

Adsorption of ^{188}Re Complexes with Aminomethylenephosphonate on Hydroxyapatite

Kazuyuki Hashimoto,* Hiromitsu Matsuoka, and Mishiroku Izumo

Department of Research Reactor, Japan Atomic Energy Research Institute, Tokai-mura, Ibaraki-ken 319-1195, Japan

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The adsorption properties of newly developed ^{188}Re complexes with aminomethylenephosphonate (amp) (EDTMP - Ethylenediamine-N,N,N',N'-tetrakis(methylenephosphonic acid), EDBMP - Ethylenediamine-N,N'-bis(methylenephosphonic acid) and NTMP - Nitritoltris(methylenephosphonic acid)) on hydroxyapatite was investigated. The adsorption of ^{188}Re complexes was strongly affected by pH and ionic strength in a solution. The adsorption coefficient (ml/g), which is defined by the ratio of the concentration of the complex per unit weight of the hydroxyapatite to the concentration in unit volume of the solution, increased with decreasing pH and ionic strength. These observations suggest that one of the causes of the adsorption of ^{188}Re -amp is considered to be the electrostatic forces between the negative ^{188}Re -amp complex and the positive HAP surface. The adsorption affinity of the ^{188}Re -amp complex for HAP increased in the order of ^{188}Re -EDBMP < ^{188}Re -NTMP < ^{188}Re -EDTMP. Furthermore, the adsorption coefficient of carrier-free ^{188}Re -EDTMP was larger than that of carrier-added one under the same conditions. From a detailed comparison of the effect of the Re carrier concentration on the adsorption coefficient of ^{188}Re -EDTMP with its structural analysis by HPLC, we concluded that the Re carrier effect on the adsorption coefficient was caused by the different distribution of the components in the ^{188}Re -EDTMP mixture between the carrier-free and the carrier-added complex.

Introduction

Rhenium-186 (^{186}Re : $T_{1/2}$, 89.24 h; E_{β} , 1.07 MeV; E_{γ} , 137 keV) and rhenium-188 (^{188}Re : $T_{1/2}$, 17.005 h; E_{β} , 2.12 MeV; E_{γ} , 155 keV) are of current interest for therapeutic applications in the field of nuclear medicine especially for the palliative treatment of metastatic bone pain, because of their energetic beta particles and gamma rays suitable for imaging.¹⁻³ Rhenium-186 with a carrier can be produced in nuclear reactors, however ^{188}Re can be obtained at carrier-free levels from a ^{188}W - ^{188}Re generator.⁴ The diphosphonate ligands such as MDP (methylenediphosphonic acid), HMDP (1-hydroxymethylene-1,1-diphosphonic acid) labeled with $^{99\text{m}}\text{Tc}$ have been widely used as bone imaging agents. Preparations of ^{186}Re and ^{188}Re complexes with diphosphonate ligands including MDP,^{5,6} HEDP (1-hydroxyethylidene-1,1-diphosphonic acid),⁷⁻¹³ AEDP (1-amino-ethylidene-1,1-diphosphonic acid),¹⁴ APD (3-amino-1-hydroxypropylidene-1,1-bisphosphonic acid)¹⁵ and ABP (4-amino-1-hydroxybutylidene-1,1-bisphosphonic acid)¹⁶ are reported and they are found to be good bone seeking agents similar to $^{99\text{m}}\text{Tc}$ diphosphonates. Among them, the ^{186}Re -HEDP is reported to show effective palliation of metastatic bone pain.¹⁷⁻²² The EDTMP, aminomethylenephosphonate derivative, labeled with ^{153}Sm has also shown to be a promising agent for effective palliative treatment of widespread skeletal metastases.²³⁻²⁶

