Speciation of N-(2-Mercaptopropionyl)glycine Zinc, Nickel, Cadmium and Lead Complexes in Aqueous Solution

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Received August 14, 1984

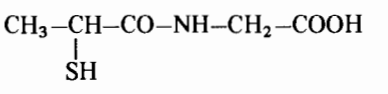
Abstract

Formation constants for the N-(2-mercaptopropionyl)glycine zinc, nickel, cadmium and lead complexes in aqueous solution have been determined at 37 °C and 150 mmol dm^{-3} Cl^- using glass electrode potentiometry. The emf data obtained have been analyzed using the ESTA programs. The major complexes formed, many of which were polynuclear, were as follows: Zn(MPG), $Zn(MPG)_2^{2-}$, $Zn(MPG)_3^{4-}$, $Ni_2(MPGH_{-1})_2^{2-}$, $Ni(MPGH_{-1})(MPG)^{3-}$, $Ni(MPG)_3^{4-}$, $Cd_2(MPG)_2H^+$, $Cd_2(MPG)_2$, $Cd_2(MPG)_3^{2-}$, $Cd_2(MPG)_4^{4-}$, $Cd(MPG)_3^{4-}$, $Pb(MPG)H^+$, Pb(MPG), $Pb_2(MPG)_3^{2-}$, $Pb(MPG)_2^{2-}$, and $Pb(MPG)_3^{4-}$. The formation constants obtained were used in a simulation model of blood plasma.

Introduction

There have been many reports concerning stability constants of metal complexes of sulphydryl molecules having biological interest but although the chelating properties of sulphur-containing amino-acids such as cysteine, glutathione, methionine, and penicillamine, have been studied in great detail, suphur-containing peptides have received scant attention.

N-(2-Mercaptopropionyl)glycine (*Thiola, Tiopronin*) (1) is a drug that is widely used as a radioprotective agent and in the treatment of chronic liver disease. It contains three electron donor groups commonly found *in vivo*—an amide, a carboxylate and a thiol group, which suggests that (a) it can be used as a sulphur-containing protein model, and (b) it might compete for metals if administered.



(1)

Examples of (a) are that the ligand has been studied as a structural model for the active sites of 'blue' copper proteins [1–9] and its iron-sulphide chelate has been considered as a model in the study of the structure and formation of non-heme iron proteins [10, 11].

N-(2-Mercaptopropionyl)glycine has been studied as an antidote to mercury poisoning and it is reported to be effective in the elimination of mercury [12–16], methylmercury [14, 17–20], ethylmercury [21, 22] and phenylmercury [14, 23] in mice tests; to accelerate the excretion of mercury from the body into the urine in man [24–28]; to remove the mercury [29, 30] and the ethylmercury [29] bound to sulphydryl groups of haemoglobin, and to prevent the teratogenic and fetotoxic effects induced by methylmercury in mice [31].

N-(2-Mercaptopropionyl)glycine has also been reported to be effective in the treatment of increased lead absorption [32] and although it has been proposed as a useful treatment for the infertility caused by cadmium intoxication [33], it has been found to increase cadmium levels in kidney and testis in common with other monothiols [34].

Because the molecule resembles D-penicillamine in some respects, N-(2-mercaptopropionyl)glycine has been studied in the treatment of Wilson's disease and in acute copper poisoning [35–39]. It has also been administered to rheumatoid arthritis patients [40].

In relation to the Angiotensin-converting enzyme inhibitory activity displayed by some mercaptoacyl amino-acids, it is interesting to note that N-(2-mercaptopropionyl)glycine has showed a hypotensive effect in rats [41] and that its inhibitory effect on Angiotensin-converting enzyme activity *in vivo* has been studied [42].

Surprisingly, in spite of displaying the aforegoing metal dependent reactions *in vivo*, very few stability constants have been published for N-(2-mercaptopropionyl)glycine–metal complexes and of those available [2, 43–46], many may be considered unreliable because they have been calculated on the assumption that only monomeric complex

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0020-1693/85/$3.30

species were formed. Thus, it is timely to undertake a detailed potentiometric study of complex formation between N-(2-mercaptopropionyl)-glycine and certain representative metal ions—nickel, zinc, cadmium and lead. The data obtained have been used to compute formation constants, which can be included in data bases needed for large computer models of biological fluids such as blood plasma. In order that the data would be applicable to biological fluids, the investigations were performed at 37 °C and in a background electrolyte of 150 mmol dm^{-3} NaCl.

Experimental

Materials

N-(2-Mercaptopropionyl)glycine was supplied by Sigma Chemical Co. *Anal.* Found: C, 36.8; H, 5.5; N, 8.5. Calc. for $C_5H_9NO_3S$: C, 36.8; H, 5.6; N, 8.6%, and was used without further purification. A freshly prepared solution was used for each titration.

Standard stock solutions of metal ions were prepared as their chloride salt (BDH Analar) and analysed by EDTA complexometric titrations for metal ion concentration [47] and by Gran plots [48] for hydrogen ion concentration.

Sodium hydroxide solution (100 mmol dm^{-3}) was prepared freshly and frequently from BDH concentrated ampoules.

To control the ionic strength, all solutions were maintained at a chloride concentration of 150 mmol dm^{-3} by the addition of sodium chloride (BDH Analar).

