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The Binding of Metal Ions by Mercaptoacids. II*. Formation Constants for the Complexes of Mercaptosuccinate with Cd(II) and Computer Simulation of its Ability to Mobilize the Low Molecular Weight Fraction of Cd(II) in Blood Plasma

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Abstract

Formation constants for cadmium(II) complexes of mercaptosuccinate in aqueous solution have been determined at 37 °C and 150 mmol dm^{-3} chloride medium by potentiometric titrations using a glass electrode. The emf data obtained have been analyzed using the ESTA computer program library. The experimental data can be explained by the formation of the complexes $Cd_3(MSA)_2$, $Cd_2(MSA)_2H^-$, $Cd_2(MSA)_2^{2-}$, $Cd_2(MSA)_2OH^{3-}$ and $Cd(MSA)_2^{4-}$. The formation constants obtained have been used in a simulation model of blood plasma to investigate the mobilization of cadmium(II) by mercaptosuccinate in normal plasma. It is shown that mobilization is unlikely at pharmacological levels of the drug.

Introduction

Although the adverse effects of cadmium overload in humans have been widely documented, to date no chelating agent has been recommended for the treatment of cadmium poisoning.

The metal tends to be accumulated by mammals in their liver and kidneys. Initially, hepatic levels are very high but then the cadmium is gradually redistributed from the liver to the kidney [2]. The relative concentrations of cadmium in the body depend on the duration and the magnitude of exposure. A steady accumulation of cadmium may be assumed for all those living in industrialized environments since mammalian species excrete cadmium very slowly. The retention of the metal ion is related to its binding within the protein called metallothionein [3].

The metal has been shown to interfere with metabolism in general. It is both a carcinogen and teratogen. Sub-acute pulmonary exposure leads to respiratory complications and long-term intake is responsible for the Japanese 'itai-itai' disease.

As early as 1946, more than forty mercaptans were examined for their ability to counter cadmium poisoning [4]. Of all the compounds examined, BAL (2,3-dimercaptopropanol) proved to be the most promising one, mainly because it was already in clinical use for other conditions rather than for its cadmium specificity. Unfortunately, its use in the treatment of patients who have been chronically exposed to cadmium has been generally contraindicated [5] because the neutrality of the resulting $Cd(BAL)^0$ complex causes the metal to accumulate in the kidney wherein it enhances the nephrotoxicity of cadmium resulting in renal damage [4, 6–8]. Nevertheless, Cherian has shown BAL to be effective in chronic cadmium poisoning, mobilizing both hepatically and renally deposited cadmium, even when the metal has been incorporated into metallothionein [9, 10]. Nephrotoxic complications are less likely when BAL is administered in acute cadmium intoxication provided that the metal is localized in the lungs and has not yet been systemically distributed [11].

The toxicity of BAL, that usually restricts its clinical applications, has been reduced in two dithiols with similar chelating properties, DMSA (2,3-dimercaptosuccinic acid) and DMPS (2,3-dimercaptopropanesulphonic acid, Unithiol), by the introduction of hydrophilic side-groups into the molecule in order to increase its water solubility. Both ligands have been shown to promote cadmium excretion *in vivo* provided therapy is administered within a very short period after intake [12–15]. A feature that is common to all mercaptans is that they seem to be of little value in mobilizing cadmium from the body since metallothionein synthesis has commenced.

*For Part I, see ref. 1.

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The polyaminopolycarboxylic acids, EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylenetriaminepentaacetic acid), have been shown to effectively mobilize cadmium *in vivo* [16–19] but they are of limited value because they are only capable of removing extracellular cadmium due to their hydrophilicity and they remove essential metals simultaneously. Their usefulness in the treatment of cadmium poisoning is further limited by their potential renal toxicity [12, 20]. Furthermore, DTPA fails to mobilize cadmium once it has been incorporated into metallothionein [12].

A recently published computer-simulation assessment of chelating drugs in the treatment of cadmium poisoning reveals that DMSA or DMPS are likely to be the most efficacious ligands in the immediate treatment of intoxication while a synergistic therapy is proposed for the treatment of chronic intoxication. The latter involves using first a lipophilic drug to mobilize intracellular cadmium, then a hydrophilic agent to promote urinary excretion of the metal [21].

