Thermodynamic Considerations in Co-ordination. Part XXIII.[1] Formation Constants for Complexes of Protons, Zinc(II), and Acid Anions and their Use in Computer Evaluation of a Better Zinc Therapeutical

By George K. R. Makar, Murray L. D. Touche, and David R. Williams,* Department of Chemistry, The University of St. Andrews, Fife KY16 9ST

Glass electrode potentiometric studies at 37 °C, I = 150mM($NaClO_4$), have been used to study a selection of ligand-zinc-proton systems. The formation constants for adding protons and zinc ions to the ligands are reported. Computer models of solution equilibria have been used to suggest the best ligands and concentrations for improved zinc therapy.

CORRECT concentrations of zinc ions are essential to human life, there being many diseases associated with zinc deficiency.[2] Further, such deficiencies can have an iatrogenic origin; administering ligands to lower the concentrations of pollutant metals can also remove some of the essential zinc from the body.[1] Both situations require zinc supplementation to prevent serious damage occurring.

By analogy to iron supplementation, zinc sulphate has been used in several clinical trials but it is hoped to improve the quantity of zinc absorbed intestinally, just as iron therapy has been greatly improved since iron sulphate was first introduced. Our studies of the solution chemistries of iron-supplementing therapeuticals have suggested that the first step, of a many stage process of metal-ion assimilation, is the formation of uncharged complexes in the intestinal solution at duodenal pH values.[3] These complexes then pass into the organic solvent-like lipid/protein cell membranes of the epithelial cells lining the intestine.

The complexes formed by zinc ions with eleven ligands of low molecular weight have been investigated in aqueous solution at 37 °C and I = 150mM and the relevant stability constants are reported and then used in the computation of pH *versus* complex species present profiles. The ligands were chosen from carboxylate and amino-acid ligands found in man and in particular those known to be, or suspected of being, involved with zinc metabolism (aspartic acid, creatine,† α-D-galacturonic acid, D,L-β-hydroxybutyric acid, L-4-hydroxyproline, L-malic acid, malonic acid, oxalic acid, L-proline, and L-tartaric acid), from ligands previously administered with zinc (Jonctum, *i.e.* *N*-acetyl-L-4-hydroxyproline [4]), and from a ligand currently being introduced as an artificial sweetener [5] ('Aspartame,' *i.e.* L-aspartyl-L-phenylalanine methyl ester, and its parent amino-acids). With the exception of oxalate, all the ligands were considered as acceptable to the body when displaced from the zinc ions.

† The rate of urinary zinc excretion during muscle catabolism is paralleled by that of creatine ($HO_2C{\cdot}CH_2{\cdot}NMe{\cdot}C(NH){\cdot}NH_2$, p$K$ values = 11.5 and 2.633) excretion suggesting that a zinc–creatinate complex is involved. However, we could detect no evidence that these two species form a complex in aqueous solution.

‡ 1M = 1 mol dm^{-3}.

[1] Part XXII, preceding paper.

[2] J. A. Halsted, J. C. Smith, and M. I. Irwin, *J. Nutrition*, 1974, **104**, 345.

[3] J. N. Cape, D. H. Cook, and D. R. Williams, *J.C.S. Dalton*, 1974, 1849.