All solutions were prepared using distilled and degassed double deionized water (resistivity higher than 2 Mohm cm).

High quality grade nitrogen (British Oxygen Co.) was passed through Fiezer's solution [49], concentrated sodium hydroxide, sulphuric acid and ionic background solution, all thermostatted at 37 °C.

Method

Protonation constants for the ligand and formation constants for the metal complexes were determined by potentiometric titrations in a double walled vessel (Pye Ingold 604) thermostatted at 37.0 ± 0.02 °C by circulating water. The free hydrogen ion concentration was measured with a Radiometer pH-64 Research pH Meter equipped with a wide-range glass electrode (Russell SW) and a saturated potassium chloride calomel electrode (Russell CR).

The cell incorporated a Teflon stirrer and a purified nitrogen atmosphere was maintained in the vessel during the titrations.

The electrode system was calibrated in terms of hydrogen ion concentrations by performing strong acid *versus* strong base titrations [50].

Experimental solutions containing ligand, metal (when appropriate) and hydrochloric acid were titrated, the hydrogen ion concentration being varied by adding sodium hydroxide to the solution from a piston-burette (Metrohm E-264) through a finely drawn glass capillary.

Protonation curves for the ligand were obtained at a variety of different total ligand concentrations and, similarly, the metal complex formation curves were obtained at different ligand:metal ratios and for different total ligand and total metal concentrations.

Data Treatment

The general equilibria involving metal M^{2+}, ligand L^{2-} (*i.e.* deprotonated N-(2-mercaptopropionyl)-glycine = MPG), and H^+ ions can be written as:

$$pM + qL + rH \rightleftharpoons M_pL_qH_r$$

The overall formation constants are defined as:

$$\beta_{pqr} = \frac{[M_pL_qH_r]}{[M]^p[L]^q[H]^r} \quad (2)$$

where the square brackets indicate the concentrations of the free metal, ligand and hydrogen ions. Charges are omitted for simplicity.

For the metal hydrolytic equilibria, values taken from the literature [51] were used. Inclusion of the proposed complexes and equilibrium constants (recalculated for 150 mmol dm^{-3} and 37 °C [52]) in the calculations showed that metal hydrolysis was negligible under our conditions.

The initial models were obtained from the shapes of the formation curves, $\bar{Z}_M(-\log[A])$ and of the deprotonation curves [53], $\bar{Q}(-\log[H])$, $\bar{Z}_M$ being defined as:

$$\bar{Z}_M = \frac{T_L - [A](1 + \Sigma_n\beta_{LH_n}[H]^n)}{T_M} \quad (3)$$

where $[A] = (T_H - [H] + [OH])/\Sigma_n\beta_{LH_n}[H])$, T_L = total ligand concentration, T_M = total metal concentration, and T_H = total hydrogen ion concentration. $\bar{Q}$ was defined as:

$$\bar{Q} = \frac{(T_H^* - T_H)}{T_M} \quad (4)$$

where T_H^* is the calculated total concentration of protons in the system at the observed $-\log[H]$ ignoring the presence of all metal complexes. Initial estimates of formation constants were obtained using the BETA task of the ESTA program [53], values for the formation constant of a single species can be calculated from the emf reading at each titration point. The final selection of species was based on graphical comparison between calculated

and experimental $\bar{Z}$ and $\bar{Q}$ curves in addition to various statistical criteria.

Formation constant refinement was performed using the ESTA optimization module with a weighted least-squares objective function based on emf residuals. Debye-Hückel corrections for changes in ionic strength were applied. The program allows for the simultaneous optimization of formation constants and other titration parameters, such as electrode slope and intercept, initial volumes and concentrations. It has been shown that this can significantly reduce the effect of systematic errors in the data [54]. The effectiveness of optimizing different sets of parameters was examined by analysing several sets of simulated titration data generated with random errors in electrode parameters, initial volumes, initial concentrations, emf, and titre volumes.

The results obtained were checked against those calculated by other least-squares programs such as MINIQUAD [55] and SCOGS [56]. Good agreement was obtained in all cases for which a valid comparison could be made.

All calculations were performed on a VAX 11/780 computer.

Results and Discussion

Formation constants obtained in the present work are listed in Table I. The quoted objective function, U, is defined as:

$$U = \frac{\Sigma_n w(\text{emf}^o - \text{emf}^c)^2}{N - n_p}$$

where N = total number of experimental titration points and n_p = total number of optimized parameters. The weight at each titration point was based on standard deviation in titre of 0.001 cm^3 and in emf of 0.05 mV. The sum of squares of residuals is based on total electrode ion concentrations.

The MPG–H^+ System

MPG shows two protonation constants (see Table I). These can be assigned to the carboxylate and thiol group. Calculations included the simultaneous optimization of electrode intercept, initial acid and inital ligand concentrations. The results obtained agree well with those found by other authors (Table II).

The Zn^{2+}–MPG–H^+ System

The analysis of the formation curve (Fig. 1a) and the deprotonation curve (Fig. 1b) for the Zn^{2+} system shows that only mononuclear species are formed since all points fall on a single curve. The species 110, 120 and 130 gave a low objective function value and the $\bar{Z}$ and $\bar{Q}$ graphical representation indicated a very satisfactory fit. Electrode intercept, intial acid concentration and initial volume concentration were optimised simultaneously with log β values. Results agree well with those previously reported (Table II).