Mercaptosuccinic acid, being similar in structure to DMSA, has also been studied in relation to cadmium poisoning; assays have been carried out in frogs [22] and in mice [23]. Although its administration to mice has been stated not to reduce cadmium levels in liver, kidney, brain or blood [24], it has proved efficient at eliminating cadmium in cultured epithelial cells [25] and thus, apparently, it does have the capabilities of penetrating into, and chelating within, a cell, *i.e.* playing the second role of the synergistic pair of drugs mentioned above. However, it was reported to be less effective than EDTA or dithiols in eliminating cadmium from erythrocytes [26]. Clearly, further analysis is highly desirable.

Thus, in the present study, the ability of mercaptosuccinic acid to bind cadmium in human blood plasma has been assessed by computer simulation. The formation constants required for the simulation were previously determined under biological conditions of temperature and ionic strength.

Experimental

Reagents

Analytical grade reagents (Merck) were used throughout. All solutions were prepared using deionized doubly-distilled water which had been boiled and cooled under nitrogen.

Mercaptosuccinic acid (Merck) was used without further purification. *Anal.* Found: C, 32.0; H, 4.03; S, 21.4. Calc. for $C_4H_6O_4S$: C, 32.0; H, 4.03; S, 21.4%. Its purity was tested by means of its reaction with mercuric chloride according to the procedure proposed by Tiwari and Verma [27]. Solutions of mercaptosuccinic acid were freshly prepared for each titration by direct weighing.

Stock solutions of cadmium were prepared from their chloride salt and were made slightly acid by adding hydrochloric acid in order to prevent hydrolysis and absorption of carbon dioxide. The metal content of the solution was determined against EDTA using xylenol orange as indicator [28]. Its mineral acid content was determined by titration with standard alkali (Gran plots) and the concentration optimized by use of the MAGEC program [29].

Sodium hydroxide solutions and hydrochloric acid solutions were prepared and standardized as previously described [1]. To control the ionic strength, all solutions were maintained at a chloride concentration of 150 mmol dm^{-3} by the addition of sodium chloride.

Experimental Procedure

Potentiometric titrations were performed at 37 °C and $I = 150$ mmol dm^{-3} Cl^- following our usual approach [1]. The metal complex formation curves were unsuccessful for this reason [30, 31] and so the for different total ligand and total metal concentrations. The metal concentrations used and the volumes of titrant added were lower than usual in order to avoid the formation of metal complex precipitates. In fact, previous attempts to study this system were unsuccessful for this reason [30, 31] and so the computation of formation constants had been precluded because of the formation of insoluble complexes. All titrations where precipitate appeared in the solution, as noted from a steady drift in the pH meter readings, were discarded.

Values of cadmium and ligand concentrations, as well as $-\lg[H]$ ranges used in calculating the formation constants, are summarized in Table I.

TABLE I. Summary of the Titration Data Used for Calculating Stability Constants[a]

Titration	Number of points	C_{Cd}	C_{MSA}	$-\lg[H]$ range
1	65	0.855	4.186	2.7–7.8
2	66	0.855	4.249	2.7–7.8
3	47	1.711	2.232	2.6–5.8
4	79	1.711	5.039	2.5–9.5
5	81	1.711	6.729	2.5–8.8
6	73	1.711	8.698	2.5–9.7
7	76	1.711	9.035	2.5–9.7
8	41	1.711	10.520	2.4–9.0
9	85	1.711	11.498	2.4–7.9
10	76	3.421	3.342	2.4–5.3
11	38	3.421	3.421	2.4–5.0

[a]Number of titration points, initial concentrations of metal (C_{Cd}) and ligand (C_{MSA}) and approximate $\lg[H]$ ranges. Concentrations are expressed in mmol dm^{-3}.

Data Treatment

In evaluating the equilibrium constants, metal M^{2+}, ligand L^{3-} (*i.e.* deprotonated mercaptosuccinic acid = MSA) and H^+ ions were chosen as components. The general three-component equilibria can be written as:

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

The overall formation constants are defined as:

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

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

For the metal hydrolysis values taken from the literature for the main hydrolysis products of cadmium: $CdOH^+$, Cd_2OH^{3+} and $Cd_4(OH)_4^{4+}$ were used. Including the proposed hydroxo complexes and equilibrium constants (recalculated for 150 mmol dm^{-3} and 37 °C [32]) in the calculations showed that metal hydrolysis was negligible under our conditions.

The mercaptosuccinate protonation constants used ($\lg \beta_{011} = 10.152$, $\lg \beta_{012} = 14.670$ and $\lg \beta_{013} = 17.754$) had been previously determined [1].