EXPERIMENTAL

The following ligands were used: *N*-acetyl-L-4-hydroxyproline (Merrell International Research Centre, Strasbourg) (Found: C, 48.15; H, 6.5; N, 7.85. Calc. for $C_7H_{11}NO_4$: C, 48.55; H, 6.4; N, 8.08%); L-aspartyl-L-phenylalanine methyl ester (G. D. Searle and Co.) (Found: C, 54.2; H, 6.25; N, 8.85. Calc. for $C_{14}H_{18}N_2O_5$: C, 57.13; H, 6.16; N, 9.51%); L-aspartic acid (B.D.H. Biochemicals) (Found: C, 35.9; H, 5.35; N, 10.45. Calc. for $C_4H_7NO_4$: C, 36.1; H, 5.3; N, 10.52%); α-D-galacturonic acid monohydrate (Sigma Chemical Company) (Found: C, 32.9; H, 5.7. Calc. for $C_6H_{12}O_8$: C, 33.96; H, 5.70%); D,L-β-hydroxybutyric acid sodium salt (Boehringer Mannheim GmbH) (Found: C, 38.0; H, 5.6. Calc. for $C_4H_7O_3Na$: C, 38.07; H, 5.55%); L-4-hydroxyproline (Koch–Light) (Found: C, 46.15; H, 7.4; N, 10.85. Calc. for $C_5H_9NO_3$: C, 45.80; H, 6.92; N, 10.68%); L-malic acid (Koch–Light) (Found: C, 35.55; H, 4.35. Calc. for $C_4H_6O_5$: C, 35.82; H, 4.51%); malonic acid (Koch–Light) (Found: C, 34.45; H, 3.9. Calc. for $C_3H_4O_4$: C, 34.62; H, 3.87%); oxalic acid (B.D.H. AnalaR) (Found: C, 19.0; H, 4.8. Calc. for $C_2H_2O_4,2H_2O$: C, 19.05; H, 4.79%); L-proline (Koch–Light) (Found: C, 50.35; H, 8.0; N, 11.85. Calc. for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17%); L-tartaric acid (Koch–Light) (Found: C, 31.95; H, 4.1. Calc. for $C_4H_6O_6$: C, 32.01; H, 4.02%). Zinc perchlorate (G. F. Smith Chemical Co.) solution was prepared and analysed as in ref. 6. Other solutions prepared as in refs. 6 and 7.

The potentiometric approach was as described in ref. 8, all studies being at 37.0 °C, I = 150mM ‡ ($NaClO_4$). Ligand protonation formation curves measured for different total ligand concentrations and computed and plotted using our RWZPLOT program were superimposable, *i.e.* only simple AH, AH_2, *etc.*, complexes are formed. However, the zinc–ligand complexes produced a pattern of formation curves as the A : Zn ratio was varied. This was taken as evidence of protonated, hydroxo, or polynuclear complexes being present and these were indeed found in SCOGS [9] and MINIQUAD [10] least-squares analyses of the data and confirmed by PSEUDOPLOT [11] plots.

The next stage involved COMPLOT [12,13] computer-simulation models of the circumstances prevailing when the

[4] J. L. Schelling, S. Muller-Hess, and F. Thonney, *Lancet*, 1973, (ii), 968.

[5] *Chem. Eng. News*, 1974, **52** (31), 5.

[6] D. R. Williams and P. A. Yeo, *J.C.S. Dalton*, 1972, 1988.

[7] A. D. Jones and D. R. Williams, *J. Chem. Soc.* (*A*), 1970, 3138.

[8] D. R. Williams, *J.C.S. Dalton*, 1973, 1064.

[9] I. G. Sayce, *Talanta*, 1968, **15**, 1397.

[10] A. Sabatini, A. Vacca, and P. Gans, *Talanta*, 1974, **21**, 53.

[11] A. M. Corrie, G. K. R. Makar, M. L. D. Touche, and D. R. Williams, *J.C.S. Dalton*, 1975, 105.

[12] D. D. Perrin and I. G. Sayce, *Talanta*, 1967, **14**, 833.

[13] A. C. Baxter and D. R. Williams, *J.C.S. Dalton*, 1974, 117.

TABLE 1

Log formation constants (β_{pqr}) * for ligand protonation at 37 °C and I = 0.15-M($NaClO_4$); n = number of experimental observations, d denotes the standard deviation, and s the site of protonation