The Ni^{2+}–MPG–H^+ System

Sugiura *et al.* [2, 44] have reported that Ni^{2+} induces amide deprotonation in MPG to yield a 1:1 complex, assumed to be mononuclear, in which the ligand is coordinated through the sulphydryl group, the deprotonated amide nitrogen and a carbo-

TABLE I. Formation Constants Determined in this Study at 37 °C and I = 150 mmol dm^{-3} Cl^-.

	p	q	r	log β_{pqr}	U	Sum of squares of residuals	No. of data points	No. of titrations
H^+	0	1	1	8.191 ± 0.001	6.52	3.237 E-07	499	8
	0	1	2	11.620 ± 0.001				
Zn^{2+}	1	1	0	5.371 ± 0.001	10.5	1.579 E-07	480	11
	1	2	0	10.086 ± 0.002				
	1	3	0	13.285 ± 0.004				
Ni^{2+}	2	2	−2	−0.326 ± 0.004	72.7	7.079 E-07	527	12
	1	2	−1	2.144 ± 0.007				
	1	3	0	12.135 ± 0.023				
Cd^{2+}	2	2	1	18.347 ± 0.027	28.8	3.949 E-07	461	11
	2	2	0	14.946 ± 0.006				
	2	3	0	20.834 ± 0.006				
	2	4	0	25.432 ± 0.010				
	1	3	0	14.988 ± 0.008				
Pb^{2+}	1	1	1	9.854 ± 0.011	10.6	1.862 E-07	474	10
	1	1	0	6.726 ± 0.003				
	2	3	0	20.846 ± 0.013				
	1	2	0	11.527 ± 0.010				
	1	3	0	14.379 ± 0.016				

TABLE II. Literature Formation Constants of Several Metal Complexes of the Ligand.

p	q	r	log β_{pqr}					Technique	Conditions	References
			H^+	Zn^{2+}	Ni^{2+}	Cd^{2+}	Pb^{2+}			
0	1	1	8.43					Potentiometry	25 °C	[43]
0	1	2	11.81						150 mmol dm^{-3} $NaClO_4$	
1	1	0		5.70		7.21	7.37			
1	2	0		10.53		12.86	12.52			
0	1	1	8.74					Potentiometry	22 °C	[2, 44]
0	1	2	12.34						100 mmol dm^{-3} KNO_3	
1	1	0		5.72	5.44	7.06				
1	2	0		10.45		13.11				
1	1	−1			−1.44					
0	1	1	8.47					Polarography	20 °C	[57]
0	1	1	8.40					Potentiometry	25 °C	[45]
									500 mmol dm^{-3} KNO_3	
0	1	1	8.33					Potentiometry	25 °C	[46]
0	1	2	11.75						200 mmol dm^{-3} KCl	

xylate oxygen. Sóvágó *et al.* [58] confirmed that MPG could act as a terdentate ligand in the presence of Ni^{2+} but reported an extensive polynuclear complex formation through sulphydryl bridges in solutions containing comparable amounts of metal ion and ligand.

The formation curve (Fig. 2a) and the deprotonation curve (Fig. 2b) suggest that polynuclear, protonated or hydroxo species are present in the system. However, the search for the best set of complexes proved difficult and over ninety different models were tested. Finally, the species 22-2, 12-1 and 130 were selected. The formation constant and, perhaps, even the existence of the species 130 can, however, be regarded as doubtful since it was not formed in most of our titrations, and even at the highest ligand:metal ratio its calculated maximum concentration only reached 22% of the total metal.

It has been demonstrated frequently that ligands containing mercaptide ions have the ability to form sulphur-bridged complexes with Ni^{2+}, and so it may be assumed that the 22-2 complex has the structure proposed by Sugiura *et al.* [44] but having two metal ions linked through sulphur bridges. As the ligand: metal ratio increases, the 12-1 complex is formed. This is in agreement with Sóvágó *et al.* [58] who have pointed to the possible existence of a complex where one ligand is bound to Ni^{2+} as a terdentate ligand whilst the second is bound only through a sulphydryl group.

The Cd^{2+}–MPG–H^+ System

Formation (Fig. 3a) and deprotonation (Fig. 3b) curves suggested the existence of polynuclear complex formation and this was confirmed by finding the species 220, 230, 240 and 130 which gave the best agreement with observed data. Including the species 221 resulted in a significant improvement in the objective function and in a better fit of the curves. It was thus included, in spite of the complex being formed to a maximum of only 10%. This is reflected in the relatively large standard deviation in the value of its formation constant. Further analysis showed that no other protonated or polynuclear complexes seemed to be formed in any appreciable amount. The same titration parameters as those for the zinc system were optimized.

The Pb^{2+}–MPG–H^+ System

The best model was found to contain the species 110, 120, 230, 130 and 111. Formation and deprotonation curves are shown in Fig. 4. The parameters optimized were the same as used previously with zinc and cadmium. It should be noted that this resulted in a high correlation between the values of the formation constants of the species 120 and 130 which, it is estimated might affect the precision of those values by as much as 0.05 log units.

