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Synthesis of a new bisphosphonic acid ligand (SEDP) and preparation of a ^{188}Re-(Sn)SEDP bone seeking radiotracer

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Abstract

The new bisphosphonate ligand SEDP (2-sulfonato-1,1-ethylidene bisphosphonic acid) has been synthesized and characterized, including the determination of the protonation constants, and used to form ^{188}Re-(Sn)SEDP from Na^{188}ReO$_4$. The title compound ^{188}Re-(Sn)SEDP shows slightly greater bone uptake and less kidney uptake than ^{188}Re-(Sn)HEDP in rat biodistribution studies. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Rhenium-188; Bisphosphonate; Diphosphonate; HEDP

1. Introduction

Rhenium-188 (β; E_{max} = 2.12 MeV; γ photon, 155 KeV; 15%; $t_{1/2}$ = 17.0 hrs) is one of the most attractive radioisotopes for radiotherapy and radioimmunotherapy since it is easily obtained carrier free as perrhenate ion (ReO_4^-) in normal saline solution [13,14] or perrhenic acid ($HReO_4$) in aqueous HCl or HNO_3 [16] from a rugged and economical alumina based W-188/Re-188 generator system. The alumina-based W-188/Re-188 generator is completely analogous to the Mo-99/Tc-99m generator, however, the W-188/Re-188 generator system has a greater shelf life, since W-188 ($t_{1/2}$ = 69 days) has a much longer half-life than Mo-99 ($t_{1/2}$ = 66 hrs). Because the ^{188}W parent is available only in low specific activity a new system of elution and concentration has been developed to provide a bolus of as much as 14.8 GBq of ^{188}ReO4$^-$ in less than 1 ml of saline. [8] The gamma emission of Re-188 allows for easy in vivo location of the radiopharmaceutical.

There is a great deal of interest in geminal bisphosphonates or bisphosphonic acids (also called diphosphonates or diphosphonic acids) [5]. These versatile molecules are characterized by a P-C-P structural element (Fig. 1), and have a variety of important uses such as metal chelation ligands in separation science [24], therapeutic use in patients with two main types of disorders, ectopic calcification and ossification, and increased bone resorption [5], and in nuclear medicine as ligands for radiometals in bone-seeking diagnostic and therapeutic agents [11,21].

One of the least understood classes of diagnostic ^{99m}Tc imaging agents is the class of skeletal imaging agents prepared with geminal bisphosphonates such as methylene diphosphonic acid (MDP) and 1-hydroxyethane-1,1-diphosphonic acid (HEDP) [2,11]. These bisphosphonate ligands can also be used to prepare the analogous ^{186}Re and ^{188}Re compounds [9]. The best-studied Re-bisphosphonate is that prepared by using HEDP to produce ^{186}Re(Sn)HEDP, which includes carrier Re [2,4,26]. This radiopharmaceutical can also be prepared using Re-188 to form ^{188}Re(Sn)HEDP, in which case carrier Re must also be added in order for the bone seeking agent to form in good yield [10,15]. Reaction conditions for the synthesis of ^{186}Re(Sn)MDP must be acidic (pH 1.4–1.6) in order to facilitate reduction of the perrhenate ion with stannous ion and to keep the reduced species from being oxidized [3], although the synthesis and stabilization of ^{186}Re(Sn)HEDP is different and can be accomplished at pH 5–8.

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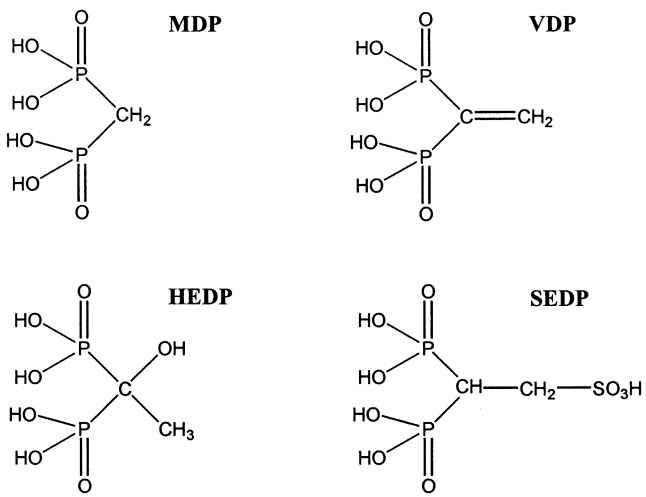


Fig. 1. Chemical formulas of selected bisphosphonates.