In vitro studies on adsorption properties of labeled compounds with phosphonate-containing ligands on bone have been limited such as $^{99\text{m}}\text{Tc}$ -diphosphonate,²⁷⁻³¹ tin ($^{117\text{m}}$, $^{119\text{m}}\text{Sn}$)-diphosphonate³² and ^{153}Sm -EDTMP.^{33, 34} The adsorption study of $^{186/188}\text{Re}$ complexes has not been so far reported. In the previous paper,³⁵ we determined optimum conditions for synthesis of ^{188}Re complexes with aminomethylenephosphonate (EDTMP, EDBMP and NTMP) as shown in Figure 1. In this paper, we describe the adsorption properties of the newly

developed ^{188}Re complexes with aminomethylenephosphonate on hydroxyapatite, which is the major constituent of the hard tissues of bone. The influence of the shaking time, pH and ionic strength on the adsorption was investigated from fundamental aspects. The difference in adsorption properties between carrier-free (cf-) and carrier-added (ca-) ^{188}Re -EDTMP was also studied.

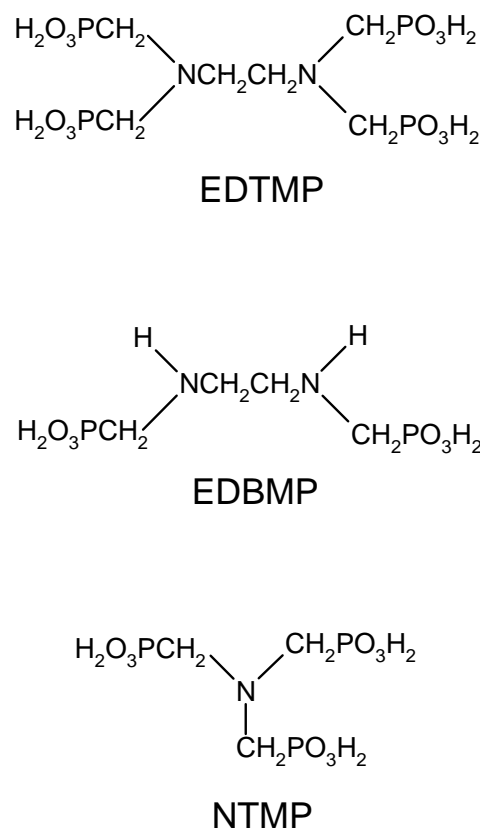


Figure 1. Structures of the aminomethylenephosphonate ligands used in this study.

*Corresponding author. E-mail: hashi@popsvr.tokai.jaeri.go.jp. FAX: +81-29-282-5932.

Experimental

Production of $^{188}\text{W}/^{188}\text{Re}$ Generator. Typically 25–35 mg of enriched ^{186}W as WO_3 (99.79% enrichment: euriso-top) was irradiated for 26–52 days in JAERI JMTR (a thermal neutron flux of $2.7 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$). The $^{188}\text{W}/^{188}\text{Re}$ generator was prepared by the alumina column system.⁴ The irradiated WO_3 was dissolved in 2 M NaOH. The pH of a part of the ^{188}W solution was adjusted to about 2 using HCl. This solution was absorbed onto the alumina column (10 mm \times 60 mm; BIO-RAD, AG-4, 100–200 mesh), which was conditioned with 0.01 M HCl. The column was then washed with normal saline. Rhenium-188 was eluted with normal saline after the equilibrium between ^{188}W and ^{188}Re had almost been reached. The chemical form of ^{188}Re obtained from the generator was checked by paper chromatography in 0.9% saline. The chemical form of ^{188}Re was found to be perrhenate ion ($^{188}\text{ReO}_4^-$) and was free from ^{188}W breakthrough. Rhenium-188 solution (2×10^5 – $1 \times 10^6 \text{ Bq ml}^{-1}$) was obtained from the generator in a saline solution and was used for labeling purposes without further purification.

Synthesis of ^{188}Re -Aminomethylenephosphonate (^{188}Re -amp) Complex. Aminomethylenephosphonate derivatives (EDTMP, EDBMP and NTMP) were purchased from Dojindo Laboratories, Japan. All other chemicals used were of guaranteed reagent grade.