The computer program ESTA [33] was used to analyse the data, develop the equilibrium model and refine the formation constants as previously described in reference [1].

The efficacies of chelating agents for complexing and removing metal ions *in vivo* may be studied by means of computer simulation. The ECCLES (Evaluation of Constituent Concentration in Large Equilibrium Systems) program [32] can be used to evaluate the effect on the low molecular weight (l.m.w.) metal ion distribution of the administration of exogenous chelating agents. The efficacy of a chelating agent for mobilizing a metal ion from the labile metal–protein complex in blood plasma is expressed in terms of a Plasma Mobilizing Index (PMI) [34]:

$$\text{PMI} = \frac{\text{total concentration of low molecular weight metal complex species in the presence of the drug}}{\text{total concentration of low molecular weight metal complex species in normal blood plasma}}$$

ESTA calculations were performed on a VAX 11/750 computer and ECCLES calculations were performed on a VAX 11/780 computer.

Results and Discussion

The Cd^{2+}–Mercaptosuccinate–H^+ System

The solution chemistry of cadmium with mercaptosuccinate is largely unexplored, probably due to the fact that insoluble complexes are formed. This fact was already observed in 1965 by Lenz and Martell [30] and corroborated later by Corrie *et al.* [31] who reported that a precipitate is formed even at a cadmium concentration as low as 1.0 mmol dm^{-3}. In the present work, precipitation could be avoided by working at metal ion concentrations lower than 2 mmol dm^{-3} since at higher concentrations only ligand:metal ratios lower than 1:1 avoid the formation of precipitates. Regardless of the metal concentration used, titrant should always be added in relatively small volumes to preclude precipitation.

Formation and deprotonation curves for the Cd^{2+}–MSA–H^+ system are shown in Fig. 1. The analysis of these curves suggests that polynuclear, protonated or hydroxo species are present in the system. Application of the usual statistical and graphical species selection criteria converges upon the species 320, 221, 220, 22–1 and 120 as being the best model. The complexes 320 and 221 are the only species found to be formed in the lowest pH range whenever complexation occurs, the extent of their formation being highly dependent on the cadmium concentration. The 220 species, which had been already reported for other metal systems such as Zn^{2+}–MSA–H^+ and Ni^{2+}–MSA–H^+ [1], becomes predominant at higher pH values accounting for the complexation of 85% of metal at pH 6.0. Finally, the mononuclear species 120 binds all the metal at pH > 9.5.

Values for the formation constants of the complexes formed in the system Cd^{2+}–MSA–H^+ are given in Table II. As already noted, very few studies have been reported for this system. Furthermore, most of them overlooked the tendency of the thiolate group to bridge metal ions into polynuclear structures. The only reliable study that can be found in the literature was performed by Antonetti [35] (25 °C and 1 mol dm^{-3} ClO_4^-) who found the complexes $Cd(MSA)^-$ ($\lg \beta = 8.45$) and $Cd(MSA)_2^{4-}$ ($\lg \beta = 13.75$) to be the major ones, and considered the polynuclear species $Cd_2(MSA)_2^{2-}$ ($\lg \beta = 19.65$) and $Cd_3(MSA)_2$ ($\lg \beta = 23.96$) to be formed to a lesser extent. A reasonably good agreement can be observed between his results and the ones presented here in spite of the differences found in the extent of formation of the complexes which can be explained by the low ranges of metal (0.100–0.575 mmol dm^{-3}) and ligand (1.62 mmol dm^{-3}) concentrations used – much lower than those used in the present work.

Figure 2 shows the species distribution for the Cd^{2+}–MSA–H^+ system computed from the constants reported in Table II for total concentration of mercaptosuccinate = 6 mmol dm^{-3} and cadmium(II) = 1 mmol dm^{-3}