Ligand	p	q	r	s	log β	d	n	Literature data (θ/°C, I/M, log β)	Ref.
Aspartamate	1	0	1	NH_2	7.365	0.005	200		
A^-	1	0	2	CO_2^-	10.327	0.010			
L-Aspartate	1	0	1	NH_2	9.266	0.004	193	25, 0.1 (KNO_3), β_1 9.63, β_2 13.34, β_3 15.28	*a*
A^{2-}	1	0	2	CO_2^-	12.865	0.007		20, 1 ($NaClO_4$), β_1 9.56, β_2 13.34, β_3 15.34	*b*
	1	0	3	CO_2^-	14.813	0.009		25, 0.1 (KCl), β_1 9.87, β_2 13.74	*c*
								25, 0.2 (KNO_3), pK_2, 3.69, pK_3 1.92	*d*
N-Acetyl-L-4-hydroxyprolinate A^-	1	0	1	CO_2^-	3.153	0.060	128		
α-D-Galacturonate A^-	1	0	1	CO_2^-	3.209	0.001	130		
D,L-β-Hydroxybutyrate	1	0	1	CO_2^-	4.489	0.016	148	31, 0.1 ($NaClO_4$), β_1 4.40	*e*
A^-								25, 0.2 (KCl), β_1 4.39	*f*
L-4-Hydroxyprolinate	1	0	1	NH	9.159	0.006	200	35, 0.1 (KCl), β_1 9.55, β_2 11.30	*g*
A^-	1	0	2	CO_2^-	10.828	0.010		20, 1 ($NaClO_4$), β_1 9.58, β_2 11.51	*b*
	(1	0	1)	O^-	11.587(pK)	0.017		25, 0.1 (?), β_1 9.46	*h*
L-Malate	1	0	1	CO_2^-	4.477	0.002	183	20, 0.1 ($NaClO_4$), β_1 4.71, β_2 7.93	*i*
A^{2-}	1	0	2	CO_2^-	7.592	0.002		30, 0.1 (KCl), β_1 4.78, β_2 8.00	*j*
								20, 0.1 ($NaClO_4$), β_1 4.72, β_2 8.00	*k*
Malonate	1	0	1	CO_2^-	5.090	0.002	200	25, 0.15 ($NaClO_4$), β_1 5.34, β_2 8.19	*l*
A^{2-}	1	0	2	CO_2^-	7.673	0.004		20, 0.1 ($NaClO_4$), β_1 5.32, β_2 7.98	*i*
								30, 0.2 ($NaClO_4$), β_1 5.10, β_2 7.70	*m*
Oxalate	1	0	1	CO_2^-	3.682	0.005	200	25, 0.15 ($NaClO_4$), β_1 2.59, β_2 3.92	*l*
A^{2-}	1	0	2	CO_2^-	4.768	0.033		25, 0.1 ($NaClO_4$), β_1 3.81, β_2 5.18	*n*
								25, 1 $NaClO_4$, β_1 3.54, $\beta_2 \sim$ 4.54	*o*
L-Prolinate	1	0	1	NH	10.165	0.006	200	37, 0.15 (KNO_3), β_1 10.34	*p*
A^-	1	0	2	CO_2^-	12.122	0.010		35, 0.1 (KCl), β_1 10.30, β_2 12.15	*g*
								25, 0.1 (?), β_1 10.41	*h*
								20, 0.03 (?), β_1 10.68, β_2 12.61	*q*
								37.5, 0 corr, pK_2 1.95	*r*
L(+)-Tartarate	1	0	1	CO_2^-	3.689	0.004	197	20, 0.1 ($NaClO_4$), β_1 3.96, β_2 6.76	*k*
A^{2-}	1	0	2	CO_2^-	6.491	0.005		25, 1.0 (KNO_3), β_1 3.77, β_2 6.37	*s*
								20, → 0, β_1 4.52, β_2 7.41	*t*
								20, ?, β_2 4.62, β_2 7.81	*u*

* β_{pqr} Refers to the general complex $A_pB_qH_r$ where A = ligand, B = metal, and H = proton. For key to references see Table 2.

zinc complexes were dissolved in stomach fluid and the pH raised to duodenal pH values (6.5—7.5). These models require the total concentrations of zinc and ligand (7.65 and 15.30mM respectively) and the formation constants from Tables 1 and 2.*

RESULTS AND DISCUSSION

To within the limits of hydrolysis and solubilities, each system was studied using a pattern of titrations where both A and B (the total concentrations of ligand and zinc respectively) were held constant and equal in the titrate and titrant, the sole difference in these solutions being their perchloric acid concentrations. For each value of B equal to 0 (protonation), 1, 2, 2.5, 5, and 10mM, the $A:B$ ratio was set to 8 : 1, 4 : 1, 2 : 1, 1 : 1, and ½ : 1. By using this approach it was possible to obtain formation constants for all the simple binary and the more complex hydroxo, polynuclear, and protonated ternary complexes present in solution.

All combinations of β_{pqr} † were tried (where p = 0,1,2,3, q = 0,1,2, and r = −2,−1,0,1,2) until a good PSEUDOPLOT fit was obtained (for example see Figure 1). In some instances even more sophisticated complexes were suggested and tried. The resultant 'best' log constants are listed in Tables 1 and 2 alongside those reported by other workers. It is noteworthy that 33 previously unreported species have now been found in this research and verified using the PSEUDOPLOT approach.

