BioMetals 1996, **9**, 351–361

Biospeciation, by potentiometry and computer simulation, of Sm–EDTMP, a bone tumor palliative agent

G. Charmaine de Witt, Peter M. May, John Webb & Glenn Hefter

School of Physical Sciences, Engineering and Technology, Murdoch University, Perth, Australia

Received 21 March 1996; accepted for publication 4 April 1996

^{153}Sm–EDTMP (ethylenediaminetetra(methylenephosphonic) acid) is of considerable interest as a bone therapeutic radiopharmaceutical but its properties in solution are not yet well characterized. The protonation constants of EDTMP and the formation constants of the complexes of Sm–EDTMP have accordingly been measured potentiometrically by glass electrode titrations at 25°C in 0.15 M NaCl. Six protonation constants ($\log \beta_{011}$ = 9.638, $\log \beta_{012}$ = 17.330, $\log \beta_{013}$ = 23.597, $\log \beta_{014}$ = 28.636, $\log \beta_{015}$ = 31.501, $\log \beta_{016}$ = 32.624) and the formation constants of the $[Sm(EDTMP)H_{-1}]^{6-}$ ($\log \beta_{11-1}$ = 4.865), $[SmEDTMP]^{5-}$ ($\log \beta_{110}$ = 12.018), $[Sm(EDTMP)H]^{4-}$ ($\log \beta_{111}$ = 17.892) and $[Sm(EDTMP)H_2]^{3-}$ ($\log \beta_{112}$ = 23.437) complexes were determined. Computer simulations indicate that the $[SmEDTMP]^{5-}$ and the hydroxy $[Sm(EDTMP)H_{-1}]^{6-}$ species are the major Sm(III) complexes formed in blood plasma, which explains the high degree of localization in the kidney and urine observed in biodistribution studies. Calcium ions are probably the maior competitor for EDTMP in blood plasma. As the presence of secondary skeletal metastases results in a high rate of bone turnover, it is possible that the high concentration of calcium at these sites encourages localization of ^{153}Sm–EDTMP.

Keywords: EDTMP, formation constants, protonation constants, samarium, speciation

Introduction

The complex of ^{153}Sm with the ligand ethylenediaminetetra(methylenephosphonic) acid, EDTMP (Figure 1), has recently attracted considerable interest as a bone therapeutic radiopharmaceutical (Collins *et al.* 1993, Bayouth *et al.* 1994, Serafini 1994, Winderen *et al.* 1995). In biodistribution studies using rabbits and rats, the complex was found to be stable *in vivo* for at least 72 h, and demonstrated a high degree of localization in the skeleton and in the urine (Goeckeler *et al.* 1987, Ketring 1987, Logan *et al.* 1987). In one study (Goeckeler *et al.* 1987), the level of activity in the blood after administration of ^{153}Sm-EDTMP was lower than those of the ^{153}Sm complexes of nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA) and *N*-hydroxyethylethylenediaminetriacetic acid (HEDTA), and cleared rapidly (approximately 99.5% within 2 h after administration). ^{153}Sm–EDTMP demonstrated a higher skeletal uptake, lower degree of tissue localization and faster vascular clearance than the commercial imaging agent, ^{99m}Tc- methylenediphosphonate (^{99m}Tc–MDP).

In radiotoxicity studies on dogs bearing spontaneous bone carcinoma, administration of ^{153}Sm-EDTMP has been found to result in pain palliation, a lack of tumor growth or regression, extended survival time and, in one case, a cure (Ketring 1987). The negative responses include a transitory rise in the levels of serum alkaline phosphatase and a depression of red and white blood cell counts, although not of a sufficient severity to cause anemia (Lattimer *et al.* 1990). Both are indications of radiation damage to the bone marrow. The damage, however, was estimated to be minimal as all

Address for correspondence: J. Webb, School of Physical Sciences, Engineering and Technology, Murdoch University, Perth, Western Australia 6050, Australia. Fax: (+61) 9 310 5005.

hematologic parameters had returned to normal within 6 weeks of the last injection of the complex.

Clinical studies on human patients with skeletal metastases resulted in substantial pain relief after administration of ^{153}Sm–EDTMP (Turner *et al.* 1989, Collins *et al.* 1993, Bayouth *et al.* 1994, Serafini 1994, Winderen *et al.* 1995). Bayouth *et al.* (1994) observed no uptake of ^{153}Sm–EDTMP in non-skeletal tissues in whole-body gamma camera images, while Turner *et al.* (1989) report that imaging studies performed up to 18 weeks post-injection revealed a reduction in the size of the metastases. In one case metastases disappeared for over 9 months. All studies reported that the patients experienced significant relief from symptoms, such as severe pain and reduced mobility, with no toxic effects in other organs. Like the canine trials, toxicity was found to be exclusively hematologic, with most of the patients exhibiting complete hematologic recovery (Collins *et al.* 1993). Similar results have been obtained with ^{90}Y-EDTMP (Serafini 1994).

Treatment with Sm–EDTMP is not restricted to those patients suffering from secondary bone metastases. Patients suffering from ankylosing spondylitis, Paget's disease and rheumatoid arthritis showed improvement after administration of the complex (Alberts *et al.* 1995). Like bone metastases, these diseases produce sites of increased osteoblastic activity and therefore provide suitable sites for localization.

In this context we have used potentiometry to determine the protonation constants of EDTMP, as well as the suite of Sm–EDTMP complexes that best represents our experimental data (i.e. the 'model') and their respective formation constants. We have employed these constants to simulate the speciation of Sm–EDTMP in blood plasma and thereby proposed an explanation for the pattern of excretion reported in *in vivo* biodistribution studies.

Materials and methods

Potentiometry

Instrumentation. Titrations were carried out in specially constructed jacketed glass vessels (Hefter 1972), thermostated to a temperature of 25.00 ± 0.02°C using a Haake N3 water bath, under an atmosphere of high purity nitrogen which had first been pre-saturated by sparging through a solution of sodium chloride (0.15 M). Potentials of a hydrogen-ion selective glass electrode (Metrohm 6.0101.000, Switzerland) were measured relative to a calomel reference electrode (Metrohm 6.0702.100) in the presence of a platinum grounding electrode (to enhance the stability of the cell potential measurements). All titrations were performed using a computerized titration system (Clare *et al.*, unpublished).