Both ^{186}Re(Sn)HEDP and carrier added ^{188}Re(Sn)HEDP have been used quite successfully [18–20] in alleviating pain and treatment of multiple metastatic foci of bone in bone cancer patients. It has also been reported [25] that the comparative instability of the ^{186}Re(Sn)HEDP radiopharmaceutical to in vivo oxidation to perrhenate is a possible advantage. The radiopharmaceutical ^{186}Re(Sn)HEDP "washes off" from normal bone faster than it does from abnormal (cancerous) bone and thus the abnormal/normal bone uptake ratio increases with time.

Besides ^{186}Re(Sn)MDP and ^{186,188}Re(Sn)HEDP, few Re-bisphosphonates have been described in the literature [9–11]. This is in stark contrast to ^{99m}Tc-bisphosphonates where many different bisphosphonate ligands have been utilized in bone imaging studies. We desired to extend the medicinal chemistry of the bisphosphonates by designing a molecule that contained a sulfonic acid moiety, as this functional group had not been reported in the literature on a gem-bisphosphonate. We knew that under in vivo conditions the sulfonic acid moiety would confer additional anionic character to any bisphosphonate it was attached to and we postulated that this may favorably influence the in vivo biocharacteristics of ^{99m}Tc and ^{186,188}Re bone seeking agents. Because even what appears to be modest structural changes can have dramatic effects on the targeting and stability of Tc and Re bisphosphonates, it is important to evaluate the effects of other analogues in order to try and optimize the biological properties of these important complexes.

In this paper we report the synthesis and characterization of a new bisphosphonate, SEDP (2-sulfonatoethylidene-1,1-bisphosphonic acid) and the synthesis and characterization of the ^{188}Re(Sn)SEDP complex and biodistribution studies in rats. The ^{188}Re(Sn)HEDP agent was also prepared and used as a control in order to provide a direct comparison of these two similar compounds.

2. Experimental

2.1. Materials and methods

The ^{31}P, ^{13}C and ^{1}H NMR spectra were recorded on a Varian 300 Mhz spectrometer with a 5mm multinuclear probe. The SEP-PAK Accell QMA cartridges were purchased from Waters Associates, Milford, Massachusetts. Unless otherwise noted, all chemicals were of reagent grade. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. High performance liquid chromatography (HPLC) analyses were performed on a Beckman Model 302 dual pump apparatus using an Aminex anion exchange column. The 1-hydroxyethane-1,1-diphosphonic acid (HEDP) ligand was obtained as a 60–70% aqueous solution from Albright & Wilson (Richmond, VA) and purified by recrystallization using glacial acetic acid according to the Merck Index. The vinylidene-1,1-diphosphonic acid (VDPA) was prepared by the method reported in a previous publication [6]. The protonation constants were determined by a potentiometric titration [17] of the sodium salt of SEDPA at a constant ionic strength of 0.1M (NaCl). The p[H] (p[H] = $-\log [H^+]$) was measured using an Orion Ross combination glass electrode calibrated by HCl/NaOH titrations between p[H] 1.5 and 12.5. All titrations were conducted in a thermostated vessel at 25.0 ± 0.1°C under an argon cap.