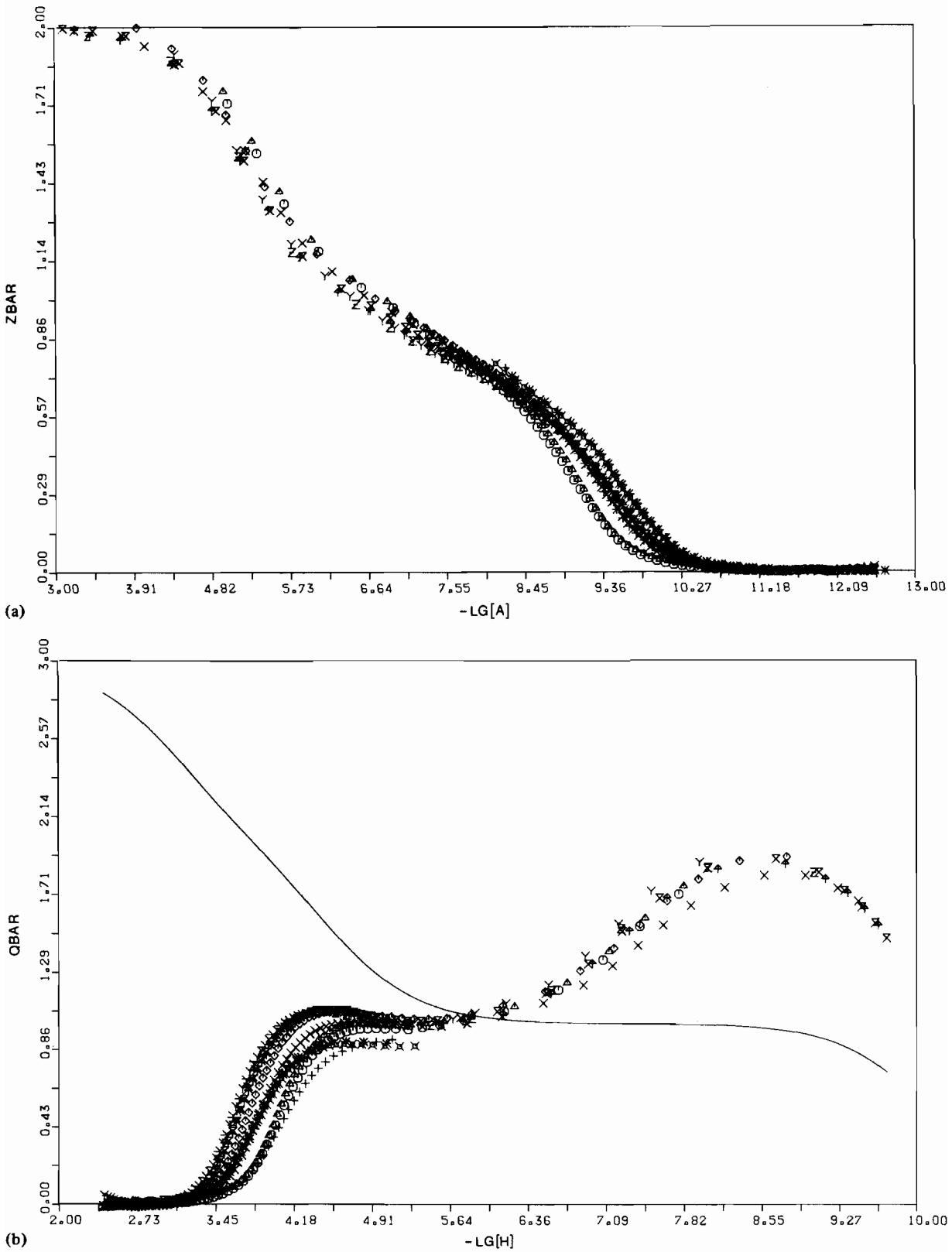

Fig. 1. Cd^{2+}–MSA–H^+ experimental (a) formation and (b) deprotonation curves. Different symbols refer to different titrations having different ligand:metal ratios and different total ligand and total metal concentrations. On each deprotonation curve, a plot of $\bar{n}$ *vs.* $-\lg[H]$ appears as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

TABLE II. Formation Constants for the Cd^{2+}–Mercaptosuccinate–H^+ System Determined in this Study at 37 °C and I = 150 mmol dm^{-3} Cl^-

Species			lg β_{pqr}	Objective function	R factor	Number of points	Number of titrations
p	q	r					
3	2	0	23.879 ± 0.012	13.9	0.001	727	11
2	2	1	24.916 ± 0.009				
2	2	0	20.236 ± 0.008				
2	2	−1	12.734 ± 0.011				
1	2	0	13.819 ± 0.007				