Solution preparation. Volumetric glassware ('A' grade), previously cleaned and rinsed with distilled deionized water, was used for all experiments. All solutions were prepared using Millipore (Milli-Q, Cole-Palmer, Niles, Illinois, USA) water. The water was boiled and purged with nitrogen prior to use in preparation of sodium hydroxide solutions. Sodium chloride (Ajax, Auburn, NSW, Australia, AR) was used to provide a background electrolyte at a chloride ion concentration of 150 mM in the test solutions. This concentration was chosen to approximate physiological conditions of osmotic pressure.

Sodium hydroxide and stock standard hydrochloric acid solutions were prepared using commercial volumetric-standard ampoules (BDH, Kilsyth, Victoria, Australia, Convol). The concentrations of the prepared sodium hydroxide solutions were checked by titration against potassium hydrogen phthalate (Ajax, AR, > 99.8%) (Bassett *et al.* 1985). The hydrogen ion concentrations of the serially diluted acid solutions were standardized by titration against the previously checked sodium hydroxide solution, with the end-point determined by Gran plot (Bassett *et al.* 1985, Rossotti 1978).

Stock solutions of metal ion were prepared by dissolution of Sm(III) oxide (Sigma, Castle Hill, NSW, Australia, minimum 99.9% rare earth content, as Sm_2O_3) in known volumes of standard hydrochloric acid solution. The resulting solutions were then filtered to remove any solid impurities and made up to an appropriate volume. The concentration of Sm(III) in the final solution was standardized by titration against EDTA (BDH, Convol) using xylenol orange (Shetty & Sathe 1976) indicator.

The ligand EDTMP was supplied by the Australian Nuclear Science and Technology Organization (Lucas Heights, NSW, Australia) as a commercially obtained preparation (Monsanto, Melbourne, Victoria, Australia, 92%, with the remaining 8% comprising moisture and minor contamination by other phosphonates) and was dried in an oven overnight prior to use. This was the same material used in relative biodistribution studies (Turner *et al.* 1989).

Solutions used in the determination of the protonation constants of the ligands were prepared by dissolving weighed amounts of solid ligand and sodium chloride in standard hydrochloric acid so as to produce a final pH in the range 1.5–1.6.

The formation of insoluble protonated metal–ligand species (Sawada *et al.* 1991) in acidic solutions required the determination of the formation constants of the Sm–EDTMP complexes to be performed by titrating an alkaline solution of the metal ion and ligand against hydrochloric acid. To minimize carbon dioxide contamination, the test solutions were prepared inside the titration vessel. An aliquot of a standard solution of EDTMP and sodium chloride was delivered into the vessel under

Table 1. Concentration of components in titration solutions ([Cl^-] = 150mM)

Solution	[H^+] (mM)	[OH^-] (mM)	[Sm] (mM)	[EDTMP] (mM)	M:L
EDTMP	12.5	–	–	5–15	–
NaOH	–	100	–	–	–
Sm–EDTMP	–	44–144	2–6	5–15	1:1–1:3
HCl	100	–	–	–	–

a blanket of nitrogen. To this was added an appropriate volume of sodium hydroxide (0.5 M) solution, delivered using a piston burette. Finally, to suppress metal hydroxide formation, the aliquot of $SmCl_3$ solution was delivered slowly and with stirring. The volumes of the individual aliquots were calculated so as to obtain a final volume of 50 ml of test solution.

The metal ion, ligand, hydrogen ion and hydroxide ion concentrations of the titrated solutions are listed in Table 1. All solutions had chloride ion concentrations of 150 mM. As the Sm–EDTMP titrations were complicated by the gradual formation of insoluble metal–EDTMP compounds in acidic solution, the titrant was delivered extremely slowly and with vigorous stirring to prevent localized high concentrations of hydrogen ions around the burette tip. Titrations were terminated at the first visual sign of precipitation.

Formation constants. Experimental data obtained from potentiometric titrations were used to calculate ligand protonation and ligand–metal ion formation constant (β) values. In general, for the equilibrium

$$pM + qL + rH \gtrless M_pL_qH_r$$

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[M]^p[L]^q[H]^r}$$

where M, L and H represent metal, ligand and hydrogen ions, respectively, and species concentrations, [ ], are expressed in units of mol L^{-1}.

The formation constants determined here follow the convention that expresses hydroxy species as $M_pL_qH_{-r}$, and not as $M_pL_q(OH)_r$. The symbols '$(OH)_r$' and 'H_{-r}' are related through the ionization constant, K_w (where $K_w = [H^+][OH^-]$). Hence, for the reaction

$$pM + qL + rOH \gtrless \text{hydroxy species}$$

$$\beta' = \frac{[\text{hydroxy species}]}{[M]^p[L]^q[OH]^r}$$

$$= \frac{[\text{hydroxy species}]}{[M]^p[L]^q K_w^r [OH]^{-r}}$$

Hence,

$$\beta_{pq-r} = \beta'_{pqr} \cdot K_w^r$$

$$= \frac{[\text{hydroxy species}]}{[M]^p[L]^q[H]^{-r}}$$

Computational procedures. The data from the potentiometric titrations were processed using the ESTA (Equilibrium Simulation for Titration Analysis) suite of programs (May *et al.* 1982, 1985, 1988, May & Murray 1988a,b).

Graphical representations were employed to examine experimental data, to provide information on the types of complexes present under experimental conditions and as a general aid in model selection. There were three types of ESTA plot function used: $\bar{Z}_H$ for ligand protonations, $\bar{Z}_M$ for metal–ligand complexation, and percentage distribution to display the relative change in composition with varying pH. The $\bar{Z}$ functions are:

$$\bar{Z}_H = \frac{T_H - H + OH}{T_L}$$

$$\bar{Z}_M = \frac{T_L - A(1 + \sum_n \beta LH_n H^n)}{T_M}$$

where

$$A = \frac{T_H - H + OH}{\sum_n \beta_{LH_n} H^n)}$$

and H is the free hydrogen ion concentration, OH is the free hydroxide ion concentration (= K_W/H) and T_i is the total concentration of species i.

