Cadmium Binding by Biological Ligands. Part 4.[1] Polynuclear Complexes of Cadmium with 2,3-Dimercaptopropane-1-sulphonic Acid

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Extensive alkalimetric anaerobic titrations of cadmium and 2,3-dimercaptopropane-1-sulphonic acid (H_3dmps) in aqueous solutions have revealed a family of polynuclear complexes. Over 750 pH measurements [glass electrode, 25 °C, I = 0.2 mol dm^{-3} (KNO_3)], in 17 separate titrations, were collected with a computer-controlled titrator. The solutions consisted of 0.6–4.0 mmol dm^{-3} cadmium nitrate and at least a 2.5-fold excess of H_3dmps. The functional form of the data was consistent with the equilibrium model [Cd_3(dmps)$_3$]$^{3-}$ (log β = 59.9), [Cd(dmps)$_2$]$^{4-}$ (log β = 28.27), [Cd_3(dmps)$_4$]$^{6-}$ (log β = 71.9), [Cd_5(dmps)$_6$]$^{8-}$ (log β = 114.3) and [Cd_7(dmps)$_8$]$^{10-}$ (log β = 156.7). Polynuclear species form below pH 2, but break down to the mononuclear bis complex above pH 7. Simulations suggest that H_3dmps can effectively compete for cadmium binding with the sulphydryl-rich protein metallothionein.

The search for therapeutic chelating agents for the treatment of chronic cadmium intoxication has been slow and largely unsuccessful.[2–4] *In vivo* studies with laboratory animals have shown that shortly after the introduction of cadmium several organs begin to produce a metal-binding, sulphydryl-rich, protein called metallothionein (H_{20}mt), which binds cadmium (4–7 Cd mol^{-1}) more strongly than do the biological ligands present initially.[5,6] After 24 h the metal ions are secured in long-term storage sites in the liver and kidney, after which chelation therapy is not particularly effective.

Aminopolycarboxylates, such as diethylenetriaminepenta-acetic acid and ethylenediaminetetraacetic acid (H_4edta), can be effective, but only if administered immediately after the metal incorporation, before any appreciable quantity of new metallothionein forms. The antidotal efficacy seems to depend directly on the values of the stability constants of the cadmium complexes.[4] Chelating agents which can mimic the polysulphydryl metal binding sites found in metallothionein[7] are anticipated to be effective antidotes for both acute and chronic cadmium intoxication. These ligands would be expected to bind cadmium with stability constants greater than 10^{20} dm^3 mol^{-1}.

Unfortunately, the stability constants of cadmium complexes with polysulphydryl ligands are either unknown or are poorly characterized. There is a pressing need to extend the measurements of binding constants to new ligands as a means of screening candidates for further studies. In particular, the study of cadmium binding with model disulphydryl ligands should prove fruitful. Of interest are two vicinal dithiols: H_2bal ('British anti-Lewisite'; 2,3-dimercaptopropan-1-ol) and H_3dmps (2,3-dimercaptopropane-1-sulphonic acid).

Although H_2bal has been successful in the treatment of heavy-metal intoxication (As, Pb, Hg, especially), inexplicably it has not been effective in the treatment of cadmium poisoning.[4,8] The other dithiol ligand, H_3dmps, has shown promise in the treatment of heavy-metal poisoning.[9–14] A water-soluble analogue of H_2bal, it is orally active in clinical applications and far less toxic than H_2bal. In mice, H_3dmps is effective in preventing acute cadmium toxicity if it is administered within 90 min of the cadmium incorporation.[3]

The reactions of cadmium with H_2bal in aqueous solution have not been examined in great detail. A comprehensive equilibrium study of zinc and H_2bal was conducted by Leussing and Tischer.[15] They reported the formation of the mononuclear species [Zn(bal)] and [Zn(bal)$_2$]$^{2-}$, and a series of 'core-plus links' polynuclear complexes Zn(bal)$_2$·[Zn(bal)]$_k$$^{2-}$ for $k > 0$.

The reactions of zinc and cadmium with H_3dmps were studied by Pilipenko and Ryabushko.[16,17] They proposed that both zinc and cadmium form 1:1 and 1:2 (metal:ligand) complexes with H_3dmps. Their model did not consider species other than the above two, and their experimental design was somewhat limited.