To 0.2 M aminomethylenephosphonate aqueous solution, distilled water, 38 mg/ml (0.216 M) l-ascorbic acid aqueous solution, 3 M HCl for pH adjustment, a ^{188}Re solution from the generator, and 38 mg/ml (0.168 M) tin(II) chloride dihydrate solution (0.6 M HCl) were added. The final concentrations of aminomethylenephosphonate, l-ascorbic acid and tin(II) chloride dihydrate in the reaction mixture were 0.1 M, 2.85 mg/ml and 0.57 mg/ml. The pH of the solution was 0.8–0.9. The final concentration of the Re varied from carrier-free levels to 20 $\mu\text{g Re/ml}$ ($1.074 \times 10^{-4} \text{ M}$) by adding NH_4ReO_4 to the ^{188}Re solution. The reaction mixture was vigorously stirred and allowed to react in boiling water for 30 min. The formation of ^{188}Re -amp was checked by silica gel TLC with acetone as described in the previous paper.³⁵ The ^{188}Re -amp complex was obtained in a more than 95% yield. The distribution of ^{188}Re in TLC was measured with a radioanalytic imaging system (AMBIS-100).

Adsorption Studies of ^{188}Re -amp Complex on Hydroxyapatite. Hydroxyapatite for liquid phase chromatography

was used as a adsorbent, supplied by Wako Pure Chemicals Co. as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$.

In a vial, 100 mg of HAP was suspended ultrasonically in 1 ml of HCl, distilled water, sodium acetate or NaOH solution for pH adjustment and 0.5 ml of 4 M NaCl solution for ionic strength adjustment. Then 0.5 ml of the ^{188}Re complex solution was added. The concentration of aminomethylenephosphonate in the final solution was $2.5 \times 10^{-2} \text{ M}$. The vial was shaken mechanically in a water bath at 37°C. At regular time intervals, the mixture was centrifuged and an aliquot of the solution was taken for radioactivity measurement. The radioactivity of the ^{188}Re complex itself in the solution was corrected by the fraction of the ^{188}Re complex measured by silica gel TLC. The adsorption coefficient (ml/g), which is defined by the ratio of the concentration of the complex per unit weight of the hydroxyapatite to the concentration in unit volume of the solution, was determined by measuring the radioactivity of the solution and the standard solution of ^{188}Re . The radioactivity of ^{188}Re was measured by a Cherenkov-counting method using a liquid scintillation counter (Beckman LS3801).³⁶

HPLC Analysis of ^{188}Re -EDTMP. The liquid chromatograph used was a Waters 2690 separations module equipped with a Waters 996 photodiode array detector and a radio-HPLC detector (Packard Radiomatic 515TR). The ^{188}Re -EDTMP was analyzed by reversed-phase ion-pair chromatography (Hypersil C18 BDS-5, 4.6 mm $\phi \times$ 150 mm) using a gradient system comprising 0.008 M tetrabutyl ammonium hydroxide (TBA) / 10% methanol (pH 6.0) (solvent A) and 0.008 M TBA / 60% methanol (pH 6.0) (solvent B). The flow rate was 1.0 ml/min and the gradient was defined by the following points (min% B): 0-0, 5-85, 30-100.

Results and Discussion

The fractions of ^{188}Re -EDTMP adsorbed on HAP increased with the elapse of time and attained an almost constant value after 24 hours within the experimental error, as shown in Figure 2. For the adsorption isotherm experiments, a time period of 24 h was used. In the previous paper,³⁵ the stability of the carrier-free (cf-) ^{188}Re -amp was found to be lower than that of the carrier-added (ca-) ^{188}Re -amp. However, the decomposition of cf- ^{188}Re -EDTMP to perrhenate was scarcely observed during the experiment, as shown in Figure 3. It was