The $\bar{Z}_H$ function calculates the average number of protons associated with the ligand at each point in the titration and is plotted against solution pH. The $\bar{Z}_M$ function calculates the average number of ligand molecules associated with a metal ion and is plotted against pA (pA = –log [A] where, in simple cases, [A] represents the concentration of free, deprotonated ligand). The functions describing the percentage distribution are defined as previously reported (May *et al.* 1985).

Speciation in blood plasma

The protonation constants of EDTMP and the formation constants of its complexes with Sm(III) were used in the computer simulations carried out using the program ECCLES (Evaluation of Constituent Concentrations in Large Equilibrium Systems) (May *et al.* 1977). This program is capable of simulating complex equilibria in systems containing up to approximately 10 000 species and was used to calculate the equilibrium concentrations of each EDTMP species, in the presence of the endogenous ligands and metal ions, in a simulated plasma. A compilation of over 100 low molecular mass organic and inorganic ligands, together with six to 10 metal ions of biological significance was used (May 1995). The database included not only the formation constants for the binary complexes for the components mentioned above but also the observed or estimated formation constants of their ternary complexes, since such species occur widely in

systems composed of metal ions in the presence of two or more different ligands. The program allows the inclusion of other metals and ligands as required.

The blood plasma model considers that metal ions in plasma appear in four distinct fractions:

(1) Incorporated into metalloproteins and therefore non-exchangeable.
(2) Bound loosely to other proteins and in labile equilibrium with similar ions in solution.
(3) Complexed with low molecular weight ligands.
(4) Free metal ions

The metal ion complexes existing in the second and third fractions are considered to be in competitive equilibrium with the free metal ions (May *et al.* 1977). Strong binding by metalloproteins means that the concentration of low molecular weight complexes is small and that of the free metal ions is even smaller. Moreover, the amount of low molecular weight complex produced is negligible compared with the amount of ligand present and has no significant effect on the free ligand concentration. The concentration of each low molecular weight species is therefore dependent on, and directly proportional to, the concentration of the free metal ion, as is the concentration of the metal ion in the low molecular weight fraction. Thus the percentage of the metal ion complexed as a given species is constant irrespective of the absolute concentration of the free metal ion. It is therefore possible to obtain meaningful information even though the absolute concentrations of the free metal ion and its low molecular weight complexes may be unknown (May *et al.* 1976, May & Bulman 1983).

The addition of exogenous ligands alters the naturally occurring equilibria by removing and complexing metal ions from labile protein complexes. The resulting increase in the low molecular weight fraction may be quantified by the plasma mobilizing index (PMI) (May & Williams 1977, Berthon *et al.* 1980, Williams 1980, May & Bulman 1983):

$$PMI = \frac{A}{B}$$

where A = total concentration of the metal low molecular mass complex species in the presence of chelating agent and B = total concentration of the metal low molecular mass complex species in normal plasma.

PMI also provides a measure of the ability of a ligand, at a given concentration, to mobilize metal ions from the labile, protein-bound fraction.

Results and discussion

Potentiometry

Protonation constants of EDTMP. Figure 1 implies that EDTMP has at least eight protonation sites, corresponding to the four phosphonate groups. Two further sites exist in principle on the central amine groups. These groups, however, would protonate only in extremely acidic solutions, outside the accessible pH range of potentiometric techniques, or exist as zwitterions.

The protonation constants and statistical results of the calculation are given in Table 2. The objective function, OBJT, provides a measure of the agreement between the observed data and that calculated from the selected model and its associated protonation or formation constants. The smaller the objective function, the closer the agreement between the observed and calculated data, and the better the selected model. Objective functions in the order of 10^{-8} or better are highly desirable.

The great number of protonations and their degree of overlap necessitated the use of two sets of calculations to determine the protonation constants of EDTMP. This was done to minimize correlations and other undesirable optimizer effects.

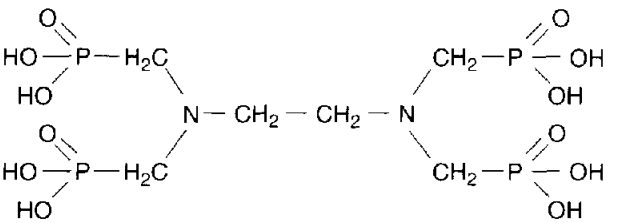

Figure 1. Structure of EDTMP.

Table 2. Protonation constants of EDTMP

Species *p q r*	Charge	Log β_{pqr}[a]	SD	OBJT	No. of titrations	No. of data points
0 1 1	−7	9.638	0.002	8.8758×10^{-9}	8	675
0 1 2	−6	17.330	0.003			
0 1 3	−5	23.597	0.003			
0 1 4	−4	28.636	0.001	6.1605×10^{-9}	8	976
0 1 5	−3	31.501	0.002			
0 1 6	−2	32.624	0.002			

[a] Defined in the text.