Two interesting features of Table 2 are that malate and malonate tris-complexes are more stable than the bis-complexes, possibly for reasons of symmetry, and so formation constants for bis-complexes were not necessary to define the systems. Similarly, the bis-β-hydroxybutyrate complex is formed in preference to the mono-complex.

Tables 3 and 4 show some aspects of the COMPLOT model search for neutral complexes, the α-D-galacturonate and β-hydroxybutyrate being the best zinc absorption promotion systems having 88.1 and 77.3% Zn^{2+} in potentially lipid/protein soluble form respectively. (Oxalate also has a high value but it is clearly too toxic for clinical studies.) The complete pH profiles of these two systems are shown in Figures 2 and 3.

The zinc complex of Jonctum has been administered to patients and its zinc absorption compared to that of zinc sulphate.[4] The report of Schelling *et al.* that Jonctum did not improve absorption is borne out by our model figures which showed no neutral zinc complexes present at duodenal pH values in this system. (It is germane to this observation that zinc–Jonctum complexing is so weak that we could not determine its formation constants.)

* The COMPLOT model outputs are to be found in Supplementary Publication No. SUP 21712 (11 pp.). For details see Notice to Authors No. 7, *J.C.S. Dalton*, 1975, Index issue.

† Throughout the paper, the term β_{pqr} refers to the formation constant of the complex $A_pB_qH_r$ where A = ligand, B = metal ion, and H = proton.

TABLE 2

Log formation constants (β_{pqr}) for the zinc complexes at 37 °C and I = 0.15-M($NaClO_4$); n = number of experimental observations and d denotes the standard deviation

Ligand	p	q	r	log β	d	n	Literature data (θ/°C, I/M, log β)	Ref.
Aspartamate	1	1	0	3.797	0.076	290		
	2	1	0	6.871	0.074			
	1	1	1	9.332	0.084			
	2	1	1	13.213	0.156			
	1	1	−1	−4.760	0.317			
	0	1	−2	−14.795				
Aspartate	1	1	0	6.011	0.072	204	30, 0.1 (KCl), β_1 5.84, β_2 10.15	*v*
	2	1	0	10.099	0.092		15, 0.005 ($ZnSO_4$), β_2 10.4	*w*
	1	1	1	11.876	0.115			
	2	1	−2	−10.410	0.177			
α-D-Galacturonate	1	1	0	1.735	0.026	180		
	2	1	0	2.617	0.057			
	1	1	1	3.932	0.098			
	1	1	−1	−3.376	0.097			
β-Hydroxybutyrate	2	1	0	5.807	0.048	35	25, 0.2 (KCl), β_1 1.06	*f*
	1	1	2	9.732	0.123			
	2	2	1	13.333	0.091			
	2	2	2	17.468	0.096			
	3	2	1	16.268	0.100			
L-4-Hydroxyprolinate	1	1	0	5.838	0.125	400	25, 0.1 (?), β_1 5.03, β_2 9.38	*h*
	2	1	0	10.270	0.149		17, 0.01 ($ZnSO_4$), β_2 9.6	*w*
	1	1	1	12.110	0.147			
	1	1	−1	−2.598	0.430			
	2	1	−1	1.027	0.191			
Malate	1	1	0	2.903	0.017	184	25, 0.2 (KCl), β_1 2.80 , $K_{101/111}$ 1.57 *	*f*
	3	1	0	4.647	0.043		25, → 0, β_1 3.32, $K_{101/111}$ 2.00 *	*x*
	1	1	1	6.244	0.030			
	1	1	−1	−3.637	0.030		20, 0.1 ($NaClO_4$), $K_{102/112}$ 1.66 †, $K_{101/111}$ 2.93 *	*i*
	1	1	2	8.870	0.044			
Malonate	1	1	0	2.637	0.079	168	20, 0.1 ($NaClO_4$), β_1 2.97, $K_{101/111}$ 1.24 *	*i*
	3	1	0	5.786	0.028		25, 0.001, β_1 3.35	*y*
	1	1	1	5.850	0.071		0—45, 0 corr., β_1 (25 °C) 3.82	*aa*
	3	1	1	10.623	0.060		25, 0 corr., β_1 3.85, β_2 5.95	*bb*
	3	1	−1	−0.983	0.089		25, 0.2 (KCl), β_1 2.78, $K_{101/111}$ 0.84 *	*f*
	3	1	2	14.703	0.134		25, 0.1, β_1 2.7	*z*
	2	2	0	7.261	0.170			
	2	2	1	10.922	0.194			
Oxalate	1	1	0	4.050	0.062	114	20, 0.1 ($KClO_4$), β_2 7.59	*cc*
	2	2	1	13.291	0.275		25, 0 corr., β_1 4.85, β_2 7.55	*bb*
							25, 1 (KNO_3) β_1 3.44, β_2 6.48, β_3 7.24	*dd*
							25, 0.1, β_1 4.9	*ee*
L-Prolinate	1	1	0	5.817	0.109	362	37, 0.15 (KNO_3), β_1 5.13, β_2 9.69, β_3 11.26, K_a-(BA) −8.35,‡ K_a (BA_2) −9.73 §	*p*
	2	1	0	10.212	0.214			
	1	1	−1	−1.974	0.066		20, 0.03 (?), β_2 10.2	*q*
	1	1	1	12.517	0.157		17, 0.01 ($ZnSO_4$), β_2 9.9	*w*
	1	1	−2	−10.888	0.156			
L(+)-Tartarate	1	1	0	2.579	0.022	155	25, 0.2 (KCl), β_1 2.68, $K_{101/111}$ 1.44 *	*f*
	2	1	0	4.499	0.029		20, 0.1 ($KClO_4$), β_1 2.69	*cc*
	1	1	1	5.589	0.032		25, 0 corr., β_1 3.31, β_2 5.16	*bb*
	2	1	1	8.221	0.026			