2.2. Synthesis

2.2.1. (SEDP) 2-sulfonatoethylidene-1,1-bisphosphonic acid

The pH of a solution of 10.0 g (0.021 mol) of VDPA tetrasodium salt decahydrate in 35 ml of water in a 100 ml Erlenmeyer flask was adjusted to pH = 8 with 0.5 *M* HCl. A solution of sodium persulfate ($Na_2S_2O_8$; 100 mg, catalytic amount) and 5.89 g (0.031 mol) of sodium metabisulfite ($Na_2S_2O_5$) dissolved in 10 ml of water was then added. The reaction mixture was adjusted to pH = 8–9, and the flask covered with a watch glass and heated to 95°C for 3.5 hours. The clear solution was then transferred to a round-bottomed flask and rotoevaporated to remove water and the white solid obtained was dried under vacuum at ambient temperature. The crude product contained no unreacted VDPA at this point (when monitored by ^{1}H and ^{31}P NMR), but did contain chloride and hydroxide salts of sodium in addition to the SEDP product. The crude product was dissolved in a minimum of water, the pH adjusted to pH = 12 with NaOH, and then recrystallized by addition of ethanol. After drying under vacuum, the product was obtained as the Na_5SEDP × 28 H_2O salt. Yield: 11.80 g, 0.0112 mol (53.3%). Anal. Calcd. for $C_2H_{59}Na_5O_{37}P_2S$: C, 2.71; H, 6.72; P, 7.00. Found: C, 2.68; H, 6.75; P, 6.97. ^{1}H NMR (D_2O) δ 3.23 ppm (dt, 2H, CH_2, 15 hz coupling H-C-C-P, 5 hz coupling H-C-C-H), δ 2.25 ppm (tt, 1H, CH, 21.4 hz coupling H-C-P, 5 hz coupling H-C-C-H); ^{13}C NMR (D_2O)

δ 49.557 (t, 15.0 hz coupling C-C-P), δ 36.224 (t, 441.7 hz coupling C-P).

2.2.2. ^{188}Re(Sn)HEDP

The same procedures described below for preparation of ^{188}Re(Sn)SEDP were followed to produce ^{188}Re(Sn)HEDP. The synthesis is essentially a slight modification of the literature preparations of ^{186}Re(Sn)HEDP [18,19].

2.2.3. ^{188}Re(Sn)SEDP

A typical preparation follows using deaerated solvents and solutions: A solution of 100 mg of SEDP (9.4×10^{-5} mol) in 3 ml of 0.003 *M* ascorbic acid was placed in a 5 ml reactivial. A solution of 0.5 mg (1.0×10^{-6} mol) of tetrabutylammonium perrhenate carrier in 0.5 ml of ethanol was then added to the vial followed by Re-188 generator saline eluate solution (1.0 ml, 8.8 mCi of Na $^{188}ReO_4$). The reaction was initiated by adding 30 mg (1.3×10^{-4} mol) of stannous chloride dihydrate to the vial, which was capped and placed in a 100°C water bath for 10 min with occasional swirling. The reaction mixture was removed and allowed to cool slowly at room temperature for 2 hrs. A prepared SEP-PAK (prepared by washing a SEP-PAK Accell QMA cartridge with 10 ml of 0.003 *M* ascorbic acid) was loaded with a dilute solution of the reaction mixture. The diluted solution was prepared by adding an additional 20 ml of 0.003 *M* ascorbic acid to the cooled contents of the Re-188 reaction mixture. The SEP-PAK was washed with 10 ml of the 0.003 *M* ascorbic acid solution. The product was then slowly eluted with a pH 7.0/0.3 *M* NaCl solution that was 0.003 *M* in ascorbic acid and 0.01 *M* in SEDP. The first 1 ml of eluent contained unreacted perrhenate (0.3 mCi) and was discarded. The product (5.65 mCi) was contained in the next 3–6 ml. Purity (>96%) was checked by use of standard paper chromatography (Whatman 3MM) using acetone to evaluate levels of $^{188}ReO_4^-$ and saline for $^{188}ReO_2$. The amount of $^{188}ReO_2$ was always less than 1% and the $^{188}ReO_4^-$ was always less than 5% or the preparation was discarded. The radiopharmaceutical was prepared by diluting the 3 ml of ^{188}Re(Sn)SEDP to 6 ml with water and filtering through a 0.22 um millipore filter into a dosing vial.