Simulation of Blood Plasma

The formation constants obtained were used to investigate the steady-state conditions which exist in bio-solutions such as blood plasma. Administered drugs like N-(2-mercaptopropionyl)glycine are sometimes capable of mobilizing metal ions from plasma proteins and tissue and of increasing the low molecular weight fraction present.

Typical species distribution plots for zinc, nickel, cadmium and lead–MPG complexing are shown in Figs. 5a, b, c and d.

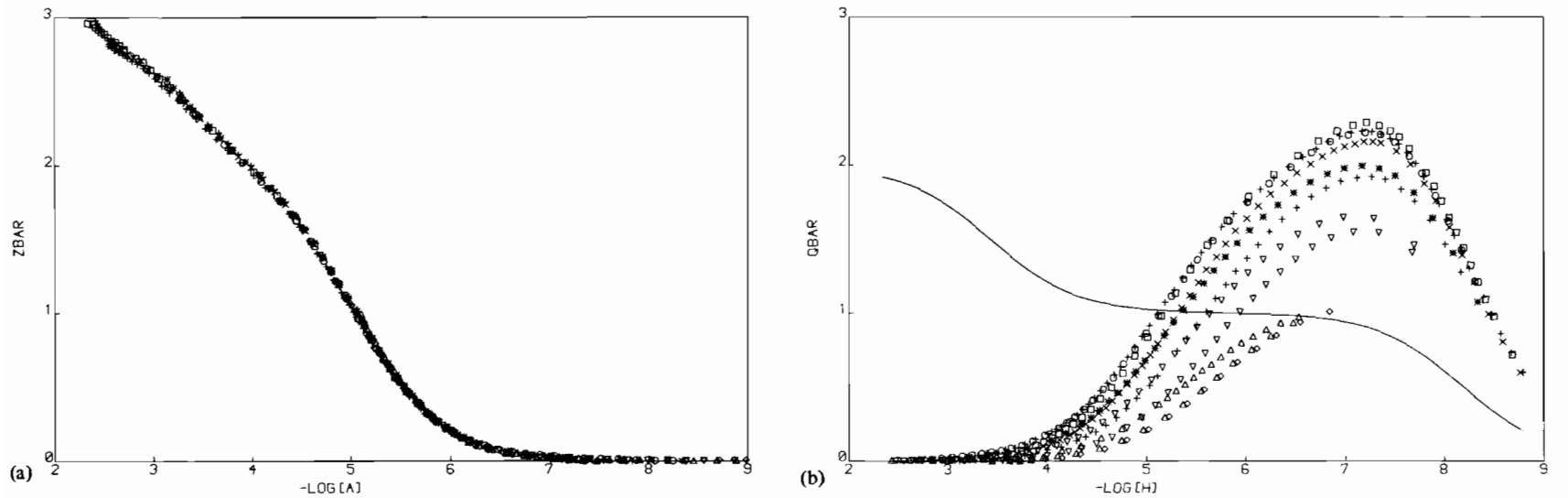


Fig. 1. Zn^{2+}–MPG–H^+ experimental (a) formation and (b) deprotonation curves. Different symbols refer to different titrations having different ligand:metal ratios and different total ligand and total metal concentrations. On each deprotonation curve, a plot of $\bar{n}$ *vs.* –log[H] appears as a solid line.

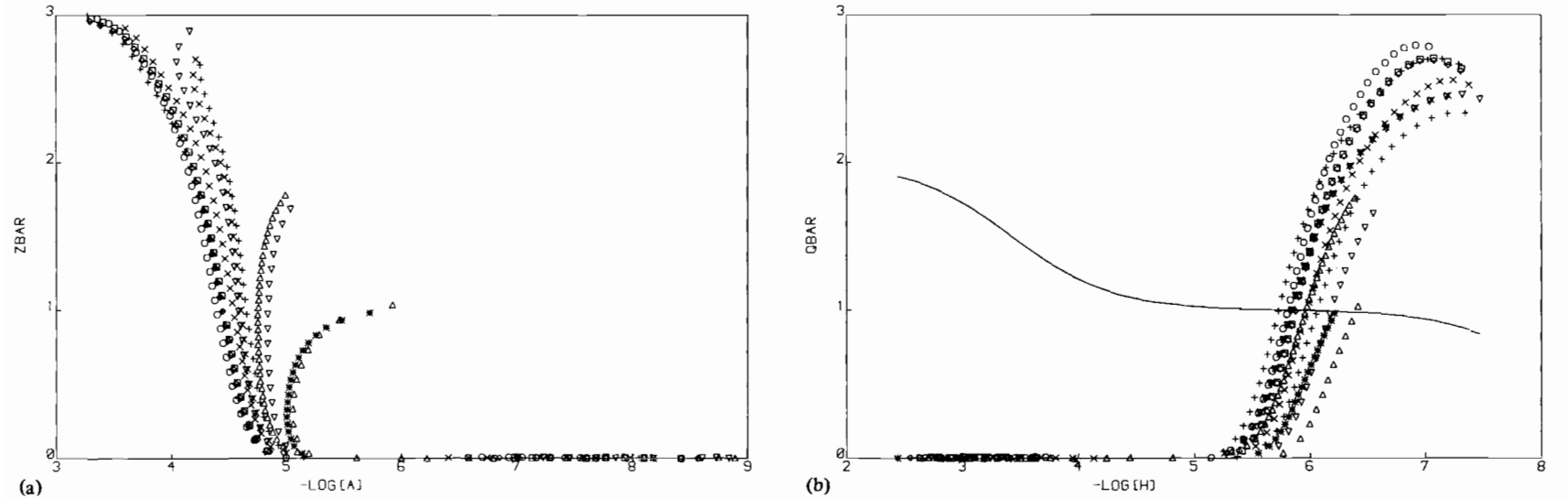
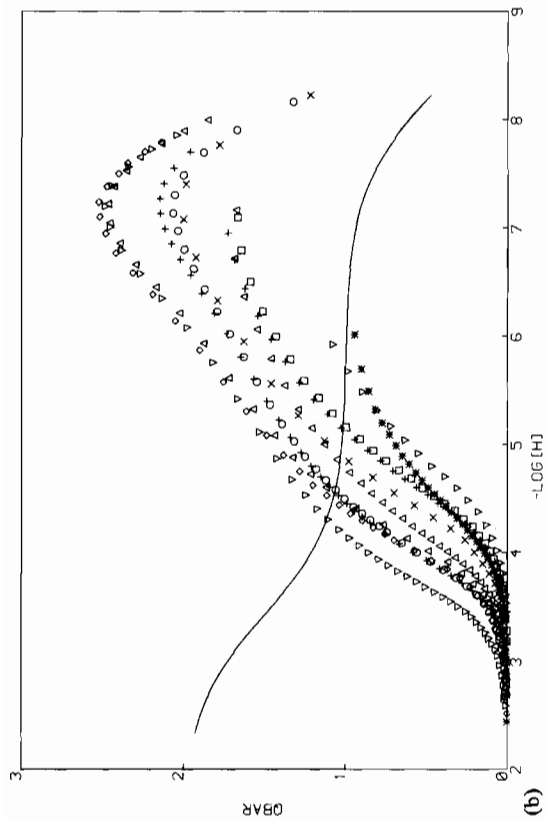


