Computer Simulation of Metal-ion Equilibria in Biofluids. Part 2.[1] Formation Constants for Zinc(II)–Citrate–Cysteinate Binary and Ternary Complexes and Improved Models of Low-molecular-weight Zinc Species in Blood Plasma †

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Equilibrium-based computer models of biofluids can be improved by establishing the exact values for the formation constants of complexes present. This work reports the compositions of the complex species and log β values for the zinc citrate, zinc cysteinate, and zinc citrate cysteinate systems at 37 °C and I = 0.15 mol dm^{-3} Na[ClO_4]. The most important low-molecular-weight zinc complexes in blood plasma are found to be $[Zn(CysO)_2]^{2-}$ and $[Zn(CysO)(HisO)]^-$ (CysO = cysteinate, HisO = histidinate).

PART 1 [1] of this series described a computer-simulated equilibrium model of low-molecular-weight (l.m.w.) metal-complexing reactions in blood plasma based on several thousand formation constants. Subsequent

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[1] Part 1, P. M. May, P. W. Linder, and D. R. Williams, *J.C.S. Dalton*, 1977, 588.

papers [2,3] have extended and justified the utility of such models in pharmaceutical research by correlating biological responses to administered chelating agents with computer calculations of the efficiency of such drugs at replenishing the l.m.w. complex fraction at the expense of protein-bound metal. The models have been extensively used to investigate the copper dependence of rheumatoid arthritis and its treatment.[4-10]

Clearly, these computations are only as accurate as the input data and it is our declared intention to check the formation constants of the most important species (*i.e.* those in Table 4 of ref. 1) if these are in any doubt. Such a problem arises with the zinc(II)–citrate-cysteinate system, literature values sometimes having an ambiguous interpretation because citrate may be taken as either a 3— or 4— anionic ligand. In this case the problem is particularly acute since computer simulations of blood plasma [1] indicate that 43% of the l.m.w. zinc is present as zinc(II) citrate cysteinate(3—). Thus, this paper examines the parent binary, and ternary, systems in some detail at 37 °C and $I = 0.15$ mol dm^{-3} Na[ClO_4], compares results with those of other workers, and then uses the constants to obtain a more reliable computation of the distribution of l.m.w. zinc complexes in plasma.

EXPERIMENTAL

Reagents.—For the binary zinc citrate system the citric acid was supplied by Prolabo R.P. (Found: C, 34.2; H, 4.70. Calc. for $C_6H_{10}O_8$: C, 34.3; H, 4.80%). In the ternary zinc cysteinate studies the citric acid was supplied by Fisons Analytical Reagents (Found: C, 34.1; H, 5.05%). Cysteine was supplied by Merck (Found: C, 29.75; H, 5.90; N, 11.4. Calc. for $C_3H_7NO_2S$: C, 29.75; H, 5.80; N. 11.55%).

Stock solutions of zinc perchlorate in acid were prepared from crystals supplied by Pierce Inorganics B.V. and the metal and mineral acid contents determined volumetrically against ethylenediaminetetra-acetate (edta) and standard alkali (Gran titration). Sodium perchlorate was supplied by Merck; solutions were purified by alkaline precipitation of hydrated oxides and ultrafiltration.

Potentiometric Equipment.—For the binary zinc citrate system Corning equipment was used (digital type 113 mV meter with cat. no. 476022 electrode, and a saturated sodium chloride calomel electrode). The electrode arrangement was as in (1). The remaining titrations employed a Radiometer meter (cat. no. pHM 52 digital voltmeter) and glass and saturated sodium chloride calomel electrodes supplied by Russell pH using the electrode arrangement (2).

Experimental Conditions.—The temperature was maintained at 37.00 ± 0.02 °C in the reaction cell by circulating thermostatted water. The ionic background to hold activity coefficients constant was $I = 0.15$ mol dm^{-3}, this being isotonic with blood plasma. All the work was performed under an atmosphere of thermostatted, scrubbed, oxygen-free nitrogen.

Both electrode systems were calibrated by determination of E_0 using readings from solutions of known [H^+]. pK_w was measured and calculated according to the method of Carpeni *et al.*[11] The initial concentrations for the titrations were as follows.*

Protonation of citrate. Six titrations, initial titrate 5×10^{-3}—10^{-2} mol dm^{-3} citrate, 10^{-2} mol dm^{-3} mineral acid, titrant 0.1—0.2 mol dm^{-3} Na[OH].

Zinc citrate. Ten titrations, initial titrate 10^{-2} mol dm^{-3} citrate, 10^{-2} mol dm^{-3} mineral acid, 1×10^{-3}—1.2×10^{-2} mol dm^{-3} Zn^{2+}, titrant 0.1—0.2 mol dm^{-3} Na[OH].