3. Animal tissue distribution studies

The animal care and use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act and were reviewed and approved by the Oak Ridge National Laboratory Animal Care and Use Committee (ACUC). The distribution of radioactivity was determined following intravenous administration (3.2 μCi of Re-188 in 500 μl) of ^{188}Re(Sn)SEDP or ^{188}Re(Sn)HEDP in a lateral tail vein of Metofane-anesthetized female Sprague-Dawley rats. The animals were allowed food and water ad libitum prior to and during the course of the experiment. The animals were anesthetized with metofane, killed by cervical fracture, and the organs removed, and placed and weighed in tared vials. Samples were counted in a Packard Minaxi 5000 sodium iodide auto gamma counter and reported as percent injected dose per gram of tissue.

4. Results and discussion

The structure assigned to the SEDP ligand was based upon the ^{13}C, ^{1}H, and ^{31}P NMR data. The ^{13}C NMR spectrum and the ^{1}H NMR spectrum of SEDP (Fig. 2) in D_2O with sodium 3-(trimethylsilyl)-1-propanesulfonate as an internal standard is indicative of the assigned structure of the bisphosphonate. The ^{1}H NMR spectrum shows that the protons on carbon "A" are equivalent with coupling to the two phosphorous nuclei to give a triplet split into doublets by the proton on carbon "B". The protons on carbon "B" resonate as a triplet of triplets with coupling to phosphorous and to the protons on carbon "A". The coupling of protons on carbon "B" to the phosphorous nuclei is large compared to their coupling with the protons on carbon "A". The ^{31}P NMR spectrum in D_2O of SEDP shows only one peak for the product at $\delta = +19.2$ ppm relative to phosphoric acid, which is consistent with a bisphosphonate where both equivalent phosphorous nuclei are in the same environment.

The synthesis of the SEDP ligand can be monitored by ^{1}H NMR spectroscopy because the VDPA starting material only has ^{1}H resonances due to the vinyl protons at 6.4 ppm (t, J = 36.1 hz). The extent of reaction is most easily monitored by ^{31}P NMR spectroscopy, however, since the phosphorous nuclei in VDPA resonate at 12.1 ppm and the phosphorous nuclei in SEDP resonate at 19.2 ppm. The VDPA was prepared by the method reported in a previous publication [6], but an alternate synthesis of VDPA has also been reported [1].

A controlled-pH synthesis study of SEDP was followed by ^{31}P NMR spectroscopy. As expected from previous studies described in the literature on bisulfite addition reactions [7,12], the synthesis of SEDP is favored at a slightly basic pH (pH = 7.5–9.5) where the HSO_3^- radical predominates in the equilibrium between the SO_3^- radical and the HSO_3^- radical, while the HSO_3^- radical reacts with the double bond in VDPA via anti-Markovnikov addition. Acidic conditions (pH = 2–6) result in only a low 25–50% conversion of VDPA to SEDP as determined by ^{31}P NMR spectroscopy. It was also determined that in the absence of the $Na_2S_2O_8$ initiator, even at slightly basic pH, VDPA does react with bisulfite to produce several as yet unidentified products. The optimum condition for synthesis of SEDP was determined to be pH = 8 with bisulfite and $Na_2S_2O_8$ initiator, which provides quantitative conversion of VDPA to SEDP.