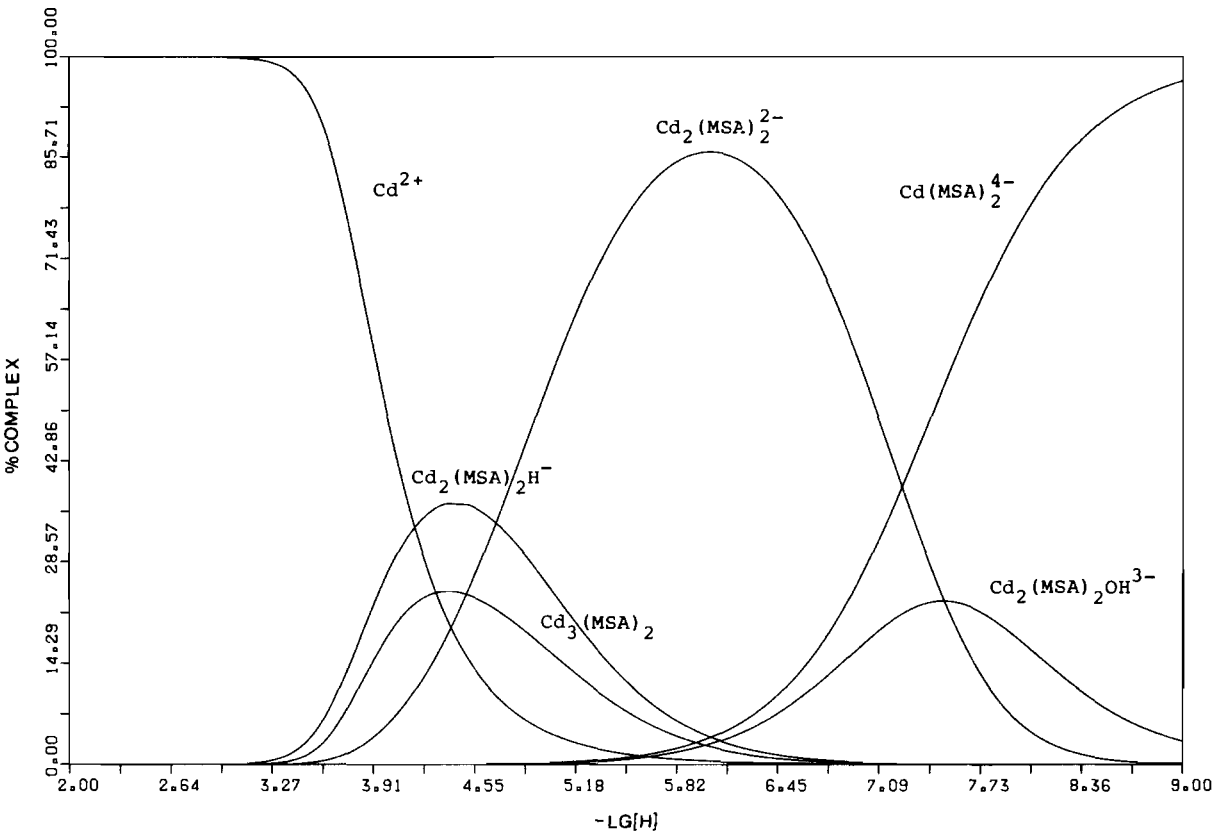

Fig. 2. Species distribution as a function of −lg[H]. Ligand concentration = 6 mmol dm^{-3}; metal concentration = 1 mmol dm^{-3}.

Simulation of Blood Plasma

The formation constants obtained were used to investigate the steady-state conditions in blood plasma after the administration of mercaptosuccinate. Since any agent capable of removing cadmium is also likely to deplete the body of zinc and nickel, their low molecular weight complex speciation was also studied using the formation constants reported previously [1].

The ability of mercaptosuccinate to mobilize metal ions can be expressed in terms of the Plasma Mobilizing Index, PMI. These values were computed at various ligand concentrations and are listed in Table III. The main low molecular weight complexes formed are shown in Table IV.

It may be seen that cadmium is indeed mobilized, as expected from the observations mentioned in the introduction although a high concentration of drug in plasma is required. At a concentration of 10^{-5} mol dm^{-3} the computer simulation shows only 0.3% of cadmium to be bound to ligand. Although this would rise to 42.8% at a mercaptosuccinate concentration of 10^{-3} mol dm^{-3}, it must be noted that such a concentration is much higher than would be produced therapeutically. The PMI curve for the mobilization of cadmium is shown in Fig. 3, and