Protonation of cysteinate. Five titrations, initial titrate 5×10^{-3}—1×10^{-2} mol dm^{-3} cysteinate, 1×10^{-2}—2.5×10^{-2} mol dm^{-3} mineral acid, titrant 0.1 mol dm^{-3} Na[OH].

Zinc cysteinate. Eight titrations, initial titrate 5×10^{-3}—1×10^{-2} mol dm^{-3} cysteinate, 1×10^{-2}—2.5×10^{-2} mol dm^{-3} mineral acid, 3×10^{-3}—1×10^{-2} mol dm^{-3} Zn^{2+}, titrant 0.1 mol dm^{-3} Na[OH].

Glass electrode | Citrate, Zn^{2+}, Na[ClO_4] (0.15 mol dm^{-3}) ‖ $K[ClO]_4$ (saturated) ‖ KCl (saturated) | Hg_2Cl_2/Hg (1)

Glass electrode | Ligand, Zn^{2+}, Na[ClO_4] (0.15 mol dm^{-3}) ‖ NaCl (saturated) | Hg_2Cl_2/Hg (2)

Zinc citrate cysteinate. Five titrations, initial titrate 1×10^{-2}—2.5×10^{-2} mol dm^{-3} citrate, 1×10^{-2}—2×10^{-2} mol dm^{-3} cysteinate, 1×10^{-2}—2.5×10^{-2} mol dm^{-3} mineral acid, 1×10^{-2}—2×10^{-2} mol dm^{-3} Zn^{2+}, titrant 0.1 mol dm^{-3} Na[OH].

Calculation of Formation Constants.—The values of optimized formation constants depend critically on the choice of complex species offered to the computer programs used to treat potentiometric-titration data.[12] Yet, in this regard, literature reports often neglect to give details of the criteria adopted. Indeed, much of the unreliability of published formation-constant data can probably be attributed to faulty model-selection procedures.[13]

Although such matters have generally been improved by the advent of high-speed computers, a completely reliable and automatic method for model selection has not been

* The data and details of the computer models are to be found in Supplementary Publication No. SUP 22324 (15 pp.). For details see Notices to Authors No. 7, *J.C.S. Dalton*, 1977, Index issue.

[2] P. M. May and D. R. Williams, *Proc. Roy. Soc. Med.*, Suppl. 3, 1977, 19.
[3] P. M. May and D. R. Williams, *F.E.B.S. Letters*, 1977, **78**, 134.
[4] G. E. Jackson, P. M. May, and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1189.
[5] A. M. Fiabane and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1195
[6] A. M. Fiabane, M. L. D. Touche, and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1201.
[7] M. Micheloni, P. M. May, and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1209.
[8] G. Arena, G. Kavu, and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1221.
[9] G. E. Jackson, P. M. May, and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1978, **40**, 1227.
[10] G. E. Jackson, P. M. May, and D. R. Williams, *J. Inorg. Nuclear Chem.*, in the press.
[11] G. Carpeni, E. Boitard, and R. Pilard, *J. Chim. phys.*, 1972, **69**, 1445.
[12] A. Vacca, *Proc. Summer School Stability Constants*, Edizioni Scuola Universitaria, Firenze, 1974, 105.
[13] M. T. Beck, *Pure Appl. Chem.*, 1977, **49**, 129.

devised.[12] Too much emphasis is still being placed on the exact value of the objective function being minimized (*e.g.* the sum of squared residuals).[14] In cases where there is little to choose between different models on the basis of criteria for ' goodness of fit ' it is important to consider the degree to which *trends* correspond in the calculated and observed data.

Comparison of experimental and theoretical potentiometric data can in principle involve any quantity which varies systematically throughout the titration. For quantitative estimates there are grounds for favouring the use of free hydrogen-ion concentration [15] but we have chosen to look at the formation function ($\bar{Z}$) because its sensitivity to species composition is well suited to a graphical display.[16] The formation function is calculated in the usual way. If T_L and T_M represent the total metal and total ligand concentrations, respectively, we obtain equation (3).

$$\bar{Z} = \{T_L - ([L] + [HL] + [H_2L] + \ldots)\}/T_M \quad (3)$$

The amount of ligand not complexed to metal is obtained from a knowledge of the free hydrogen-ion concentration and the ligand-protonation constants. The calculation is independent of the metal complex species existing in solution. (Were solely simple mononuclear non-protonated and non-hydroxo-complexes present $\bar{Z}$ would represent an average number of ligands bound per metal ion and plots for different total ligand and total metal concentrations would be superimposable. More complicated systems cannot be interpreted in this way: increases in the value of the formation function essentially reflect the liberation of protons due to the presence of the metal ion.)