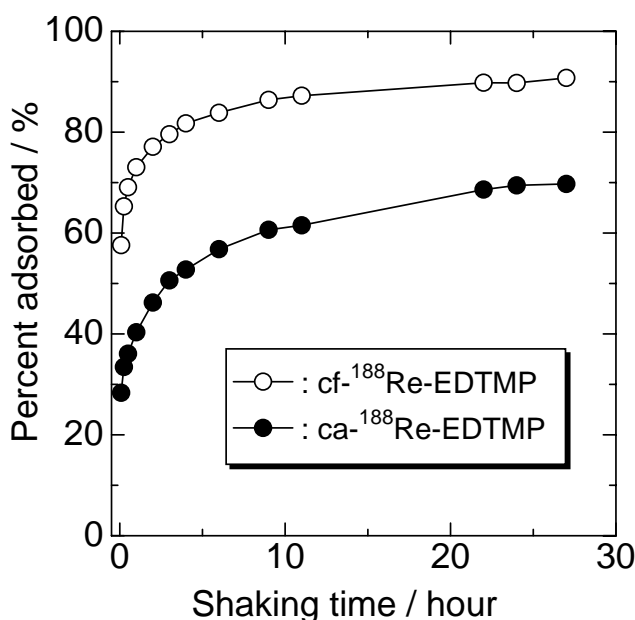


Figure 2. Typical time variation in the adsorption of carrier-free (cf-) and carrier-added (ca-) ^{188}Re -EDTMP (pH 3.5, HAP 100 mg, $I=1.0$).

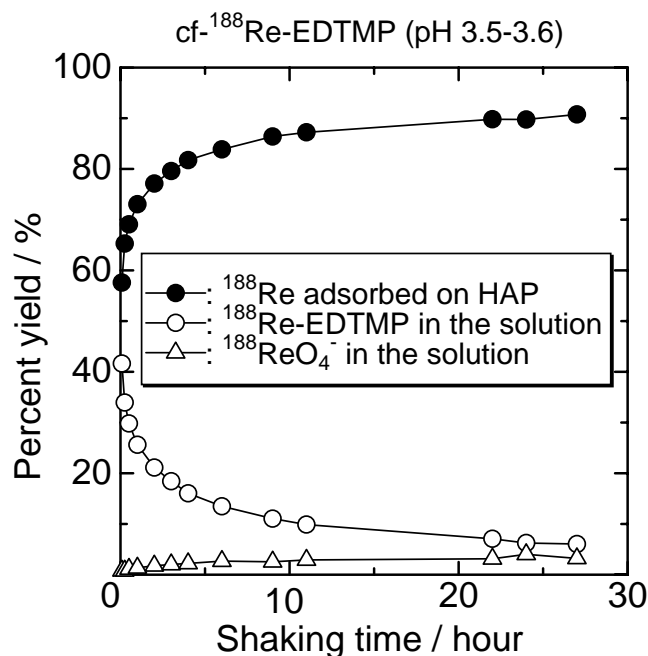


Figure 3. Chemical species during the adsorption experiment using carrier-free ^{188}Re -EDTMP (pH 3.5-3.6, HAP 100 mg, $I=1.0$).

less than 3–4% even after 24 hours. Furthermore, no adsorption of perrhenate to HAP was observed.

A plot of the concentration of ^{188}Re -EDTMP in the adsorbent against that in the solution indicated a linear isotherm as shown in Figure 4. Linearity of the plot is equivalent to the constant adsorption coefficient in the range of the complex concentration in this experiment.

The influence of pH and ionic strength on the adsorption coefficient was investigated. Figure 5 showed the influence of pH on the adsorption coefficient. The adsorption coefficient increased with decreasing pH. This result is consistent with the adsorption behavior of $^{99\text{m}}\text{Tc}(\text{Sn})$ pyrophosphate on $\text{Ca}_3(\text{PO}_4)_2$.³¹ It was observed that the adsorption of $^{99\text{m}}\text{Tc}(\text{Sn})$ pyrophosphate at pH 4.0 was higher than at pH 7.4. The pH influenced the charge of surface (zeta potential) of HAP. The surface of HAP is heterogeneous, with both positive and negative charge, but with the charge density dependent on pH. When pH is higher than point of zero charge (pzc), the zeta potential is negative and the negative charge on the HAP surface will dominate; when pH is lower than pzc, the