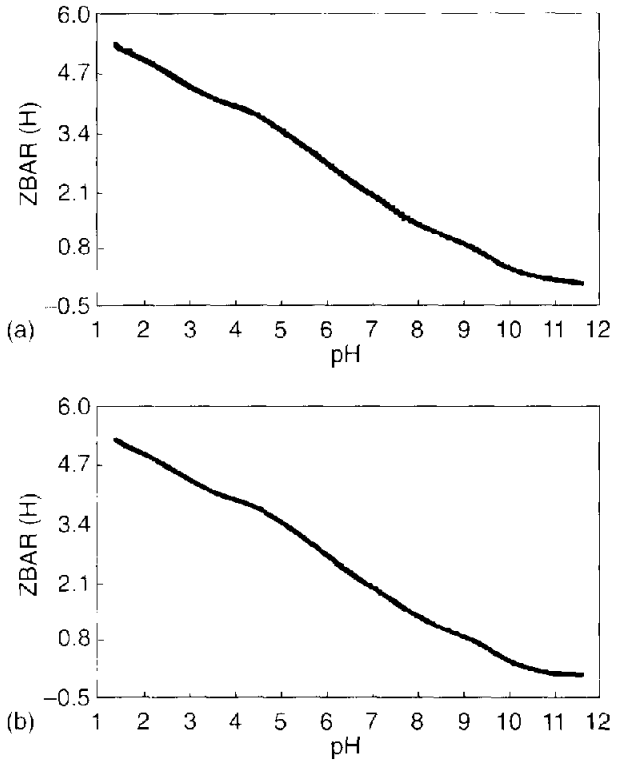

Figure 2. $\overline{Z}_{H}$ curves for the protonation of EDTMP. **(a)** $\overline{Z}_{H}$ curve obtained from experimental data. **(b)** $\overline{Z}_{H}$ curve derived from calculated protonation constants.

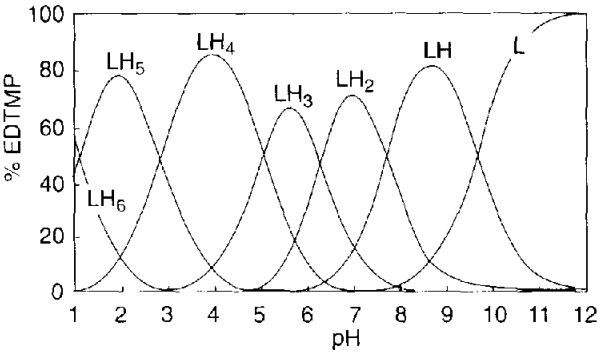

Figure 3. Percentage distribution of EDTMP as a function of pH. The symbols on the figure represent the $[EDTMPH_6]^{2-}$ **(LH_6)**, $[EDTMPH_5]^{3-}$ **(LH_5)**, $[EDTMPH_4]^{4-}$ **(LH_4)**, $[EDTMPH_3]^{5-}$ **(LH_3)**, $[EDTMPH_2]^{6-}$ **(LH_2)** [EDTMPH]$^{7-}$ **(LH)** and [EDTMP]$^{8-}$ **(L)** species.

The first subset was created with data obtained at pH 4.3–11.5. From the $\overline{Z}_{H}$ and percentage distribution plots (Figures 2a and 3, respectively), it can be seen that the major species existing in this range are the L, LH, LH_2 and LH_3 species, with some LH_4 forming in the more acidic part of the region. Hence, the protonation constants of all four species (i.e. β_{011} to β_{014}) were calculated using this data subset. The resulting values of β_{011}, β_{012} and β_{013} were then held constant while the remaining constants were calculated using the full data set. The $\overline{Z}_{H}$ plot derived from the calculated protonation constants (Figure 2b) shows excellent agreement with the experimental data.

Table 3 compares our protonation constants with published values measured under similar conditions. Agreement of the β_{011} and β_{012} values is fair but the rest of the constants diverge increasingly from the previous values, up to nearly 9 log units for β_{016}. This is mainly a reflection of the difficulty of calculating constants when there are so many overlapping equilibria. As there are no distinct protonations (i.e. no plateaux in the $\overline{Z}_{H}$ plot), no single protonation constant may be calculated with a high degree of certainty and subsequently small errors in the constants of the initial protonations are magnified in the values of the successive constants. In the case of the values published by Jarvis *et al.* (1995), these differences may also be partially attributed to an experimental temperature of 37°C compared to 25°C, used in our experiments.

Table 3. Protonation constants of EDTMP: comparison with other published values

Complex	Species	Log β_{pqr}[a]		
	p q r	This work [25°C, 0.15 M NaCl]	Vasil'ev and Zaitseva (1986) [25°C, 0.1 M NaCl]	Jarvis *et al.* (1995) [37°C, 0.15 M NaCl]
LH	0 1 1	9.638	10.33	10.67
LH_2	0 1 2	17.330	19.41	20.14
LH_3	0 1 3	23.597	27.01	27.77
LH_4	0 1 4	28.636	33.39	34.08
LH_5	0 1 5	31.501	38.47	39.16
LH_6	0 1 6	32.624	41.46	41.99

[a]Defined in the text.

The preparation and purity of the EDTMP can affect the values of the equilibrium constants obtained. Motekaitis *et al.* (1976) suggest that the method of synthesis can produce samples of variable composition, thereby affecting the results obtained. Capewell and May (unpublished) have found that a commonly used method of synthesizing EDTMP (Moedritzer & Irani 1966) probably produces a tri(methylenephosphonate) derivative (i.e. ethylenediaminetri(methylenephosphonic) acid) as well as the desired tetra(methylenephosphonate) derivative. The purity of the EDTMP used by Vasil'ev & Zaitseva (1986) is not mentioned in their paper. Jarvis *et al.* (1995) also do not specify the percentage purity of their sample. As their method of synthesis can lead to incomplete production of the tetra(methylenephosphonate) derivative, this may account for their differing values.

The disagreement between the present results and those of Vasil'ev & Zaitseva (1986) and Jarvis *et al.* (1995) is reflected in other reported protonation constants (Westerback *et al.* 1965, Kabachnik *et al.* 1967, Motekaitis *et al.* 1971, Rizkalla & Zaki 1979, Vasil'ev *et al.* 1987, Sawada *et al.* 1993). Even under identical experimental conditions (25°C, 0.1 M KNO_3) variations between β_{011} values of up to 3 log units are reported. This effect increases with successive protonations until reported values of β_{016} vary over a range of 7 log units.

Although some publications (Westerback *et al.* 1965, Motekaitis *et al.* 1971, Rizkalla & Zaki 1979, Levin *et al.* 1981) have reported up to eight protonation constants, only six were detected in our experiments (Figure 2) over the pH range 1–12. At physiological pH, EDTMP exists predominantly as $[(EDTMP)H_2]^{6-}$ and $[(EDTMP)H]^{5-}$.