Until recently, studies of cadmium reactions with sulphydryl ligands had led to oversimplified models of the nature of the complexes present in solution. The stoichiometric consequences of the tendency of thiolate groups to bridge metal ions (to form polynuclear complexes) had been often neglected in the development of equilibrium models. Factors (such as pH, total cadmium ion concentration, ionic strength and the presence of other non-sulphydryl ligands) governing the stoichiometry and the extent of formation of polynuclear complexes in aqueous solution are thus poorly understood.

In Parts 1 and 2 of this series we applied novel potentiometric techniques to study the equilibrium mechanisms of cadmium binding with simple monosulphydryl ligands: penicillamine (3-mercaptovaline)[18] and cysteamine (2-aminoethanethiol).[19] Both model systems were found to form tri- and tetra-nuclear ternary complexes under mildly acidic conditions (pH 4–6). In Part 3 we determined the cadmium binding constants of apo-metallothionein at pH 6 and 7.[1]

In view of our past studies it was apparent that the reactions of cadmium with H_3dmps in aqueous solution were incompletely characterized. We therefore pursued a comprehensive study of the system, the results of which are reported here.

Experimental

Reagents.—All solutions were prepared and stored under N_2 in a rigorously maintained inert-atmosphere glove-box (Vacuum Atmospheres) and were free of detectable O_2 and CO_2. A standardized acidified stock solution of 2,3-dimer-

Table 1 Compositions of titrated solutions

Data set	Total concentration (mmol dm^{-3}) Cd(NO$_3$)$_2$	Na(dmps)	HNO$_3$	KNO$_3$	c_L/c_M[a]	N[b]
1	0.00	4.65	9.12	190.2		41
2	0.00	5.62	14.56	171.2		43
3	0.00	5.63	13.93	173.6		44
4	0.00	5.95	9.23	190.5		43
5	0.00	6.83	15.45	166.0		45
6	0.00	6.95	15.77	169.8		45
7	0.00	7.45	9.14	190.7		44
8	2.50	6.39	16.40	168.4	2.6	43
9	2.50	7.67	17.60	165.9	3.1	43
10	2.50	10.23	20.05	160.8	4.1	45
11	2.50	12.78	22.37	155.7	5.1	45
12	2.50	15.34	24.11	150.5	6.1	47
13	0.67	10.23	18.48	168.0	15.4	42
14	1.67	10.23	19.11	163.9	6.1	47
15	2.50	10.23	19.67	160.8	4.1	44
16	3.33	10.23	19.87	157.3	3.1	45
17	4.00	10.23	20.09	154.6	2.6	45

[a] Ratio of total ligand to total metal concentrations. [b] Number of pH measurements.

Table 2 Refined equilibrium constants

Equilibrium	log constant[a]
$\mathrm{dmps^{3-} + H^+ \rightleftharpoons Hdmps^{2-}}$	11.62 ± 0.01[b]
$\mathrm{Hdmps^{2-} + H^+ \rightleftharpoons H_2dmps^-}$	8.525 ± 0.004[c]
$\mathrm{3\,Cd^{2+} + 3\,dmps^{3-} \rightleftharpoons [Cd_3(dmps)_3]^{3-}}$	59.9 ± 0.1
$\mathrm{Cd^{2+} + 2\,dmps^{3-} \rightleftharpoons [Cd(dmps)_2]^{4-}}$	28.27 ± 0.05[d]
$\mathrm{3\,Cd^{2+} + 4\,dmps^{3-} \rightleftharpoons [Cd_3(dmps)_4]^{6-}}$	71.9 ± 0.1
$\mathrm{5\,Cd^{2+} + 6\,dmps^{3-} \rightleftharpoons [Cd_5(dmps)_6]^{8-}}$	114.3 ± 0.2
$\mathrm{7\,Cd^{2+} + 8\,dmps^{3-} \rightleftharpoons [Cd_7(dmps)_8]^{10-}}$	156.7 ± 0.3

[a] At 25 °C, 0.2 mol dm^{-3} KNO$_3$. Ligand constants: g.o.f. = 0.97, data sets 1–7, pH 3–12, N_o = 257. Metal–ligand constants: g.o.f. = 1.83, data sets 8–17, pH 1.8–8.0, N_o = 289. [b] log β 11.939 at 25 °C and 0.1 mol dm^{-3} KNO$_3$,[25] 11.197 at 20 °C and 0.1 mol dm^{-3} KNO$_3$.[26] [c] log β 8.655[25] and 8.841[26] (conditions as in footnote *b*). [d] Ref. 17; 20 °C, 0.1 mol dm^{-3} KNO$_3$.