Fig. 2. Ni^{2+}–MPG–H^+ experimental (a) formation and (b) deprotonation curves.



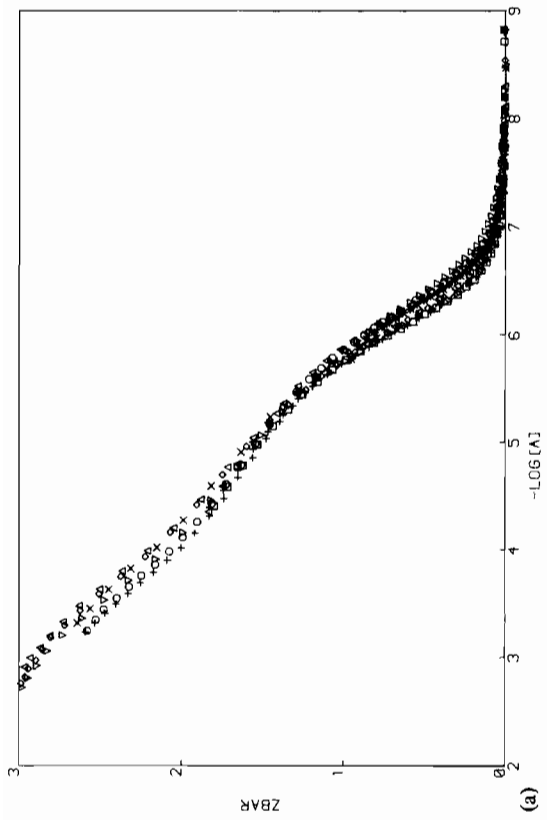
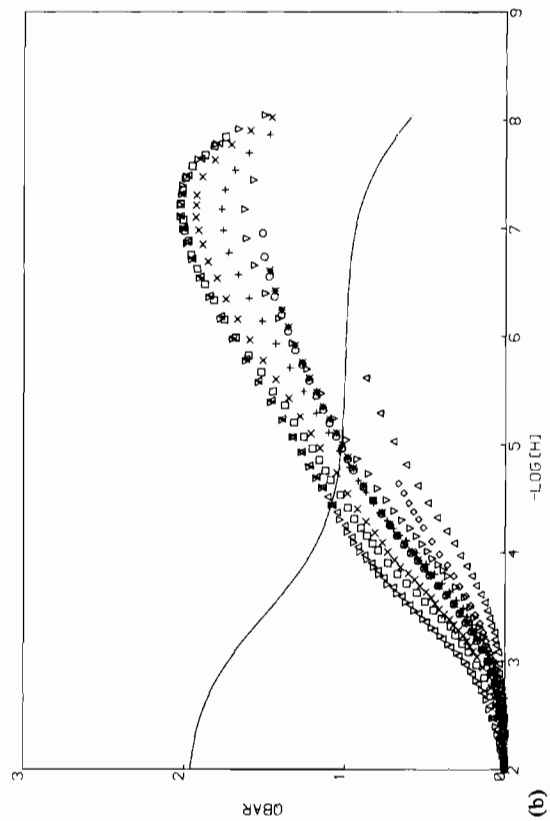


Fig. 3. Cd^{2+}–MPG–H^+ experimental (a) formation and (b) deprotonation curves.