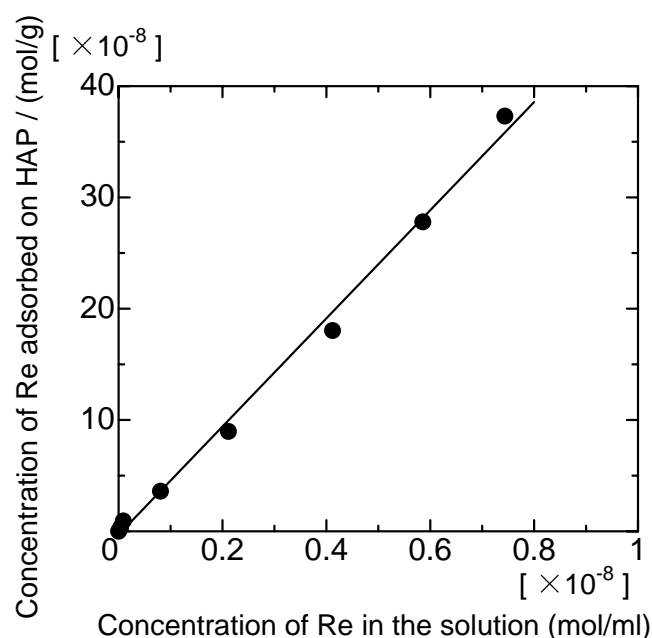


Figure 4. Adsorption isotherm of ^{188}Re -EDTMP (Re final concentration: 1.36×10^{-11} mol/l (carrier-free; 92.5 kBq/ml) $\sim 2.85 \times 10^{-5}$ mol/l). Correlation coefficient $R=0.997$.

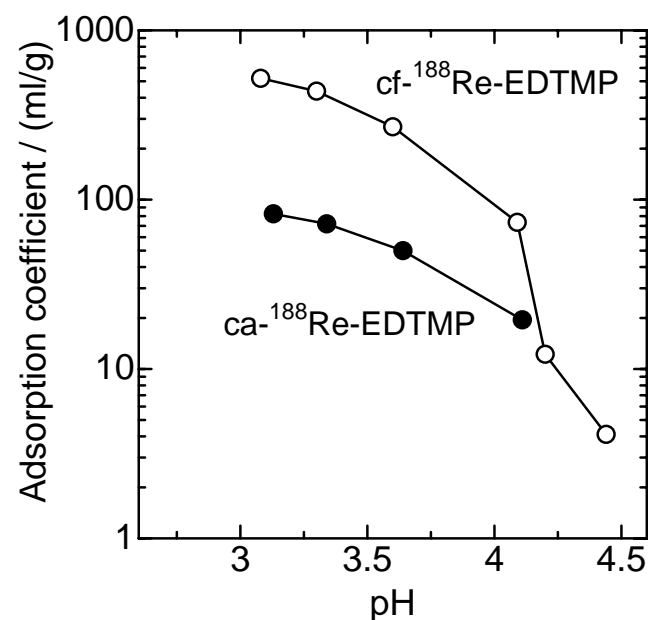


Figure 5. Influence of pH on the adsorption coefficient of ^{188}Re -EDTMP (HAP 100 mg, $I=1.0$, 24 h).

zeta potential is positive and the positive charge on the HAP will dominate. The pzc on HAP has been found to lie between pH 6.4 and 8.5.³⁷⁻⁴¹ Therefore, the charge of HAP in the pH range (3–4.5) of the present study will be positive because the pH was lower than the pzc. Taking into consideration that the ^{188}Re -amp complexes had negative charges analyzed by electrophoresis,³⁵ one of the causes of the adsorption of ^{188}Re -amp is considered the electrostatic forces between the negative ^{188}Re -amp complex and the positive HAP surface.

Figure 6 showed the influence of ionic strength on the adsorption coefficient. The adsorption coefficient decreased with increasing ionic strength. These results also show that the adsorption is mainly electrostatic. That is, it is conceivable that the adsorption coefficient decreased due to the electrostatic shielding effect and to the competitive adsorption between them by adding NaCl.