captopropane-1-sulphonic acid was prepared from the sodium salt (Aldrich). The preparation and standardization of HNO$_3$, KOH, ethylenediamine (used for electrode calibration) and Cd(NO$_3$)$_2$ stock solutions have been described elsewhere.[1,18–20]

Titration Methods.—Acidified solutions containing variable amounts of cadmium and H$_3$dmps were prepared from the stock solutions and titrated with standard KOH, using a computerized titrator.[18] Over 750 pH measurements were recorded. During all titrations (which were conducted in a capped cell outside the glove-box), premoistened N$_2$ was passed gently over the solutions. The temperature was maintained at 25 °C. Each titrated solution contained enough KNO$_3$ to maintain the ionic strength roughly constant at 0.2 mol dm^{-3}.

No precipitates were observed during the titrations; the solutions remained colourless. Solutions that are exposed to air turn pink.

Table 1 lists the concentrations of all solutions prepared for this study. Seven solutions were prepared containing H$_3$dmps (4.5–8.0 mmol dm^{-3}) and no cadmium (Group I). The titration data from this group were used to determine accurately the H$_3$dmps stock concentration and ligand pK_a values. A second group of five solutions was prepared which comprised the metal-varied set (Group II). These solutions ranged from 0.6 to 4.0 mmol dm^{-3} in total metal concentration with ligand concentration held at a constant 10.2 mmol dm^{-3}. The third group of five solutions prepared was designated the ligand-varied set (Group III). Ligand concentrations ranged from 6.4 to 15.3 mmol dm^{-3} while the total metal concentration was held constant at 2.5 mmol dm^{-3}.

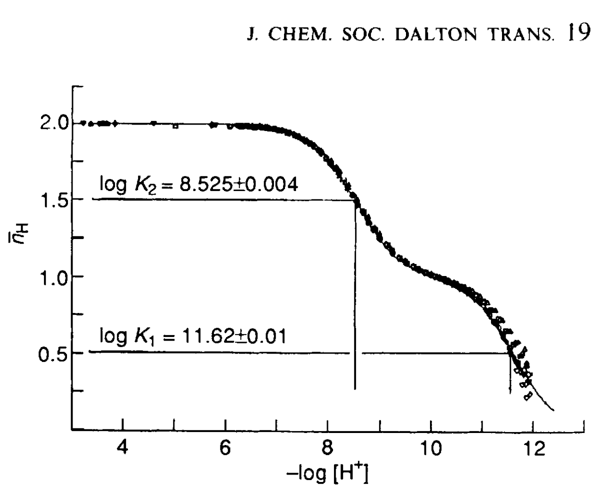


Fig. 1 Protonation curves for H$_3$dmps constructed from Group I data (sets 1–7, Table 1); 25 °C, I = 0.2 mol dm^{-3} KNO$_3$

Methods of Calculation.—The extensive FORTRAN library of routines, STBLTY,[21] was used to reduce the data, develop the equilibrium model and refine the equilibrium constants.

The pK_a values of H$_3$dmps were estimated from Group I data using Bjerrum plots,[22,23] $\bar{n}_H$ *vs.* pH, where $\bar{n}_H$ is the average number of dissociable protons bound to the ligand at a given pH. It is the property of Bjerrum plots that the pH values at half-integral $\bar{n}_H$ points roughly equal the pK_a values.

The initial metal–ligand stability constants were also estimated with the aid of Bjerrum plots.

Given a set of constants and stoichiometric coefficients (the 'equilibrium model'), along with the total reagent concentrations (corrected for dilution), it was possible to calculate pH values. Refinement of the equilibrium model had the objective of making as close a match as possible between the calculated dependent variables pH and those which were measured. The devised Gauss–Newton non-linear weighted least-squares refinement procedure specifically minimized the function (1)

$$S = \sum^{No}(\mathrm{pH^{obs}} - \mathrm{pH^{calc}})^2/\sigma^2 \quad (1)$$

where N_o is the number of points considered in the refinement (≈300) and σ^2 are the estimated variances.[24] Using only Group I data, ligand pK_a values were refined separately from the metal–ligand constants. The refinement of the metal–ligand constants included the ligand pK_a values as fixed parameters. The 'goodness-of-fit', g.o.f., an index of how precisely a model predicts the observed data, is given by expression (2) where N is

$$\text{g.o.f.} = [S/(N_o - N)]^{\frac{1}{2}} \quad (2)$$

the number of refined constants; g.o.f. < 2 generally implies a good fit.[1,18,19] For example, we obtained a g.o.f. = 0.97 in the refinement using the seven ligand sets, Group I data. Given that our weights were constructed assuming σ_{pH} = 0.02,[24] the above g.o.f. value suggests that *on the average* the difference between the observed pH curves and ones calculated with the pK_a model is approximately 0.02 pH unit.