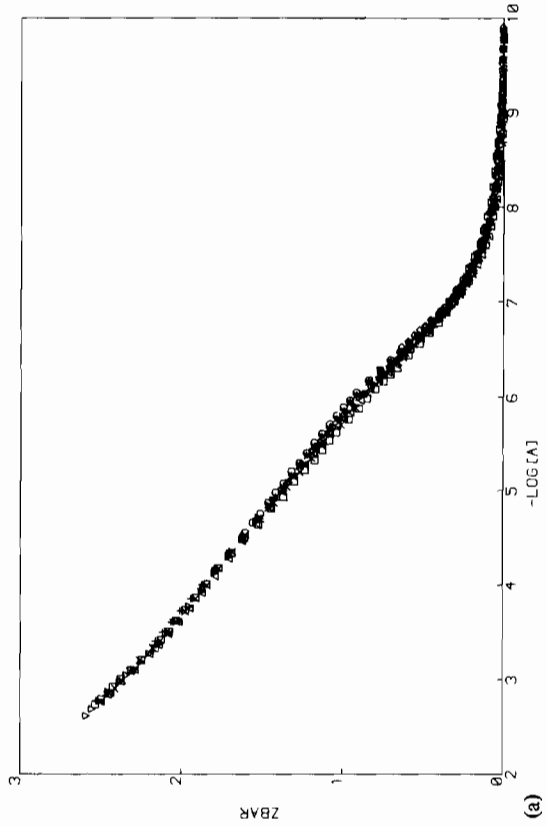


Fig. 4. Pb^{2+}–MPG–H^+ experimental (a) formation and (b) deprotonation curves.

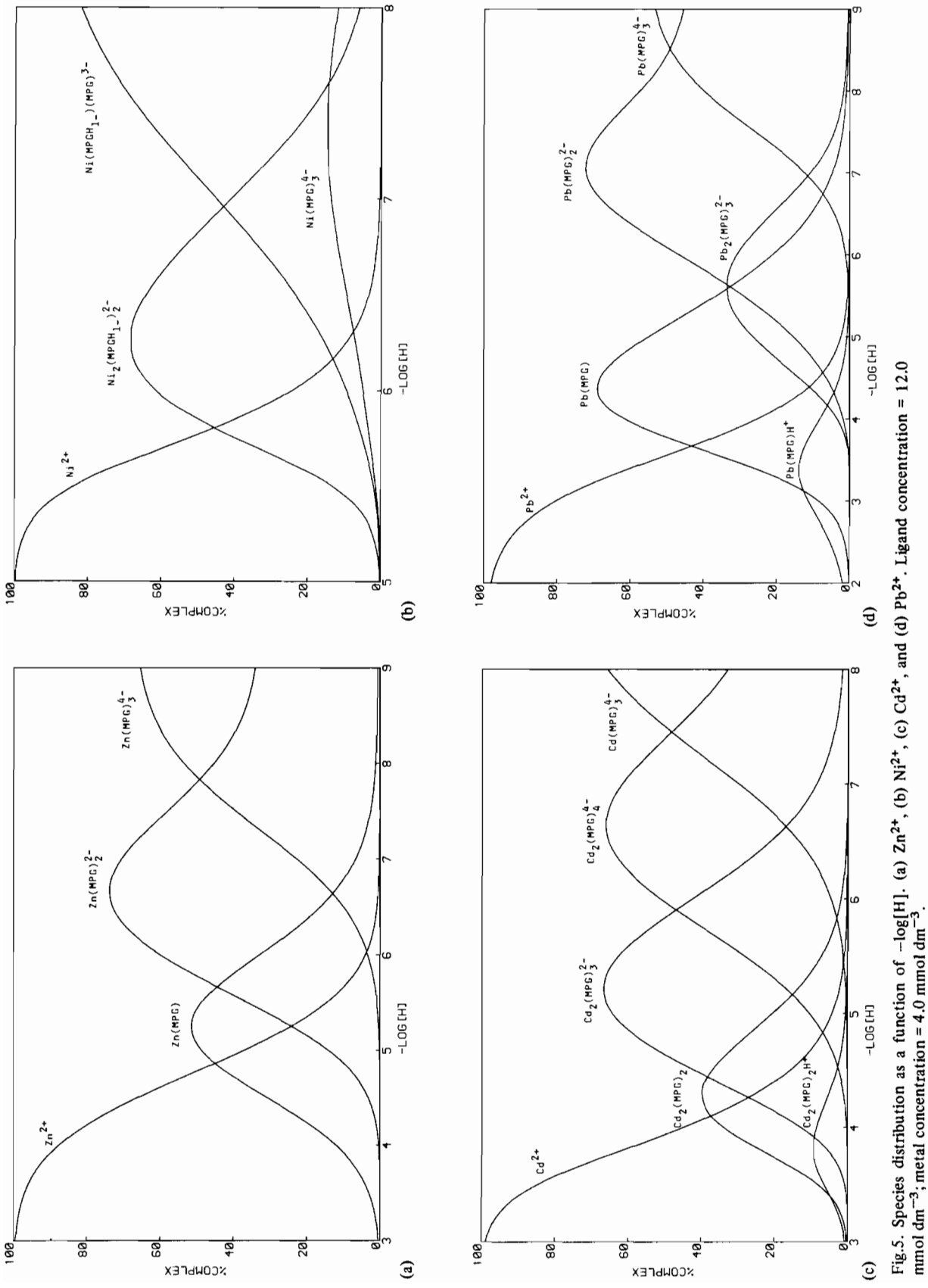


Fig.5. Species distribution as a function of $-\log[H]$. (a) Zn^{2+}, (b) Ni^{2+}, (c) Cd^{2+}, and (d) Pb^{2+}. Ligand concentration = 12.0 mmol dm^{-3}; metal concentration = 4.0 mmol dm^{-3}.