The protonation constants (log K_a) of SEDP (Table 1) were calculated from the potentiometric titration curves using the program Psequad [27]. The first protonation constant (pKa_1) for the proton on the sulfonic acid moiety of

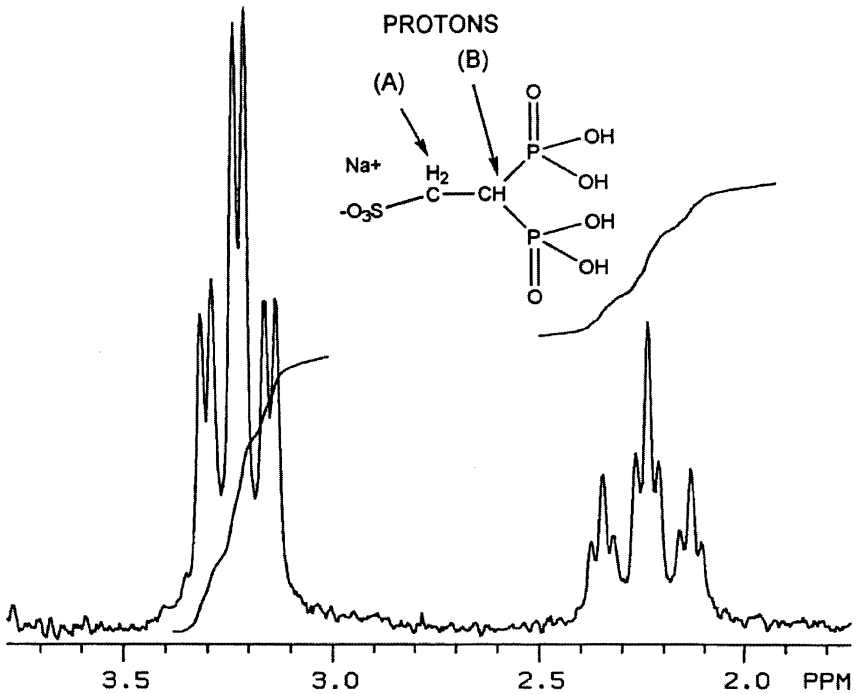


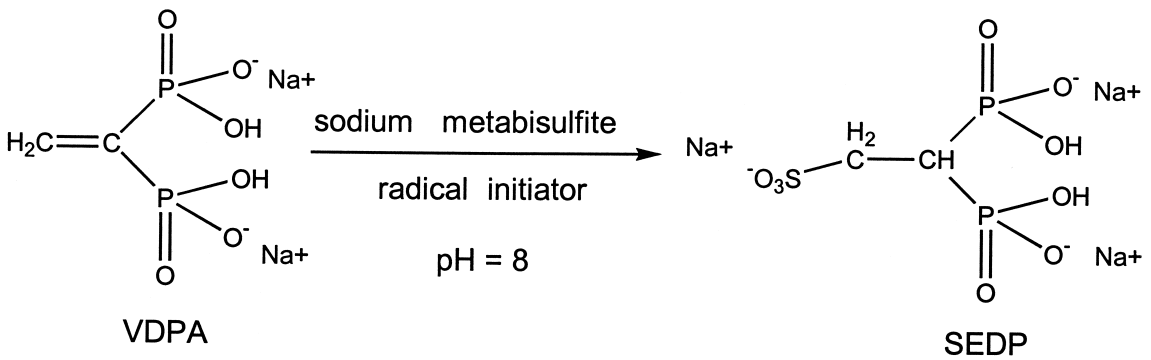
Fig. 2. The ^{1}H NMR spectrum of Na_5SEDP in D_2O. The spectrum shows chemical shifts (in ppm) for the protons on carbon (A) and carbon (B) with the subsequent coupling to each other and the two equivalent phosphorous nuclei.

SEDP can not accurately be determined, and SEDP (as H_5L) is deprotonated above pH = 1 to form the species H_4L^-. The pKa_1 values for the other bisphosphonic acids (Table 1) correspond to the loss of a proton from the bisphosphonic acid group since they contain no sulfonic acid group, and subsequently have higher values. The pKa_2 for SEDP corresponds to the loss of the first proton from the bisphosphonic acid group, and therefore the data shows that the bisphosphonic acid group on SEDP is less acidic than those of the compared bisphosphonates in Table 1. This is not surprising since the sulfonate anion on SEDP should increase the basic character of the molecule.