Results

*Ligand p*K$_a$ *Values.*—The Bjerrum protonation curves ($\bar{n}_H$ *vs.* pH) are shown in Fig. 1. The constants for H$_3$dmps, estimated from these curves (half-$\bar{n}_H$ method) and subsequently refined, are listed in Table 2, along with literature comparisons.

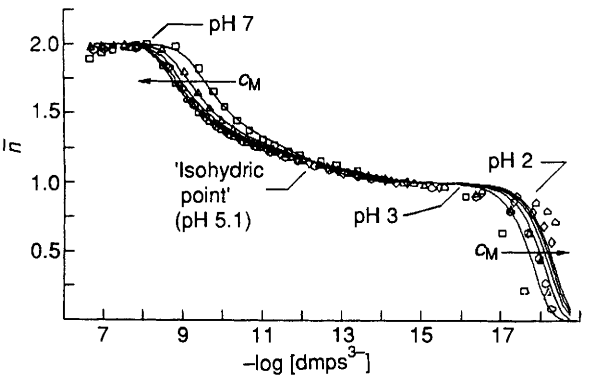


Fig. 2 Cadmium–dmps Bjerrum formation curves constructed from Group II data (varied metal concentration); c_L = 10.23 mmol dm^{-3}. Total metal concentration, c_M: 0.67 (□), 1.67 (△), 2.50 (○), 3.33 (◇) or 4.00 mmol dm^{-3} (⌂). Other conditions as in Fig. 1

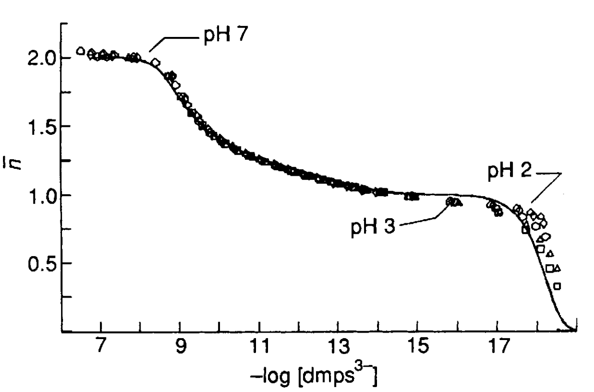


Fig. 3 Cadmium–dmps Bjerrum formation curves constructed from Group III data (varied ligand concentration); c_M = 2.50 mmol dm^{-3}. Total ligand concentration, c_L: 6.39 (□), 7.67 (△), 10.23 (○), 12.78 (◇) or 15.34 mmol dm^{-3} (⌂). Other conditions as in Fig. 1

Metal–Ligand Bjerrum Plots.—The presence of polynuclear complexes can be shown using Bjerrum plots. Without such species, $\bar{n}$, the Bjerrum metal–ligand formation function,[19] refers to the average number of bound ligands per metal ion, and is only a function of the free-ligand concentration, p(dmps) = $-\log[\text{dmps}^{3-}]$. From the plot of $\bar{n}$ *vs.* p(dmps) it is possible to determine approximate values of the stepwise metal binding constants by the half-$\bar{n}$ method. Under such conditions (*i.e.* no polynuclear or protonated complexes) the plots are *independent* of the total metal and ligand concentrations.

Inspection of Bjerrum plots for Group II data (metal varied, ligand constant) in Fig. 2 decidedly shows pronounced dependence on the total metal concentration, as seen by the systematic spreading in the formation curves. In the low pH region (<3), curves associated with higher total metal concentrations appear to suggest more stable complexation, which is characteristic of the presence of polynuclear species. Conversely, in the higher pH region, 5–7, there is a reversal of this concentration dependence, pivoting about an 'isohydric point'[18,19] at pH 5 (which marks the point of maximum condensation[27,28]). Thus polynuclear complexes begin to dissociate above pH 5.

With Group III data (metal constant, ligand varied), polynuclear species, however, are not expected to produce total concentration dependence in Bjerrum plots, *provided* protonated metal–ligand complexes are not present. Such appears to be the case in the Cd–dmps system, as shown by the curves in Fig 3. The small spread below pH 2 is likely due to non-linear pH-electrode effects in the solutions at very low pH.