* $K_{101/111} = K[B^{2+} + HA^- \rightleftharpoons BHA^+]$. † $K_{102/112} = K[B^{2+} + H_2A \rightleftharpoons BH_2A^{2+}]$. ‡ $K_a(BA) = K[BA^+ + H_2O \rightleftharpoons BA(OH) + H^+]$. § $K_a(BA_2) = K[BA_2 + H_2O \rightleftharpoons BA_2(OH)^- + H^+]$.

[a] J. H. Ritsma, G. A. Wiegers, and F. Jellinek, *Rec. Trav. chim.*, 1965, **84**, 1577. [b] D. D. Perrin, *J. Chem. Soc.*, 1958, 3125. [c] I. M. Batgaeu, S. V. Larionov, and V. M. Shul'man, *Russ. J. Inorg. Chem.*, 1961, **6**, 75. [d] I. Feldman and L. Koval, *Inorg. Chem.*, 1963, **2**, 145. [e] C. A. Crutchfield, jun., W. M. McNabb, and J. F. Hazel, *J. Inorg. Nuclear Chem.*, 1962, **24**, 291. [f] R. K. Cannan and A. Kibrick, *J. Amer. Chem. Soc.*, 1938, **60**, 2314. [g] B. S. Sekhon and S. C. Chopra, *Thermochim. Acta*, 1973, **7**, 151. [h] Ting Po I, E. J. Burke, J. L. Meyer, and G. H. Nancollas, *Thermochim. Acta*, 1973, **5**, 463. [i] E. Campi, *Ann. Chim. (Italy)*, 1963, **53**, 96. [j] M. Cefola, A. S. Tompa, A. V. Celiano, and P. S. Gentile, *Inorg. Chem.*, 1962, **1**, 290. [k] C. F. Timberlake, *J. Chem. Soc.*, 1964, 5078. [l] H. J. DeBruin, D. Kairaitis, and R. B. Temple, *Austral. J. Chem.*, 1962, **15**, 457. [m] V. T. Athavale, N. Mahadevan, P. K. Mathur, and R. M. Sathe, *J. Inorg. Nuclear Chem.*, 1967, **29**, 1947. [n] A. McAuley and G. H. Nancollas, *Trans. Faraday Soc.*, 1960, **56**, 1165. [o] T. Sekine, *J. Inorg. Nuclear Chem.*, 1964, **26**, 1463; *Acta Chem. Scand.*, 1965, **19**, 1476; R. G. Seys and C. B. Monk, *J. Chem. Soc.*, 1965, 2452. [p] C. W. Childs and D. D. Perrin, *J. Chem. Soc. (A)*, 1969, 1039. [q] A. Albert, *Biochem. J.*, 1950, **47**, 531. [r] P. K. Smith, A. T. Gorham, and E. R. B. Smith, *J. Biol. Chem.*, 1942, **144**, 737. [s] K. S. Rajan and A. E. Martell, *J. Inorg. Nuclear Chem.*, 1964, **26**, 1927. [t] V. Frei, *Coll. Czech. Chem. Comm.*, 1965, **30**, 1402. [u] V. Frei and A. Solcova, *Coll. Czech. Chem. Comm.*, 1965, **30**, 961. [v] S. Chaberek, jun., and A. E. Martell, *J. Amer. Chem. Soc.*, 1952, **74**, 6021. [w] D. J. Perkins, *Biochem. J.*, 1953, **55**, 649. [x] R. P. Bell and G. M. Waind, *J. Chem. Soc.*, 1951, 2357. [y] D. J. G. Ives and H. L. Riley, *J. Chem. Soc.*, 1931, 1998. [z] M. Yasada, K. Yamasaki, and H. Ohtaki, *Bull. Chem. Soc. Japan*, 1960, **33**, 1067. [aa] V. S. K. Nair, *J. Chem. Soc.*, 1965, 1450. [bb] D. L. G. Rowlands and C. B. Monk, *Trans. Faraday Soc.*, 1966, **62**, 945. [cc] J. Stary, *Analyt. Chim. Acta*, 1963, **28**, 132. [dd] Y. Kanemura and J. I. Watters, *J. Inorg. Nuclear Chem.*, 1967, **29**, 1701. [ee] E. Gelles and R. W. Hay, *J. Chem. Soc.*, 1958, 3673, 3684, 3689.