TABLE III. Log PMI Values for MPG in Blood Plasma Computed Using ECCLES.

MPG concentration in blood plasma	Metal ion			
	Zn^{2+}	Ni^{2+}	Cd^{2+}	Pb^{2+}
10^{-5} mol dm^{-3}	0.01	0.00	0.00	0.00
10^{-4} mol dm^{-3}	0.10	0.00	0.00	0.01
10^{-3} mol dm^{-3}	0.13	0.00	0.82	0.32

TABLE IV. Percentage Distribution of Low Molecular Weight Zn^{2+} Coordinated to MPG in Blood Plasma (Concentration of Drug = 10^{-4} mol dm^{-3}).

Complex	% of l.m.w. Zn^{2+}
$Zn(MPG)(Cys)^{2-}$	13.21
$Zn(MPG)His)^{-}$	2.9
$Zn(MPG)^{0}$	1.6
$Zn(MPG)_2^{2-}$	1.2
$Zn(MPG)(Cys)H^{-}$	0.5
$Zn(MPG)(Cis)H^{-}$	0.5

The ability of N-(2-mercaptopropionyl)glycine at various concentrations to mobilize metal ions can be expressed in terms of a plasma mobilizing index (PMI) [59]. These values have been computed [52] and listed in Table III. The mobilization of cadmium and lead expected from the biological observations mentioned earlier is found although a high concentration of drug in plasma is required. This suggests that decorporation therapy must either be prolonged or that sources other than plasma are tapped by N-(2-mercaptopropionyl)glycine.

In common with many heavy metal mobilizing agents, N-(2-mercaptopropionyl)glycine produces significant PMI values for zinc, an essential biometal. The new low molecular weight complexes formed are shown in Table IV. Caution must therefore be exercized to replace any zinc lost during extensive therapy involving this drug.

Acknowledgements

We gratefully acknowledge the assistance of Dr. K. Murray and Dr. P. M. May with the computer calculations (ESTA program). One of us (M.F.) thanks the C.I.R.I.T. (Generalitat de Catalunya) for a maintenance grant.

References

1 Y. Sugiura, Y. Hirayama, H. Tanaka and K. Ishizu, *J. Am. Chem. Soc., 97*, 5577 (1975).
2 Y. Sugiura and Y. Hirayama, *Inorg. Chem., 15*, 679 (1976).
3 Y. Sugiura and Y. Hirayama, *J. Am. Chem. Soc., 99*, 1581 (1977).
4 Y. Sugiura, *Eur. J. Biochem., 78*, 431 (1977).
5 H. Rupp and U. Weser, *Biochim. Biophys. Acta, 446*, 151 (1976).
6 M. Younes, W. Pilz and U. Weser, *J. Inorg. Biochem., 10*, 29 (1979).
7 M. Younes, S. Zienau, H. J. Hartmann and U. Weser, *Metalloproteins, Autumn Meet. Ger. Biochem. Soc., 43*, 1979.
8 N. S. Ferris, W. H. Woodruff, D. B. Rorabacher, T. E. Jones and L. A. Ochrymowycz, *J. Am. Chem. Soc., 100*, 5939 (1978).
9 G. McLendon, *J. Inorg. Nucl. Chem., 40*, 755 (1978).
10 Y. Sugiura and H. Tanaka, *Biochem. Biophys. Res. Commun., 46*, 335 (1972).
11 Y. Sugiura, M. Kunishima, H. Tanaka and H. H. Dearman, *J. Inorg. Nucl. Chem., 37*, 1511 (1975).
12 E. Ogawa, S. Suzuki and H. Tsuzuki, *Kitakanto Igaku, 24*, 299 (1974).
13 S. Maruyama, M. Hachisu, H. Iwanaga, Y. Ino, S. Ogasawara and S. Yamada, *Showa Igakkai Zasshi, 37*, 449 (1977).
14 H. Kitoh and N. Toshioka, *Jpn J. Pharmacol., 28*, 423 (1978).
15 K. Minowa, H. Keki, H. Konno and G. Ohi, *Tokyo-toritsu Eisei Kenkyusho Kenkyu Nempo, 29*, 349 (1978).
16 M. A. Basinger, J. S. Casas, M. M. Jones, A. D. Weaver and H. N. Weinstein, *J. Inorg. Nucl. Chem., 43*, 1419 (1981).
17 E. Ogawa, H. Tsuzuki and Y. Yamazaki, *Radioisotopes, 25*, 19 (1976).
18 H. Konno, H. Seki, K. Minowa, K. Yunome, H. Yagyu and G. Ohi, *Tokyo-toritsu Eisei Kenkyusho Kenkyu Nempo, 27*, 297 (1976).
19 T. Fujisaki, H. Ishii and S. Kogima, *Acta Med. Univ. Kagoshima, 19*, 83 (1977).
20 E. Ogawa, *Med. Biol., 91*, 295 (1975).
21 E. Ogawa, S. Suzuki, H. Tsuzuki and M. Kawajiri, *Kitakanto Igaku, 24*, 229 (1974).
22 E. Ogawa, *Igaku to Seibutsugaku, 101*, 269 (1980).
23 E. Ogawa, S. Suzuki, H. Tsuzuki, M. Kawajiri and H. Kito, *Kitakanto Igaku, 24*, 57 (1974).
24 T. Tsubaki and K. Shirakawa, *Shinyaku-to Rinsho, 15*, 1471 (1966).
25 M. Ogata, Y. Eguchi, T. Kinami and Y. Ohta, *J. Okayama Med. Soc., 80*, 297 (1968).
26 R. Hamada, H. Nakamura and A. Igata, *Igaku-no-Ayumi, 91*, 285 (1974).
27 H. Takahashi, K. Hirayama and Y. Hukushima, *Clin. Report (Tokyo), 9*, 1347 (1975).
28 K. Shirakawa, K. Hirota, T. Katagiri, T. Tsubaki and H. Saito, *Niigata Igakkai Zasshi, 90*, 495 (1976).
29 Y. Sugiura, Y. Hojo and H. Tanaka, *Radioisotopes, 19*, 184 (1970).
30 H. Sakurai and S. Takeshima, *Inorg. Chim. Acta, 56*, L29 (1981).
31 T. Fujimoto, M. Fuyuta, E. Kiyofuji and S. Hirata, *Teratology, 20*, 297 (1979).

