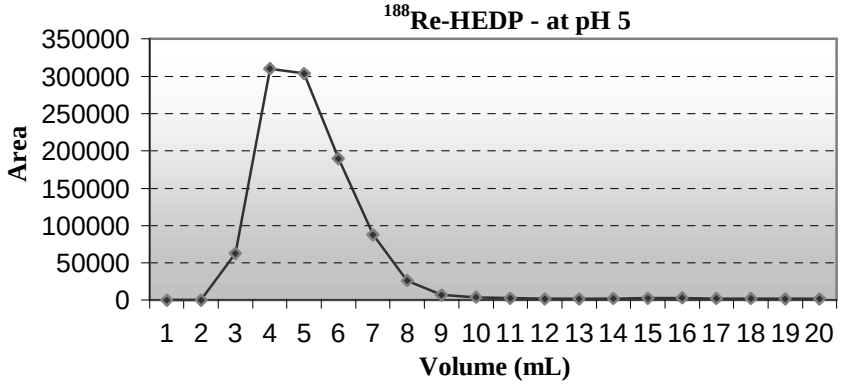

Figure 2. Elution profile of radiolabeled ^{188}Re-HEDP at pH 5 after the addition of 0.3 M sodium acetate trihydrate.

These results are similar to those described by Gasiglia [6], that used this procedure for the RCP of ^{153}Sm-HEDP, that was the basis and motivation of the present work.

The results using Sephadex were compared with those obtained by ITLC and PC and are shown in Table 1.

Table 1. Labeling yields measured with different chromatography techniques: PC, ITLC and Sephadex gel.

Experiments (n)	*E(%)*	*Y(%)*	R_{Area}*(%)*	*Counts (%)*
1	97.73	96.53	97.29	98.12
2	98.51	96.86	97.04	97.42
3	98.47	97.31	98.00	98.25
4	98.87	94.62	97.92	97.71
5	96.38	95.31	95.93	95.98
6	97.95	95.04	97.57	98.85
7	97.90	96.41	97.38	97.94
8	98.11	96.23	ND	ND
9	98.03	96.97	ND	ND
10	97.50	95.22	ND	ND
Mean	***97.95***	***96.05***	***97.30***	***97.75***
SD	0.68	0.93	0.70	0.90
CV	0.70	0.97	0.71	0.92

* ND – not determined.

There is a good agreement with the different techniques employed for the determination of the RCP of ^{188}Re-HEDP.

4. CONCLUSIONS

The labeling conditions to obtain ^{188}Re-HEDP have been standardized and the quality control tests using Sephadex showed that it has applicability because the results are in agreement with the conventional techniques of ITLC and PC. The system can separate different species of ^{99m}Tc, based in the size exclusion theory, but one can not forget the weak cation exchange characteristic of the gel to explain the overall behavior.

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