Formation constants of Sm–EDTMP complexes. The $\overline{Z}_M$ plot depicting the interaction of Sm(III) with EDTMP (Figure 4a) exhibits the characteristic 'curl-back' feature indicative of the presence of hydroxy species. There is no strongly defined plateau at $\overline{Z}_M = 1$. This suggests that the ML species is not a major component of the system although it may exist over a relatively small pH range. This is reflected in the percentage distribution plot (Figure 5) which shows that, while the ML complex is present over the range pH 4.2–10, it coexists with significant levels of the MLH_{-1}, MLH and MLH_2 species over much of this range.

The model of the suite of complexes of Sm(III) with EDTMP, their formation constants and other results of the associated calculations is given in Table 4. The $\overline{Z}_M$ plot calculated from these values

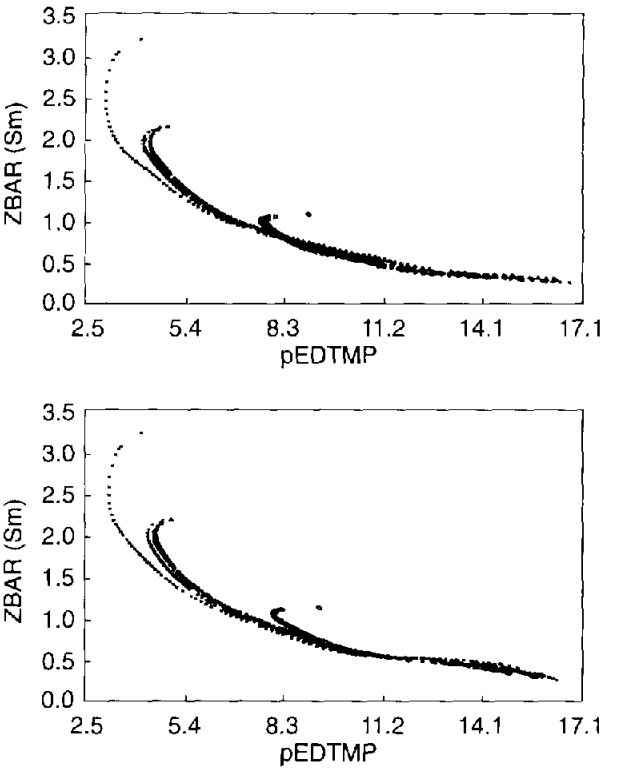

Figure 4. $\overline{Z}_M$ curves for the Sm-EDTMP complexes **(a)** $\overline{Z}_M$ curve obtained from experimental data. **(b)** $\overline{Z}_M$ curve derived from calculated model and associated formation constants.

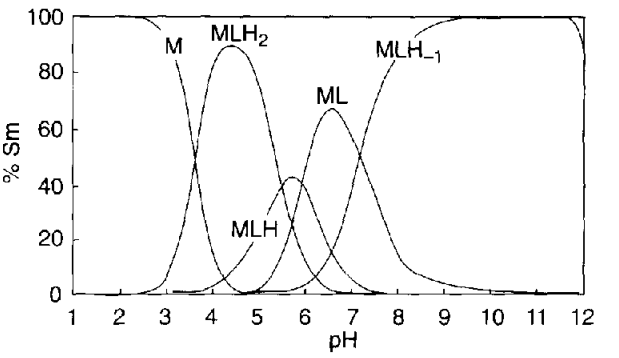

Figure 5. Percentage distribution of the Sm–EDTMP complexes as a function of pH. The symbols on the figure represent the Sm^{3+} **(M)**, $[SmEDTMPH_2]^{3-}$ **(MLH_2,)** $[SmEDTMPH]^{4-}$ **(MLH)**, $[SmEDTMP]^{5-}$ **(ML)** and $[SmEDTMPH_{-1}]^{6-}$ **(MLH_{-1})** species.

(Figure 4b) agrees well with that of the observed data.

Table 5 compares our formation constants with those previously published. The constants published by Kabachnik *et al.* (1967) for the MLH and MLH_2 complexes are defined differently from the convention used in the present study, i.e.

Table 4. Formation constants of Sm–EDTMP complexes

Species			Charge	Log β_{pqr}[a]	SD	OBJT	No. of titrations	No. of data points
p	*q*	*r*						
1	1	−1	−6	4.865	0.021	7.8417×10^{-8}	6	617
1	1	0	−5	12.018	0.017			
1	1	1	−4	17.892	0.019			
1	1	2	−3	23.437	0.014			

[a]Defined in the text.

Table 5. Formation constants of Sm–EDTMP complexes: comparison with other published values

Complex	Species			Log β_{pqr}[a]			
	p	*q*	*r*	This work [25°C, 0.15 M NaCl]	Kabachnik *et al.* (1967) [25°C, 0.1 M KCl]	Sawada *et al.* (1991) [25°C, 0.1 M KNO_3]	Jarvis *et al.* (1995) [37°C, 0.15 M NaCl]
MLH_{-1}	1	1	−1	4.865	–	–	–
ML	1	1	0	12.018	22.39	–	14.44
MLH	1	1	1	17.892	17.56[b] (*cf. 8.254*)	7.34[b] (*cf.5.874*)	21.57
MLH_2	1	1	2	23.437	13.68[b] (*cf. 6.107*)	6.29[b] (*cf. 5.545*)	27.57

[a] Defined in the text.
[b] These constants have not been calculated according to the conventions described in the text. The italicized figures in parentheses are our β values, converted to the forms used by the respective authors.

$$K_n = \frac{[MLH_n]}{[M][LH_n]}$$

Our converted values of β_{111} and β_{112} (Table 5), and our value of log β_{110}, differ greatly from those of Kabachnik *et al.* As previously mentioned, this may be due to the purity of the sample of ligand used. In addition, Kabachnik *et al.* (1967) performed a sequential calculation of the stability constants of the MLH_5 complex through to the ML complex, using the solubility products of the insoluble protonated complexes. Errors in either the analysis of the composition of the insoluble species or the determination of K_{sp} would be propagated and magnified in the values of the subsequently calculated stability constants. Thus, the level of agreement between the present and previously published values of log K_2 is greater than that of the log K_1 values and far greater than that of the log K_0 values.