The comparisons of the adsorption coefficient of the ^{188}Re -amp complexes are shown in Figure 7. The adsorption coefficient increased in the order: ^{188}Re -EDBMP $<$ ^{188}Re -NTMP $<$ ^{188}Re -EDTMP under the same conditions. However, it is thought that the adsorption coefficients of the ^{188}Re -amp complexes are also influenced by the excess aminomethylene-

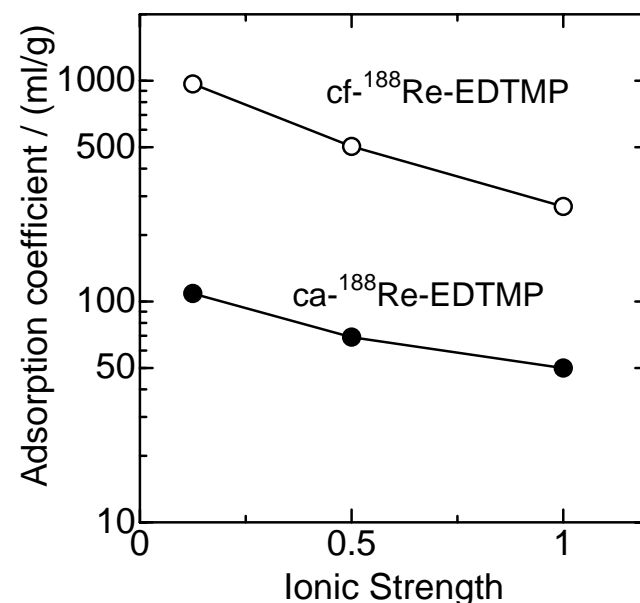


Figure 6. Influence of ionic strength on the adsorption coefficient of ^{188}Re -EDTMP (pH 3.6, HAP 100 mg, 24 h).

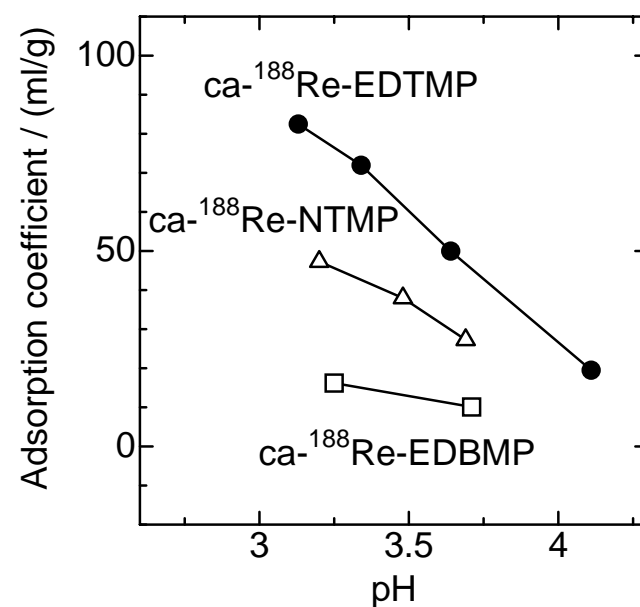


Figure 7. Comparison of the adsorption coefficient of the carrier-added ($20 \mu\text{g Re ml}^{-1}$) ^{188}Re -amp complex (HAP 100 mg, $I=1.0$, 24 h).