A species distribution curve (Fig. 3) from the log K_a data has been calculated and graphed. The curve shows that at pH 1 the SEDP species H_4L^- predominates in the protonation equilibria, at pH 4.5 the SEDP species H_3L^{2-} predominates in the equilibria, at pH 7.1 the SEDP species H_2L^{3-} predominates in the equilibria, and at pH 9.6 the SEDP species HL^{4-} predominates in the equilibria.

Conditions for synthesis of the $^{188}Re(Sn)SEDP$ radiopharmaceutical are very similar to formation of the corresponding $^{188}Re(Sn)HEDP$ analog. The $^{186}Re(Sn)HEDP$ compound has been described and characterized as a mixture of as yet unidentified components [4,23] and this has been demonstrated through use of HPLC analyses. With an Aminex anion exchange column using conditions reported in the literature we have also seen very similar HPLC tracings for both $^{188}Re(Sn)SEDP$ and $^{188}Re(Sn)HEDP$. These data clearly illustrate that $^{188}Re(Sn)SEDP$ is not a single molecular compound, but contains a mixture of components similar to that reported for $^{186}Re(Sn)HEDP$.

Biodistribution data for $^{188}Re(Sn)SEDP$ with Sprague-



Scheme 1. Synthesis of SEDP from VDPA in aqueous solution.

Table 1
Acid dissociation constants for selected bisphosphonic acids [25°C, ionic strength $\mu = 0.1$ (0.1 M NaCl or KCl)]

Compound	pKa_1	pKa_2	pKa_3	pKa_4	pKa_5
MDP[a]	1.30	2.35	6.34	8.90	—
VDPA[a]	1.41	2.06	6.01	8.60	—
HEDP[a]	1.56	2.20	6.20	9.12	—
SEDP[a]	—	2.85 ± 0.07	6.33 ± 0.06	7.78 ± 0.06	11.27 ± 0.05

[a] Entries are from [22].
[b] The quoted error is +1 SC.

Dawley rats is shown in Table 2. The compound ^{188}Re(Sn)HEDP was also prepared and used in the same study in order to provide a direct comparison of the two similar compounds. The data reveal that the two compounds have very similar biodistribution patterns in rats. Although the ^{188}Re(Sn)SEDP compound seems to have slightly less uptake in all of the non-target organs such as heart, blood, kidney, liver and lung than ^{188}Re(Sn)HEDP, because of the inherent errors in the study, the data actually show the biodistribution data for the two compounds are comparable. However, the bone uptake (% injected dose/gram of bone) of ^{188}Re(Sn)SEDP is greater than that of ^{188}Re(Sn)HEDP after 60 minutes (2.75 compared to 2.65), and after 1440 minutes (24 hours) it is significantly greater (2.47 compared to 1.94).

The sulfonic acid moiety is a stronger acid than the phosphonic acid moieties on SEDP and is in the form of the sulfonate anion in aqueous solution above pH = 1. The fact that the sulfonate group confers additional anionic character to ^{188}Re(Sn)SEDP compared to ^{188}Re(Sn)HEDP may help explain the observation in this study that ^{188}Re(Sn)SEDP has a larger bone uptake than ^{188}Re(Sn)HEDP in rats after 24 hours.

5. Conclusions

The new bisphosphonate SEDP ligand is easily and quantitatively synthesized in one step via anti-Markovnikov addition of bisulfite to VDPA at slightly basic pH (pH = 8). We have determined the log K values (pK_a's) for the bisphosphonic acid protons on SEDP, and in general they are larger than the log K values for the corresponding protons on HEDP. The synthesis of ^{188}Re(Sn)SEDP is carried out in an almost identical fashion to that of ^{188}Re(Sn)HEDP, and is easily purified for use as a radiopharmaceutical using literature methods commonly used for ^{186}Re(Sn)HEDP.