The formation constants published by Sawada *et al.* are defined by the form

$$K_{MLH_n} = \frac{[MLH_n]}{[H][MLH_{n-1}]}$$

Our values of β_{111} and β_{112} were converted to this form by employing our values of β_{110}, β_{111} and β_{112} and are listed in Table 5. Sawada *et al.* (1991) calculated their constants based on an estimated rather than an experimentally determined value of β_{110} because they did not believe that the data collected in the acidic region was sufficiently reliable. Any error in their estimation would be reflected in the values of their constants, which could account for the differences between the values of K_{MLH} in Table 5. Their value of K_{MLH_2} would be less directly affected by the estimated value of β_{110} and, hence, agrees more closely with our constant.

The background electrolyte used in both of the previously mentioned publications (i.e. KCl and KNO_3) differed from that used in this work. Differences in the extent of competitive binding by the background electrolyte (i.e. Na^+ compared with K^+ ions) to the ligand could cause large differences in log β values and may further account for the discrepancy between our formation constants and those of Kabachnik *et al.* (1967) and Sawada *et al.* (1991).

In comparison, the log values of β_{110}, β_{111} and β_{112} reported by Jarvis *et al.* (1995) agree well with our

own constants. The differences between the two sets of values may be attributed to differences in experimental temperature and in the composition of the EDTMP sample used.

No reference corroborating the existence of an MLH_{-1} species could be found in the literature. This is due to the fact that most work is carried out in the range pH 0–8 (Kabachnik *et al.* 1967, Tananaev *et al.* 1981, Sawada *et al.* 1991). According to the percentage distribution plot (Figure 5), the MLH_{-1} complex is a relatively minor species in solutions of pH < 8 and, as such, may not have been detected. Graphical and numerical evidence, however, strongly indicates that such a species exists. Omission of these species from the proposed suite of complexes results in a calculated $\bar{Z}_M$ plot that noticeably differs from the experimental plot, and in objective functions and standard deviations significantly higher than those obtained when it is included.

From Figure 5 it can be seen that, at physiological pH, Sm(III) is complexed by EDTMP predominantly as the hydroxy species $[Sm(EDTMP)H_{-1}]^{6-}$ and the $[SmEDTMP]^{5-}$ species and, to a very minor extent, as the $[Sm(EDTMP)H]^{4-}$ species.

Speciation in blood plasma

Interaction of Sm(III) with EDTMP in vivo. The interaction of Sm(III) with EDTMP under physiological conditions was modeled using ECCLES. Among its several applications, ECCLES has been used to explain, in chemical terms, the relative abilities of a number of chelation agents to promote trace-metal excretion (May & Williams 1977) and, more recently, the distribution of Gd(III) species in blood plasma (Jackson & du Toit 1992). As it also provides information regarding the types of complexes formed between the ligands and metal ions of interest under physiological conditions, it should also be possible to interpret the results of the biodistribution studies from a chemical standpoint.

The PMI curves for the metal with EDTMP and HEDTA (for comparison) are shown in Figure 6 and reflect the ligands' relative ability to bind Sm(III) *in vivo*. The PMI of EDTMP begins to increase at much higher ligand concentrations than HEDTA. This clearly indicates that EDTMP mobilizes samarium far less readily than HEDTA. Mobilization of the metal does not occur below ligand concentrations of 10^{-2} M. Below this concentration ECCLES analysis indicates that EDTMP is almost completely (i.e. > 93%) bound by Ca(II) as protonated 1:1 complexes. Small amounts (approximately

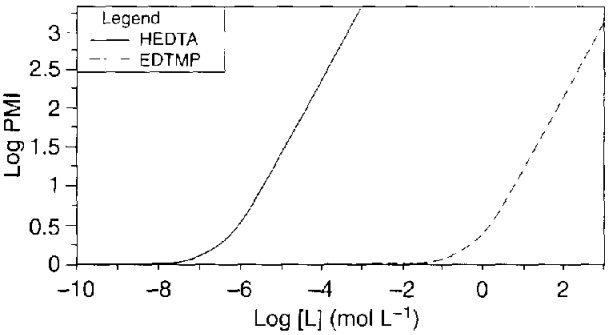

Figure 6. PMI curves for Sm–HEDTA and Sm–EDTMP.

6%) of protonated, 1:1 Mg(II) complexes are also formed.

At these low concentrations of EDTMP most of the Sm(III) is bound as 1:1 and 1:2 citrate species (Table 6). Less than 10% of the Sm(III) forms 1:1 complexes with the amino acids glycine and alanine, and with the lactate and oxalate ions. As the ligand concentration is increased, Sm(III) is increasingly bound by EDTMP, mainly as the hydroxy species $[Sm(EDTMP)H_{-1}]^{6-}$ and $[SmEDTMP]^{5-}$, with minor amounts of $[Sm(EDTMP)H]^{4-}$.

Blood plasma modeling and biodistribution studies. The results obtained from the blood plasma model enable the observed patterns of excretion and localization to be interpreted. Excretion of intravenously administered ^{153}Sm complexes occurs via the kidneys as ionic waste products or via the liver as particulate matter or electronically neutral species (Turner *et al.* 1987). The relative difference between the degree of localization in the two organs depends upon the ligand with which samarium is administered.