Development and Refinement of the Equilibrium Model.—The 'species competition' bootstrap method of Sillen[29] was used to develop a minimum-set equilibrium model consistent with the functional dependence of the Bjerrum plots. Equilibrium models encompassing Sillen's 'core-plus-links'[30] species were examined as well.

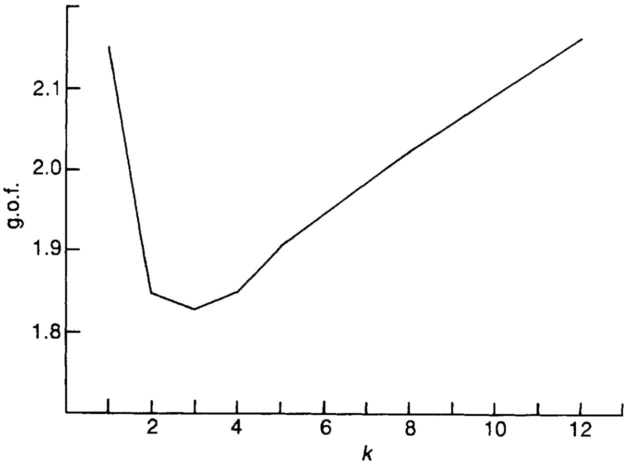


Fig. 4 Sensitivity of the refinement to the order of polymerization of the k:k series of complexes $[\text{Cd}_k(\text{dmps})_k]^{k-}$. The trimer gives the best fit, but only marginally better than the dimer or tetramer

Mononuclear model. We started our search by refining Ryabushko's[17] two-species model with our data (sets 8–17). The constants for the mononuclear complexes converged (g.o.f. = 4.30) to log $\beta[\text{Cd(dmps)}^-]$ = 18.1 (lit.,[17] 16.7) and log $\beta[\text{Cd(dmps)}_2{}^{4-}]$ = 28.1 (lit.,[17] 25.3). The fit was not good.

'Core-plus-links' models. Our effort next turned to the Leussing–Tischer[15] 'core-plus-links'[29] model of the zinc–H_2bal system [Zn(bal)$_2$ core, Zn(bal) link]. The structural similarities of H_2bal and H_3dmps (both vicinal dithiols) prompted the testing of the above zinc model with our cadmium data.

We were only able to refine the k = 2 and 3 components: log $\beta[\text{Cd}_3(\text{dmps})_4{}^{6-}]$ = 71.7 and log $\beta[\text{Cd}_4(\text{dmps})_5{}^{7-}]$ = 92.4, along with the two mononuclear species (g.o.f. = 2.25). The $k > 3$ and $k = 1$ components were not stable to refinement. The above model still did not adequately describe the functional form of the Bjerrum plots, particularly in the p(dmps) range 10–14.

The basis for the metal dependence in the region below pH 3 was still unexplained. Despite the reported[17] formation of the $[\text{Cd(dmps)}]^-$ complex, we observed better fit by incorporating oligomers of the complex. We tested each species of the series $[\text{Cd}_k(\text{dmps})_k]^{k-}$, k = 1 to 12; $[\text{Cd}_3(\text{dmps})_3]^{3-}$ produced the lowest g.o.f. Fig. 4 illustrates how the g.o.f. depended on the value of k. The formation of the trimer accounts for the systematic spread in the formation curves at low pH.

We eventually found that the gradual rise in the formation curves from $\bar{n}$ = 1.0 to 1.2 could be explained better by a modified 'core-plus-links' series: $\text{Cd(dmps)}_2\cdot[\text{Cd}_2(\text{dmps})_2]_k{}^{(2k+4)-}$.