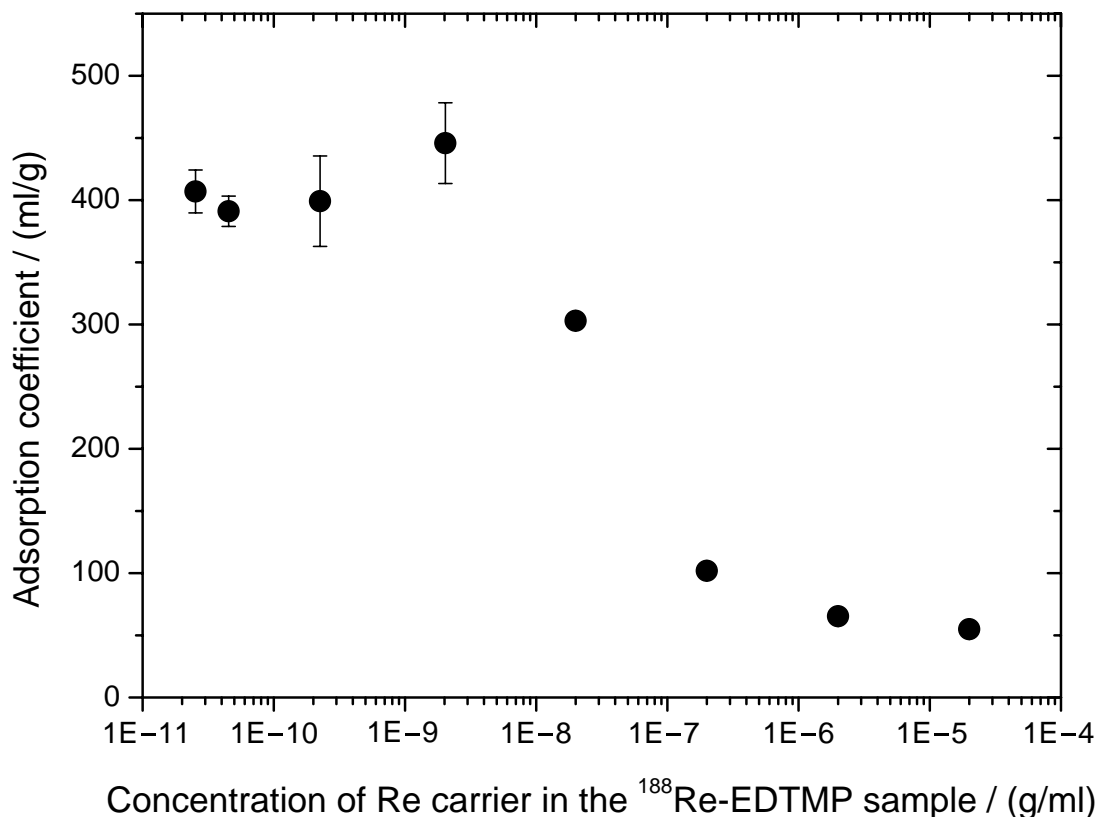


Figure 8. Effect of the carrier concentration on the adsorption coefficient of ^{188}Re -EDTMP (HAP 100mg, pH 3.6, 24 h).

phosphonate in the solution. Two competitive adsorption reactions were occurred in this experiment. One is adsorption between ^{188}Re -amp and HAP, another is adsorption between aminomethylenephosphonate and HAP. In order to estimate the effect of aminomethylenephosphonate on the adsorption to HAP, the adsorption coefficient of the ^{188}Re complex was measured by adding further aminomethylenephosphonate solution. The adsorption coefficient of ^{188}Re -amp decreased by adding aminomethylenephosphonate solution. The magnitude of the decrease was in the order $\text{EDBMP} < \text{NTMP} < \text{EDTMP}$, that is, the adsorption affinity of aminomethylenephosphonate for HAP increased in the order $\text{EDBMP} \leq \text{NTMP} < \text{EDTMP}$. Taking into account the order of the adsorption affinity of aminomethylenephosphonate for HAP, the adsorption affinity of the ^{188}Re -amp complex for HAP increased in the order ^{188}Re -EDBMP $<$ ^{188}Re -NTMP $<$ ^{188}Re -EDTMP. The order of the adsorption affinity of the ^{188}Re -amp complex obtained in this study corresponds to the number of phosphonate groups in aminomethylenephosphonate, supporting that the interaction between phosphonate groups in the ^{188}Re -amp complex and calcium ions on the surface of HAP was one of major factors in terms of the adsorption affinity of ^{188}Re -amp for HAP.