Table 6. Simulated biodistribution of Sm(III) in blood plasma with increasing EDTMP concentration

[EDTMP] (mol l^{-1})	Bound samarium (%)				
	EDTMP	Citrate	Oxalate	Lactate	Amino acids
10^{-5}	–	91.2	6.8	0.1	1.5
10^{-4}	–	91.2	6.8	0.1	1.5
10^{-3}	0.2	91.0	6.8	0.1	1.5
10^{-2}	1.4	89.8	6.7	0.1	1.5
10^{-1}	12.3	79.8	6.0	0.1	1.4
10^{0}	58.5	37.8	2.8	–	0.7
10^{1}	93.4	6.0	0.5	–	0.1
10^{2}	99.3	0.7	–	–	–
10^{3}	100.0	–	–	–	–

In the case of Sm–EDTMP, excretion occurs almost exclusively via the kidneys. Within the first 15 min after administration of the complex, localization in the kidney is high compared with that among the other organs. Moreover, within 30 min most of the activity is contained in the urine (Turner *et al.* 1989). This is in accord with the simulation results, which indicate that the major samarium complexes formed are the $[SmEDTMP]^{5-}$ complex and the hydroxy species, $[Sm(EDTMP)H_{-1}]^{6-}$. Although most of the administered Sm(III) is bound as EDTMP complexes, much of the EDTMP, which is often administered in excess, is bound as calcium complexes. This and the demonstrated rapid skeletal uptake of ^{153}Sm, when administered with EDTMP, suggests that localization in bone may involve bone turnover.

In general, the patients participating in the trial carried out by Turner *et al.* (1989) possessed secondary bone metastases arising from either prostatic or breast carcinomas. In metastases arising from breast carcinomas, the tumor cells exist in the bone marrow and, directly or indirectly, act so as to increase bone resorption. In metastases arising from prostatic carcinomas, osteoblastic activity is increased although the bone formed may not be normal. In both cases, the secondary bone metastasis causes an increase in bone turnover. Sites at which bone turnover is high tend to be highly vascularized. Uptake of radioactive phosphate compounds at such sites may occur by ion exchange through replacement of hydroxy groups (Wang *et al.* 1993). It is possible that a similar exchange may occur with phosphonate groups, which are known to bind to bone, causing a localization of ^{153}Sm at those sites. Perivascular calcification, the calcification of soft tissue, may also occur around the site of the tumor. As this involves the precipitation of amorphous calcium phosphate, the high concentration of calcium at these sites would encourage localization of EDTMP.

A similar study (Jarvis *et al.* 1995), incorporating data for the samarium transferrin interaction, has suggested that zinc binding is somewhat more significant than calcium binding to EDTMP. This does not, however, readily account for the observed preference for EDTMP and its complexes to accumulate in bone.

An understanding of the biospeciation of Sm–EDTMP complexes thus explains their biological fate. Potentiometry provides useful information about the formation constants of the Sm–EDTMP complexes and the species formed in aqueous solution under physiological conditions of pH and osmotic pressure. The latter information, however, should not be assumed to apply directly *in vivo*. Competition from endogenous ligands and metal ions can alter both the type of Sm–EDTMP complexes formed and their distribution. On the other hand, computer programs such as ECCLES, which simulate the distribution of these complexes in biofluids, provide information about both the types of Sm–EDTMP complexes formed, thereby rationalizing their excretion pattern, and the major sources of competition for both the metal ion and the ligand.

Acknowledgements

The authors would like to thank Dr B.W. Clare for valuable discussions and for technical assistance in the presentation of some of the data and Dr G.E. Strong for assistance in the preparation of this paper. We also thank Mr P. Sorby and the Australian Nuclear Science and Technology Organization for the gift of EDTMP.

References

Alberts AS, Brighton SW, Kempff P, *et al.* 1995 Samarium-153–EDTMP for palliation of ankylosing spondylitis, Paget's disease and rheumatoid arthritis. *J Nucl Med* **36**, 1417–1420.

Bassett J, Denney RC, Jeffery GH, Mendham J, eds. 1985 *Vogel's Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis*. London: Longman Group.

Bayouth JE, Macey DJ, Kasi LP, Fossella FV. 1994 Dosimetry and toxicity of samarium-153–EDTMP administered for bone pain due to skeletal metastases. *J Nucl Med* **35**, 63–69.

Berthon G, Matuschansky C, May PM. 1980 Computer simulation of metal ion equilibria in biofluids. Part 3. Trace metal supplementation in total parenteral nutrition. *J Inorg Biochem* **13**, 63–73.

Collins C, Eary JF, Donaldson G, *et al.* 1993 Samarium-153 EDTMP in bone metastases of hormone refractory prostate carcinoma – a phase-I/II trial. *J Nucl Med* **34**, 1839–1844.

Goeckeler WF, Edwards B, Volkert WA, Holmes RA, Simon J, Wilson D. 1987 Skeletal localization of samarium-153 chelates: potential therapeutic bone agents. *J Nucl Med* **28**, 495–504.

Hefter GT. 1972 The use of ion-selective electrodes in the determination of mixed stability constants. *J Electroanal Chem* **39**, 345–355.

Jackson GE, du Toit JG. 1992 Gadolinium(III) *in vivo* speciation. Part 2. A potentiometric study of gadolinium (III) amino acid complexes. *S Afr Tydskr Chem* **45**, 82–85.

Jarvis NV, Wagener JM, Jackson GE. 1995 Metal-ion speciation in blood plasma as a tool for elucidating the *in vivo* behaviour of radiopharmaceuticals containing ^{153}Sm and ^{166}Ho. *J Chem Soc Dalton Trans*, 1411–1415.

Kabachnik MI, Dyatlova IM, Medved TA, Belugin YF, Sidorenko VV. 1967 Complex-forming properties of ethylenediamine-*N,N,N′,N′*-tetra(methylenephosphonic) and diethylenetriamine-*N,N,N′,N″,N″*-penta (methylenephosphonic) acids. *Proc Acad Sci USSR* **175**, 621–623.

Ketring AR. 1987 ^{153}Sm–EDTMP and ^{186}Re–HEDP as bone therapeutic radiopharmaceuticals. *Nucl Med Biol* **14**, 223–232.

Lattimer JC, Corwin LA, Stapleton J, *et al.* 1990 Clinical and clinicopathologic effects of samarium-153 EDTMP administered intravenously to normal beagle dogs. *J Nucl Med* **31**, 586–593.