The fit with the modified model was very good. Further attempts were made to improve the fit by testing the species $[\text{Cd}_3(\text{dmps})_5]^{9-}$, $[\text{Cd}_3(\text{dmps})_3\text{H}]^{2-}$, $[\text{Cd}_3(\text{dmps})_3\text{H}_2]^-$ and $[\text{Cd}_2(\text{dmps})_3\text{H}]^{4-}$. No improvements were achieved. The equilibrium model at this point seemed complete. Refinement of ten metal–ligand titration sets incorporating the species $[\text{Cd}_3(\text{dmps})_3]^{3-}$, $[\text{Cd(dmps)}_2]^{4-}$, $[\text{Cd}_3(\text{dmps})_4]^{6-}$, $[\text{Cd}_5(\text{dmps})_6]^{8-}$ and $[\text{Cd}_7(\text{dmps})_8]^{10-}$ yielded a g.o.f. value of 1.83. This value corresponds to an average difference of 0.037 between the observed pH values (in the 10 sets) and those calculated with the model. If we delete the heptameric species from our model, for example, the g.o.f. value increases to 2.07. If we take out the pentamer as well, the g.o.f. jumps to 4.08, indicating a doubling of errors in our fit. Thus our data seemed best described by the model incorporating species up to the heptamer. Table 2 lists the refined constants.

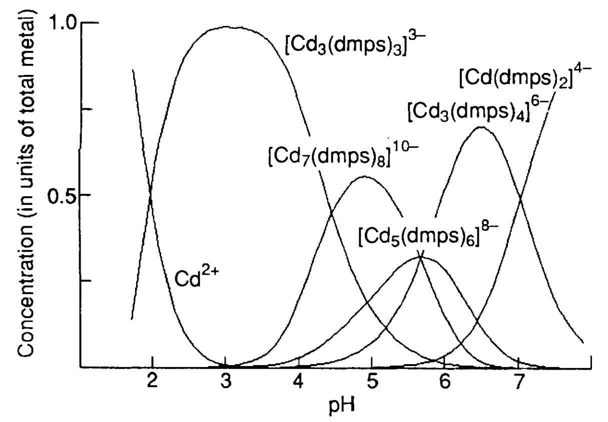


Fig. 5 Species distribution curves for cadmium–dmps in concentrated solution; [Cd] = 5, $[dmps^{3-}]$ = 10 mmol dm^{-3}

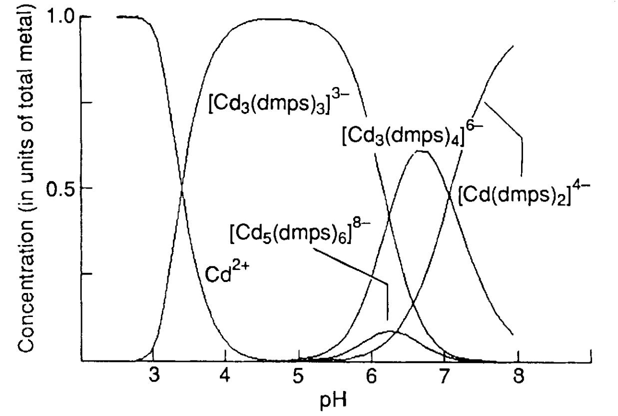


Fig. 6 Species distribution curves for cadmium–dmps in dilute solution; [Cd] = 0.1, $[dmps^{3-}]$ = 0.2 mmol dm^{-3}

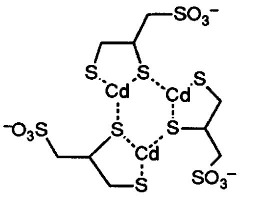
STBLTY[21] does not have a provision to refine the model in the specific core + links formalism. Consequently, the members of the family had to be individually refined, and their values are those found in Table 2. If one takes the differences between successive members, the 'stepwise core + link' equilibrium constants (log form) are 43.6, 42.4 and 42.4. The links are almost identical in strength. That is, the monomer acquires the first link ($2\ Cd^{2+} + 2\ dmps^{3-}$) to form the trimer with nearly the same strength as does the trimer to form the pentamer, and so on.

Distribution of Species.—Species distribution curves as a function of pH are shown in Figs. 5 and 6. To view the effect of the total metal concentration two different sets of concentrations were selected. The formation of higher orders of polynuclear complexes is markedly enhanced by an increase in the total metal concentration of the solution, as decidedly shown in the figures for the species $[Cd_7(dmps)_8]^{10-}$ and $[Cd_5(dmps)_6]^{8-}$. At low pH, $[Cd_3(dmps)_3]^{3-}$ is the predominant complex. As the pH increases, the 'core-plus-links' family of species begins to form. The first complex to appear is $[Cd_7(dmps)_8]^{10-}$ (pH 4–5). Later members form with the removal of a 'link' $[Cd_2(dmps)_2]^{2-}$; $[Cd_5(dmps)_6]^{8-}$ forms at pH 5–6, $[Cd_3(dmps)_4]^{6-}$ at pH 6–7 and finally $[Cd(dmps)_2]^{4-}$ predominates at pH > 7.