Like its ^{188}Re(Sn)HEDP analog, the ^{188}Re(Sn)SEDP compound consists of a mixture of as yet unidentified components as seen by HPLC analysis. The biodistribution of ^{188}Re(Sn)SEDP is comparable to ^{188}Re(Sn)HEDP in most organs of the rat but has slightly higher uptake in bone after 24 hours. Further studies need to be done on SEDP and ^{188}Re(Sn)SEDP, possibly including further animal or human studies, to determine the exact nature of the bone incorporation properties of the sulfonate moiety in SEDP and ^{188}Re(Sn)SEDP. New studies should also be initiated to synthesize and test the ^{99m}Tc analog of this promising new ligand.

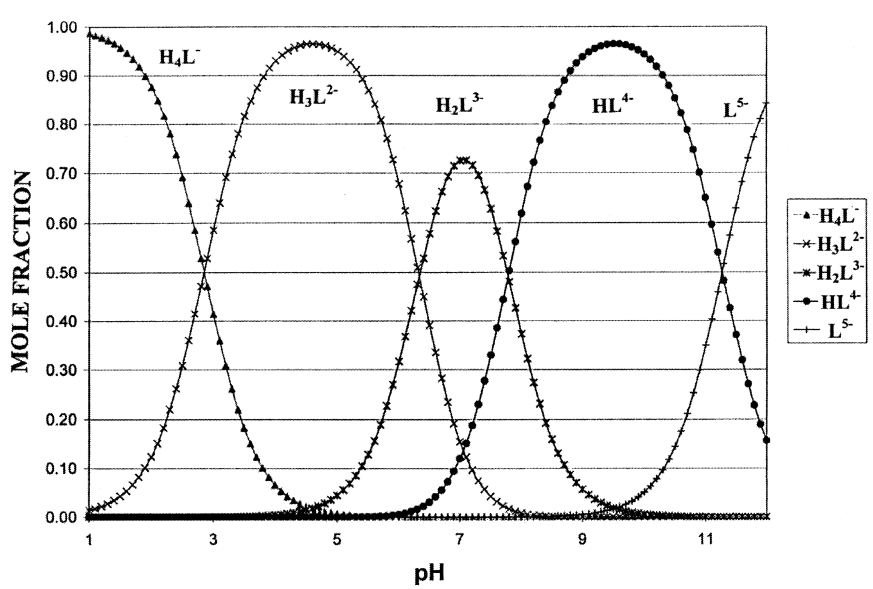


Fig. 3. Species distribution curve for SEDP.

Table 2
Biodistribution of ^{188}Re(Sn)HEDP and ^{188}Re(Sn)SEDP in rats as a function of time[a]

^{188}Re(Sn)HEDP			Organ	^{188}Re(Sn)SEDP		
5 min	60 min	1440 min		5 min	60 min	1440 min
2.16 ± 0.33	0.46 ± 0.04	0.06 ± 0.03	Blood	1.83 ± 0.51	0.53 ± 0.03	0.01 ± 0.01
0.64 ± 0.13	0.19 ± 0.03	0.02 ± 0.01	Liver	0.34 ± 0.11	0.13 ± 0.01	0.02 ± 0.01
4.64 ± 1.44	1.36 ± 0.07	0.68 ± 0.08	Kidney	3.25 ± 1.18	1.20 ± 0.05	0.69 ± 0.07
0.73 ± 0.13	0.15 ± 0.01	0.02 ± 0.01	Heart	0.60 ± 0.18	0.16 ± 0.01	0.01 ± 0.01
1.22 ± 0.21	0.27 ± 0.03	0.02 ± 0.01	Lung	0.99 ± 0.30	0.29 ± 0.01	0.03 ± 0.01
1.54 ± 0.21	2.65 ± 0.23	1.94 ± 0.16	Bone	1.46 ± 0.53	2.75 ± 0.38	2.47 ± 0.23

[a] Table entries are average % injected dose/gram of organ at the listed time of assay. The quoted error is +1 SD. Five rats were studied at each time. The radiopharmaceuticals were injected in 0.3 ml of a 0.155 M NaCl. Data are corrected for physical decay of ^{188}Re.

Acknowledgments

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