A wide range of zinc doses has been reported in the literature.[14,15] The standard dose taken for our models

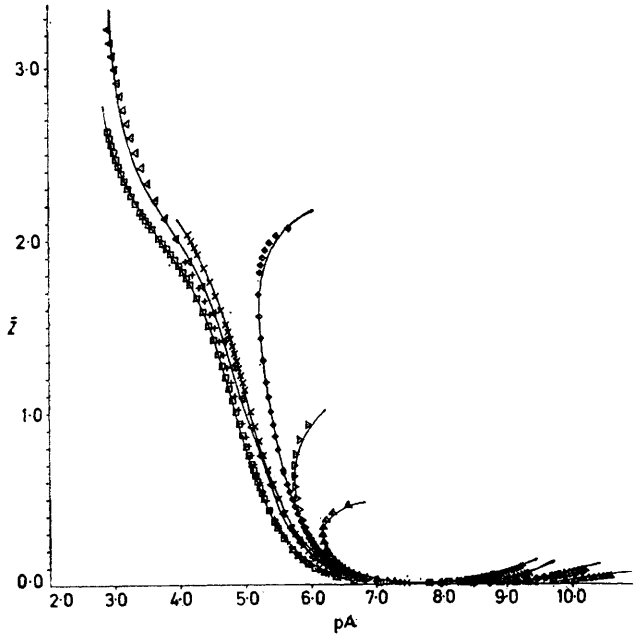

FIGURE 1 PSEUDOPLOT curves for the best set of β values of zinc prolinate plotted on the experimental ZPLOT points. Concentrations of A and B (in mM) are +, 20.3, 4.7; ×, 10.0, 2.3; ⊡, 20.2, 2.3; ◇, 5.0, 2.3; ▷, 5.0, 4.7; ◁, 10.0, 1.2; △, 2.35, 4.7

TABLE 3

Percentage of zinc complexed as neutral species by each ligand at small-intestinal pH = 6.5

Ligand	Species 110	Species 210	Species 11–1	Species 220
'Aspartamate'		27.2	0.3	
Aspartate	56.6			
α-D-Galacturonate			88.1	
β-Hydroxybutyrate		77.3		
L-4-Hydroxyprolinate		18.6	0.6	
Malate	48.3			
Malonate	46.9			16.4
Oxalate	98.8			
L-Prolinate		1.3	1.4	
Tartrate	51.0			