Levin VI, Yakovlev VG, Kodina GE, Medvedeva EI, Smolin MD. 1981 Determination of the dissociation constants of polybasic acids. *Russ J Inorg Chem* **26**, 637–641.

Logan KW, Volkert WA, Holmes RA. 1987 Radiation dose calculations in persons receiving injection of samarium-153 EDTMP. *J Nucl Med* **28**, 505–509.

May PM. 1995 Modelling metal–ligand equilibria in blood plasma. In: Berthon G, ed. *Handbook of Metal–Ligand Interactions in Biological Fluids. Bioinorganic Chemistry 2.* New York: Marcel Dekker; 1184–1194.

May PM, Bulman RA. 1983. The present status of chelating agents in medicine. *Progr Med Chem* **20**, 225–336.

May PM, Murray K. 1988a The use of glass electrodes for the determination of formation constants – IV. *Talanta* **35**, 927–932.

May PM, Murray K. 1988b The use of glass electrodes for the determination of formation constants V. *Talanta* **35**, 933–941.

May PM, Williams DR, 1977 Computer simulation of chelation therapy. *FEBS Lett* **78**, 134–138.

May PM, Linder PW, Williams DR. 1976 Ambivalent effect of protein binding on computed distributions of metal ions complexed by ligands in blood plasma. *Experientia* **32**, 1492–1494.

May PM, Linder PW, Williams DR. 1977 Computer simulation of metal-ion equilibria in biofluids: models for the low-molecular-weight complex distribution of calcium(II), magnesium(II), manganese(II), iron(III), copper(II), zinc(II), and lead(II) ions in human blood plasma. *J Chem Soc Dalton Trans* 588–595.

May PM, Williams DR, Linder PW, Torrington RG. 1982 The use of glass electrodes for the determination of formation constants – I *Talanta* **29**, 249–256.

May PM, Murray K, Williams DR. 1985 The use of glass electrodes for the determination of formation constants – II. *Talanta* **32**, 483–489.

May PM, Murray K, Willaims DR. 1988 The use of glass electrodes for the determination of formation constants – III *Talanta* **35**, 825–830.

Moedritzer K, Irani RR. 1966 The direct synthesis of α-aminomethylphosphonic acids. Mannich-type reactions with orthophosphorous acid. *J Org Chem* **31**, 1603–1606.

Motekaitis RJ, Murase I, Martell AE. 1971 New multidentate ligands. XIII. Ethylenediaminetetra(methylenephosphonic) acid. *Inorg Nucl Chem Lett* **7**, 1103–1107.

Motekaitis RJ, Murase I, Martell AE. 1976 Equilibria of ethylenediamine-*N,N,N′,N′*-tetrakis(methylenephosphonic) acid with Cu(II), Zn(II), Mg(II), Ca(II), and Fe(III) ions in aqueous solution. *Inorg Chem* **15**, 2303–2306.

Rizkalla EN, Zaki MTM. 1979 Metal chelates of phosphonate-containing ligands I. Stability of some *N,N,N′,N′*-ethylenediaminetetra(methylenephosphonic) acid metal chelates. *Talanta* **26**, 507–510.

Rossotti H. 1978 *The Study of Ionic Equilibria: An Introduction.* London: Longman.

Sawada K Kuribayashi M, Suzuki T, Miyamoto H. 1991 Protonation equilibria of nitrilotris(methylenephosphonato)- and ethylenediaminetetrakis(methylenephosphonato)-complexes of scandium, yttrium, and lanthanoids. *J Sol Chem* **20**, 829–839.

Sawada K, Miyagawa T, Sakaguchi T, Doi K. 1993 Structure and thermodynamic properties of aminopolyphosphonate complexes of the alkaline-earth metal ions. *J Chem Soc Dalton Trans* 3777–3784.

Serafini AN. 1994 Current status of systemic intravenous radiopharmaceuticals for the treatment of painful metastatic bone disease. *Int J Radiat Oncol Biol Phys* **30**, 1187–1194.

Shetty SY, Sathe RM. 1976 Sequential determination of thorium and rare earths with EDTA and kojic acid. *Talanta* **23**, 46–47.

Tananaev IV, Beresnev EN, Tereshin GS, Kuznetsova OB, Goeva LV. 1981 Lanthanide ethylenediaminetetrakismethylphosphonates. *Russ J Inorg Chem* **26**, 151–152.

Turner JH, Martindale AA, de Witt GC, Webb J, Sorby P, Boyd RE. 1987 Samarium-153 chelate localization in malignant melanoma. *Eur J Nucl Med* **13**, 432–438.

Turner JH, Martindale AA, Sorby P. 1989 Samarium-153 EDTMP therapy of disseminated skeletal metastasis. *Eur J Nucl Med* **15**, 784 795.

Vasil'ev VP, Zaitseva GA. 1986 Acid–base equilibria in an aqueous solution of ethylenediaminetetrakis[methylphosphonic acid]. *Zh Obshch Khim* **56**, 949–953.

Vasil'ev VP, Zaitseva GA, Pateleeva IE. 1987 Dissociation of ethylenediaminetetra(methylphosphonic) acid in an aqueous solution. *Izv Vyssh Uchebn Zaved Khim Khim Tekhnol* **30**, 16–18.

Wang DC, Kottamassu SR, Karvelis K. 1993 Scintigraphy in metabolic bone disease. In: Favus MJ, Christakos S, Gagel RF, *et al.*, eds. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* New York: Raven Press; 129–136.

Westerback S, Rajan KS, Martell AE. 1965 New multidentate ligands. III. Amino acids containing methylenephosphonate groups. *J Am Chem Soc* **87**, 2567–2572.

Williams DR. 1980 Trends towards the study of low molecular weight complexes in biological systems. In: Banerjea D, ed. *Coordination Chemistry 20*. Oxford: IUPAC/Pergamon Press; 129–139.

Winderen M, Kjonniksen I, Fodstad O. 1995 Pronounced therapeutic effect of samarium 153–ethylenediaminetetramethylene phosphonate in an orthotopic human osteosarcoma tibial tumor model. *J Natl Cancer Inst* **87**, 221–222.