Discussion

Comparisons with Other Studies of Cadmium–Sulphydryl Systems.—Under the conditions of this study, 2,3-dimercaptopropane-1-sulphonic acid forms anionic polynuclear species with cadmium. Unlike previous ligands we have studied, it does not form protonated polynuclear complexes in solution. Penicillamine and cysteamine have been reported to form protonated complexes with cadmium. The site of protonation in these ligands is likely either at the nitrogen or oxygen. In H_3dmps there are no nitrogen or oxygen protonation sites available. This system appears to exhibit a large chelate effect and complexation begins to occur at very low pH.

Possible Structures.—Two possible structures, **1** and **2**, for the 3:3 complex may be proposed. Structure **1** has each cadmium



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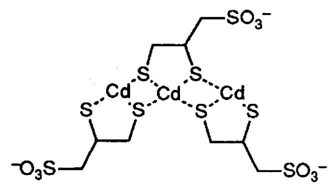

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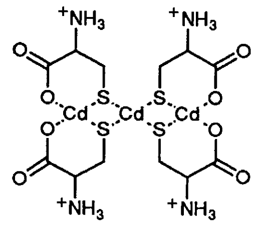
co-ordinated by three sulphur atoms. Also, three sulphur atoms bridge the metal ions into a six-membered cluster ring. In metallothionein[7] the B cluster has such a ring structure, except that each cadmium is tetrahedrally co-ordinated. The second possible structure **2** is a mixture of metal sites bound by three and four sulphur atoms.

The structure **3** for the trimeric cadmium cysteine complex proposed by Shindo and Brown[31] may be suggestive of the possible structures for the dmps 'core-plus-links' species **4**. Each



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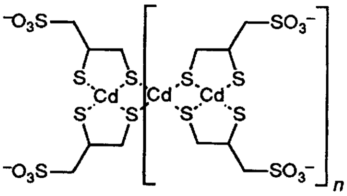

4 *n* = 1, 2 or 3

cadmium atom is tetrahedrally co-ordinated in the above linear-chain model. As the links progressively dissociate with increasing pH the bis complex can be viewed as the residual species.

Relative Binding Strengths.—The bis complex is a very stable species. To illustrate this one can calculate the distribution of species in a solution containing cadmium and comparable amounts of H_3dmps and H_4edta (known for its ability to bind cadmium strongly). Fig. 7 shows that H_4edta {log $\beta[Cd(edta)^{2-}]$ = 16.36[32]} is able to suppress effectively the formation of polynuclear complexes of H_3dmps, but not the bis complex. At pH 7 the concentration of $[Cd(edta)]^{2-}$ is comparable to that of $[Cd(dmps)_2]^{4-}$. At higher pH, the dmps complex predominates.

We did similar simulation calculations with penicillamine[18] and cysteamine.[19] All dmps species (including polynuclear) predominate at pH 2–9. Decidedly, the disulphydryl ligand is a stronger cadmium binder, due to the dithiol chelate effect,

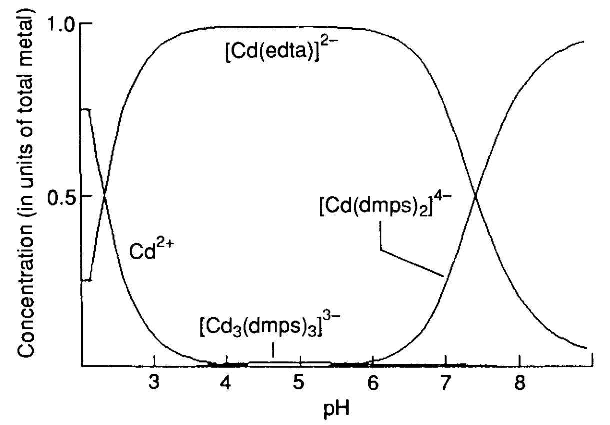

Fig. 7 Species distribution curves for cadmium–dmps and –edta; [Cd] = 0.1, [dmps^{3-}] = 0.2 and [edta^{4-}] = 0.1 mmol dm^{-3}