TABLE 4

Percentage of zinc complexed as neutral species by one of the ligands at pH = 6.5 as ligand : zinc and total zinc concentrations are varied

Ligand	[Zn]/mM	Ratio A : B	% as 210 species	% as 11–1 species
'Aspartamate'	7.65	1 : 2	2.6	0.26
		1 : 1	9.5	0.38
		2 : 1	27.2	0.34
		3 : 1	39.4	0.23
		4 : 1	45.7	0.16
		6 : 1	51.2	0.10
	1.91	2 : 1	3.2	0.10
	3.82	2 : 1	9.9	0.19
	11.49	2 : 1	46.7	0.46
	15.30	2 : 1	68.1	0.57

was 7.65mM (*i.e.* equivalent to a 50-mg zinc sulphate tablet) and then we optimised the ligand concentration by varying the $A : B$ ratio from $\frac{1}{2} : 1$ to 6 : 1. Secondly, for a given $A : B$ ratio of 2 : 1, we varied B from 1.91 to 15.3mM. These results are given in Table 4 and they suggest that increased absorption in the intestine could arise from an increase in zinc or an increase in ligand. We prefer the latter since high doses of zinc salts have an emetic effect.

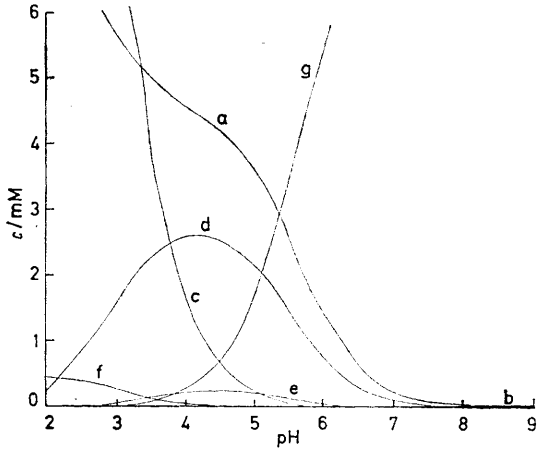

FIGURE 2 COMPLOT calculation of pH dependence for complexes present in the zinc (B)–galacturonate (A)–proton system when A = 15.3mM and B = 7.65mM: (a), Zn^{2+}; (b), $Zn(OH)^{+}$; (c), AH; (d), ZnA^{+}; (e), ZnA_2; (f), $ZnAH^{2+}$; (g), ZnA(OH)

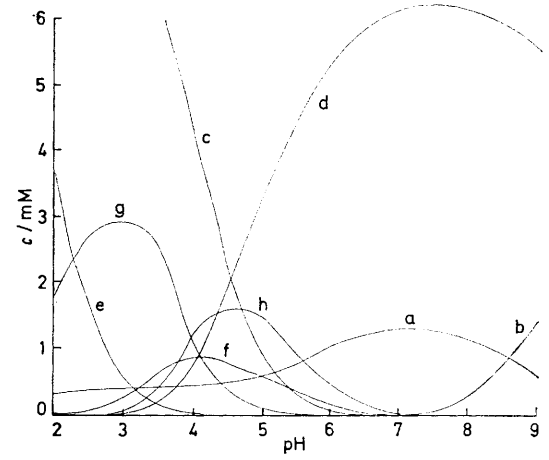

FIGURE 3 COMPLOT calculation of pH dependence for complexes present in the zinc (B)–β-hydroxybutyrate (A)–proton system when A = 15.3mM and B = 7.65mM: (a), Zn^{2+}; (b), $Zn(OH)^{+}$; (c), AH; (d), ZnA_2; (e), $ZnAH_2^{3+}$; (f), $Zn_2A_2H^{3+}$; (g), $Zn_2A_2H_2^{4+}$; (h) $Zn_2A_3H^{2+}$

We thank the British Council for a grant covering University fees (to G. K. R. M.), the University of St. Andrews for the award of a maintenance grant (to M. L. D. T.), and G. D. Searle and Co. and the Merrell International Research Centre, Strasbourg, for the donation of chemicals.

[5/1615 *Received*, 15*th August*, 1975]

[14] P. Schechter, Merrell International Research Centre, personal communication.

[15] L. Fodor, J. Eschner, W. Dick, and F. W. Ahnefeld, *Anaesthetist*, 1972, **21**, 456.