32 F. Candura, G. Franco, T. Malamani and L. Scalisi, *The Lancet*, 330 (1979).
33 A. Suzuki, N. Kajimoto, T. Yanagawa, J. Sugimoto and M. Nagata, *J. Toxicol. Sci., 3*, 313 (1978).
34 E. Ogawa, S. Suzuki and H. Tsuzuki, *Jpn J. Pharmacol., 22*, 275 (1972).
35 V. Chavdarova and Ts. Stoichev, *Izv. Inst. Fiziol., Bulg. Akad. Nauk., 16*, 297 (1974).
36 Ts. Stoichev, *Proc. Eur. Soc. Toxicol., 16*, 252 (1975).
37 K. Gibbs and J. M. Walshe, *Clin. Sci. Mol Med., 53*, 317 (1977).
38 F. Planas-Bohne, *Toxicol. Appl. Pharmacol., 50*, 337 (1979).
39 M. M. Jones, A. D. Weaver and M. A. Basinger, *J. Inorg. Nucl. Chem., 43*, 2175 (1981).
40 B. Amor, C. Mery and A. de Gery, *Revue du Rhumatisme, 47*, 157 (1980).
41 P. J. Schulze, *Arzneim.-Forsch., 22*, 1433 (1972).
42 Y. Funae, T. Komori, D. Sasaki and K. Yamamoto, *Jpn J. Pharmacol., 28*, 925 (1978).
43 Y. Funae, N. Toshioka, I. Mita, T. Sugihara, T. Ogura, Y. Nakamura and S. Kawaguchi, *Chem. Pharm. Bull., 19*, 1618 (1971).
44 Y. Sugiura, Y. Hirayama, H. Tanaka and H. Sakurai, *J. Inorg. Nucl. Chem., 37*, 2367 (1975).
45 G. Terzian, M. Asso, R. Panossian, M. Guiliano and D. Benlian, *J. Chim. Phys., 79*, 181 (1982).
46 B. Harman and I. Sóvágó, *Inorg. Chim. Acta, 80*, 75 (1983).
47 A. I. Vogel, 'A Textbook of Quantitative Inorganic Analysis', Longman, London, 1978.
48 D. Dyrssen, D. Jagner and F. Wengelin, 'Computer Calculation of Ionic Equilibria and Titration Procedures', Wiley, London, 1968.
49 L. F. Fieser, *J. Am. Chem. Soc., 46*, 2639 (1924).
50 P. M. May, D. R. Williams, P. W. Linder and R. G. Torrington, *Talanta, 29*, 249 (1982).
51 R. M. Smith and A. E. Martell, 'Critical Stability Constants. Volume 4. Inorganic Complexes', Plenum Press, New York, 1976.
52 P. M. May, P. W. Linder and D. R. Williams, *J. Chem. Soc., Dalton Trans.*, 588 (1977).
53 K. Murray and P. M. May, 'ESTA. Equilibrium Simulation for Titration Analysis', UWIST, Cardiff, 1984.
54 K. Murray and P. M. May, *Anal. Proc.*, (at press).
55 A. Sabatini, A. Vacca and P. Gans, *Talanta, 21*, 53 (1974).
56 I. G. Sayce, *Talanta, 15*, 1397 (1968).
57 E. Cságoly, A. Sántha and M. Horváth, *Acta Biochim. Biophys. Acad. Sci. Hung., 11*, 51 (1976).
58 I. Sóvágó and R. B. Martin, *J. Inorg. Nucl. Chem., 43*, 425 (1981).
59 P. M. May and D. R. Williams, *FEBS Lett., 78*, 134 (1977).