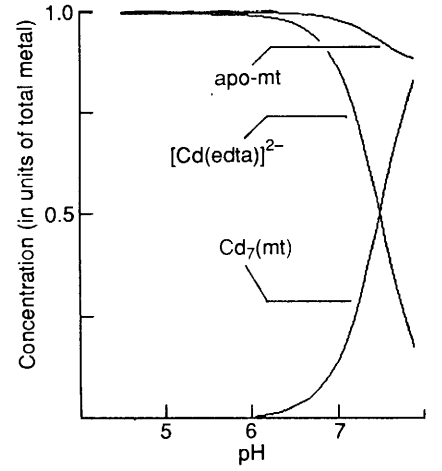

Fig. 8 Species distribution curves: metallothionein in competition with edta for cadmium binding; [Cd] = [edta^{4-}] = [mt^{20-}] = 0.1 mmol dm^{-3}; apo-mt = uncomplexed metallothionein

compared with either of the above monosulphydryl ligands. Distribution curves in the simulation calculation are identical to those shown in Fig. 5 and 6.

The most interesting comparison is that with metallothionein. We used the pH 6 constants for metallothionein from ref. 1: log β(H$_{24}$mt^{4+}) = 209.7, log β[Cd$_7$(mt)H$_4$$^{2-}$] = 156 and log β[Cd$_5$(mt)H$_{13}$$^{3+}$] = 191, and calculated the effect of edta on mt. Fig. 8 shows that [Cd(edta)]$^{2-}$ is the predominant complex below pH 7. Above pH 7, metallothionein is able to retain its metal ions in the Cd$_7$ form.

When a solution consisting of 0.1 mmol dm^{-3} Cd^{2+}, 0.1 mmol dm^{-3} apo-mt and 0.2 mmol dm^{-3} dmps^{3-} is allowed to react the predicted distribution of species is quite complicated, as shown in Fig. 9. At pH 6, mt is able to preserve much of the cadmium in the Cd$_5$ and Cd$_7$ forms. There are significant amounts of cadmium dmps complexes, however. The balance tips in favour of dmps quite dramatically when the dmps concentration is doubled, as shown in Fig. 10. Metallothionein is no longer able to compete effectively for the cadmium ions.

Uniqueness of the Model.—A study such as ours in principle cannot prove that the model we propose is the 'unique' model. Under our choice of concentrations (and concentration ratios), our model is the one that is 'best' supported by the data, within the scope of its range. If we had chosen metal concentrations ten times higher than those used other higher-order polymeric species might have been revealed. As careful a screening of possible compounds was made as we were able to conceive. Many possibilities were ruled out, as mentioned above, because of lack of agreement with the reasonably general structure of the data collected. At least our study can be a thoughtful springboard for other studies, using such structurally sensitive techniques as cadmium nuclear magnetic resonance, for example.

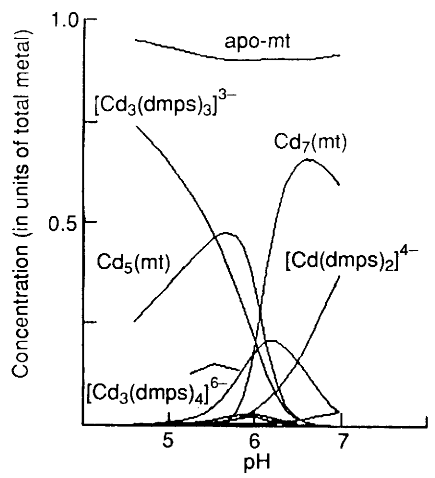

Fig. 9 Species distribution curves for metallothionein (0.1 mmol dm^{-3}) in competition with dmps (0.2 mmol dm^{-3}) for cadmium (0.1 mmol dm^{-3}) binding; apo-mt = uncomplexed metallothionein

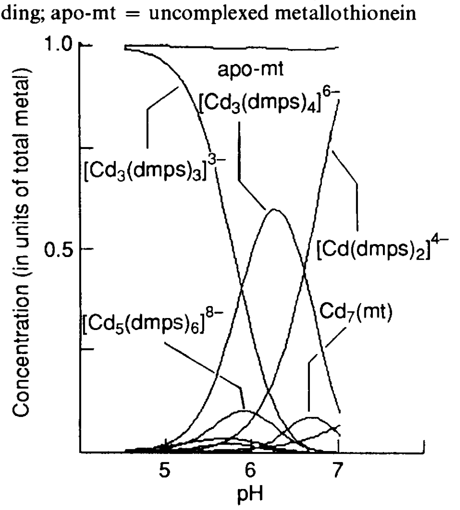

Fig. 10 Species distribution curves for metallothionein in competition with dmps for cadmium binding; concentration of dmps is twice that in Fig. 8

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