As shown in Figures 5 and 6, the adsorption coefficient of carrier-free ^{188}Re -EDTMP was larger than that of carrier-added one (20 $\mu\text{g}/\text{ml}$) under the same conditions. From the linear isotherm as shown in Figure 4, the adsorption coefficient of the ^{188}Re complex should be independent of the Re carrier concentration. In order to clarify the effect of carrier, the influence of the concentration of the Re carrier on the adsorption coefficient of ^{188}Re -EDTMP was examined in detail. The adsorption coefficient was constant in the range from carrier-free to 0.002 $\mu\text{g}/\text{ml}$, but decreased with increasing the concentration of the Re carrier in the ^{188}Re -EDTMP sample over 0.002 $\mu\text{g}/\text{ml}$ to 20 $\mu\text{g}/\text{ml}$, as shown in Figure 8. Rhenium-diphosphonate complexes, as well as technetium complexes, are thought to be a mixture of polymeric complexes containing tin (Sn) by HPLC analysis^{5, 8} and EXAFS data.⁴² And the ratio of these components of technetium-diphosphonates is known to depend

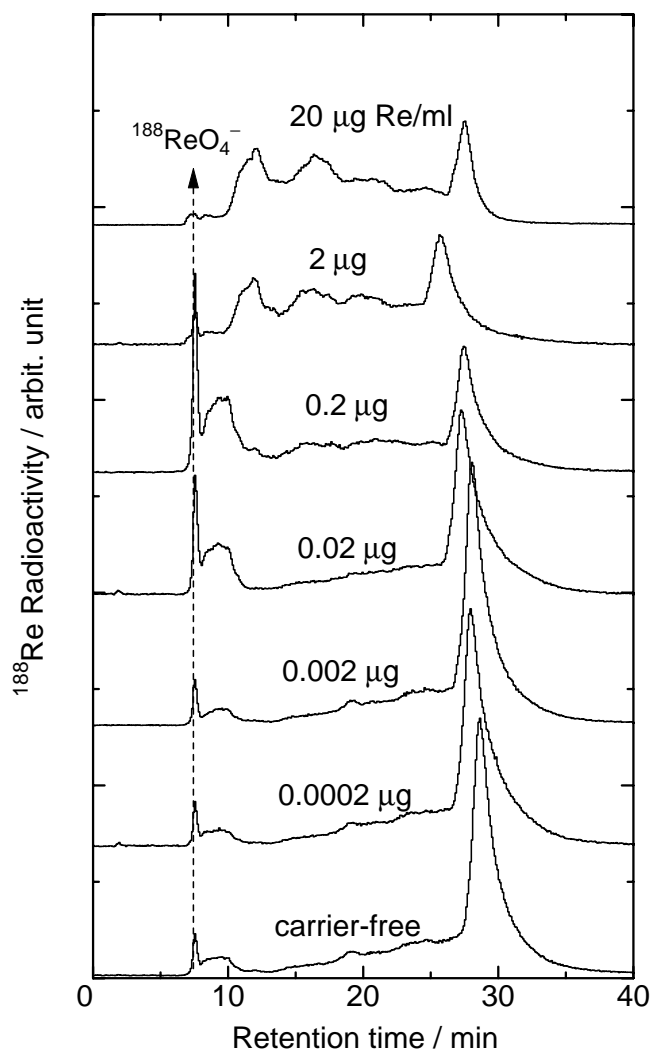


Figure 9. Effect of the carrier concentration on the ion-paired reversed phase HPLC chromatogram of ^{188}Re -EDTMP.

on the formation conditions such as pH, adding the carrier, the concentration of diphosphonate and the difference of reductant (SnCl₂ or NaBH₄).⁴³⁻⁴⁶ And, the analysis of ¹⁸⁸Re-EDTMP by ion-paired reversed phase chromatography showed that the chromatogram of carrier-free complex was different from that of carrier-added complex.⁴⁷ The effect of the concentration of Re on the chromatogram of ¹⁸⁸Re-EDTMP was examined by ion-paired reversed phase chromatography, as shown in Figure 9. The chromatogram was almost constant in the range from carrier-free to 0.002 µg/ml, but changed gradually with increasing the concentration of the Re carrier over 0.002 µg/ml to 20 µg/ml. The change of the chromatogram corresponded to the change of adsorption coefficient. In conclusion, the effect of the concentration of Re on the adsorption coefficient was caused by the different distribution of the components in the ¹⁸⁸Re-EDTMP mixture between the carrier-free and the carrier-added complex.

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