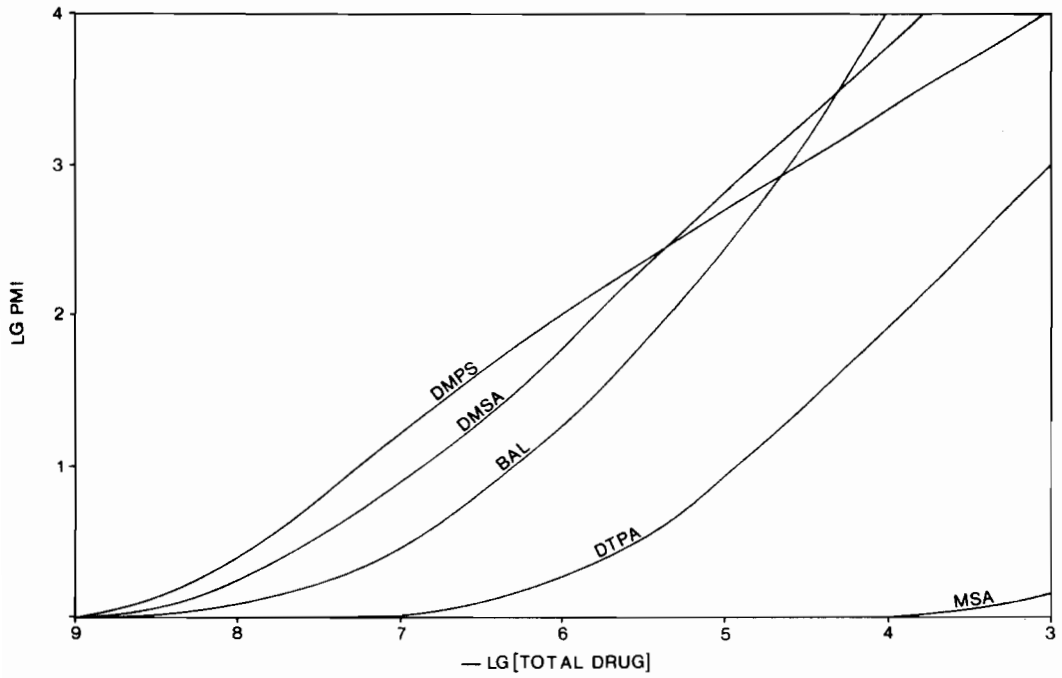

Fig. 3. Curves for lg Cd(II) Plasma Mobilizing Index (PMI) *vs.* – lg drug concentration.

TABLE III. Lg PMI Values for Mercaptosuccinate in Blood Plasma Computed Using ECCLES

Ligand concentration in blood plasma (mol dm^{-3})	Metal ion		
	Zn^{2+}	Ni^{2+}	Cd^{2+}
10^{-5}	0.02	0.00	0.00
10^{-4}	0.21	0.01	0.01
10^{-3}	1.14	0.05	0.23

PMI curves for DMSA, DMPS, BAL and DTPA are also shown for comparison.

In common with many heavy metal mobilizing agents, mercaptosuccinate brings about significant PMI values for zinc, an essential biometal and, in consequence, metal supplementation must be considered in all therapies involving the use of mercaptosuccinate, be they for cadmium removal or other conditions, such as in the treatment of rheumatoid arthritis with sodium aurothiomalate [36].

TABLE IV. Computed Low Molecular Weight Complexes of Mercaptosuccinate in Human Blood Plasma[a]

Species	Metal bound (%) at drug concentration (mol dm^{-3})		
	10^{-5}	10^{-4}	10^{-3}
Zinc(II)			
$Zn(MSA)_2^{4-}$	0.0	4.7	54.5
$Zn(MSA)(Cys)^{3-}$	3.6	23.6	26.8
$Zn(MSA)(His)^{2-}$	0.8	5.1	5.9
$Zn(MSA)(Cys)H^{2-}$	0.1	0.9	1.0
$Zn(MSA)(Cis)H^{-}$	0.1	0.9	1.0
Nickel(II)			
$Ni(MSA)(His)^{2-}$	0.1	0.9	8.2
$Ni(MSA)(Cys)^{3-}$	0.0	0.4	3.8
Cadmium(II)			
$Cd(MSA)_2^{4-}$	0.0	0.4	25.7
$Cd(MSA)(Cys)^{3-}$	0.2	2.0	11.5
$Cd(MSA)(Cys)H^{2-}$	0.1	0.8	4.7

[a]Abbreviations: Cys, cysteinate; His, histidinate; Cis, cystinate.

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