Accordingly, the final selection of the sets of formation constants shown in Table 1 has been strongly influenced by comparisons made between the ZBAR function $\bar{Z}$ and our theoretical function PSEUDOPLOT.[16] The latter is evaluated from pseudo-titration data (calculated [17] using the proposed set of formation constants) in exactly the same way as ZBAR is obtained from the experimental data. Formation-constant refinement was performed using the MINIQUAD program.[18] SCOGS [19] was used additionally as noted below.

TABLE 1

Formation constants obtained from these studies. The computational strategy used for each set of constants is explained in the text. The formula of the general complex is $[Zn_r(citrate)_p(CysO)_qH_s]$. n = Number of experimental observations, S = sum of squares of residuals, NDP = number of dissociable protons on the parent acid

	System	p	q	r	s	log β	S	n	Notes
(A)	Proton citrate, NDP = 3	1	0	0	1	5.539 ± 0.003	5.054E-07	144	$\bar{n}_h > 0$
		1	0	0	2	9.775 ± 0.003			
		1	0	0	3	12.644 ± 0.005			
(B)	Proton cysteinate, NDP = 2	0	1	0	1	10.110 ± 0.003	8.664E-07	191	
		0	1	0	2	18.078 ± 0.006			
		0	1	0	3	20.050 ± 0.008			
(C)	Zinc citrate	1	0	1	0	4.715 ± 0.015	1.141E-05	199	
		1	0	1	1	8.441 ± 0.030			
		2	0	1	0	7.361 ± 0.074			
		2	0	2	−2	−2.214 ± 0.036			
(D)	Zinc cysteinate	0	2	1	0	17.913 ± 0.010	1.130E-07	135	
		0	1	1	1	14.544 ± 0.024			
		0	2	1	1	23.813 ± 0.056			
		0	3	2	0	29.826 ± 0.086			
		0	3	2	1	36.392 ± 0.037			
		0	3	2	2	41.748 ± 0.037			
(E)	Zinc cysteinate	0	2	1	0	17.905 ± 0.008	0.852E-07	135	
		0	1	1	1	14.604 ± 0.015			
		0	2	1	1	24.114 ± 0.016			
		0	4	3	0	42.278 ± 0.080			
		0	4	3	1	48.313 ± 0.073			
		0	4	3	2	54.082 ± 0.030			
(F)	Zinc cysteinate	0	1	1	0	8.600 ± 0.109	0.676E-07	135	
		0	2	1	0	17.909 ± 0.008			
		0	1	1	1	14.449 ± 0.039			
		0	2	1	1	24.022 ± 0.027			
		0	2	1	2	29.013 ± 0.132			
		0	4	3	0	42.362 ± 0.076			
		0	4	3	1	48.168 ± 0.099			
		0	4	3	2	53.949 ± 0.041			
(G)	Zinc citrate cysteinate	1	1	1	0	12.363 ± 0.067		163	SCOGS (deviation in titre = 0.095 4 cm³)
		1	1	1	2	24.021 ± 0.119			
(H)	Zinc citrate cysteinate	1	1	1	0	12.348 ± 0.067		163	SCOGS (deviation in titre = 0.094 8 cm³)
		1	1	1	2	24.039 ± 0.115			
(I)	Zinc citrate cysteinate	1	1	1	2	23.502 ± 0.317	0.334E-05	163	MINIQUAD
(J)	Zinc citrate cysteinate	1	1	1	2	23.520 ± 0.301	0.323E-05	163	MINIQUAD

Ionisation of water. pK_w was measured to be −13.38.

Protonation of citrate. The citrate anion was assumed to have three negative charges [*i.e.* number of dissociable protons of the parent acid (NDP) = 3]. The protonation constants listed in Table 1 [system (A)] were obtained.

Protonation of cysteinate. See Table 1 [system (B)].

Zinc citrate. With polydentate ligands there is a wide range of complexes that might be formed. A total of

[14] A. Vacca, A. Sabatini, and M. A. Gristina, *Co-ordination Chem. Rev.*, 1972, **8**, 44.

[15] L. G. Sillén, *Acta Chem. Scand.*, 1962, **16**, 159.

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

[17] N. Ingri, W. Kakolowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261.

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

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

17 sets of complexes was examined, the results of which may be found in SUP 22324. Many were rejected when negative values for the constants were produced, when the sum of squares ' was large, or when constants had large standard deviations. The three sets remaining were examined in greater detail using PSEUDOPLOT (see data deposition) and the best is listed in Table 1 [system (C)].

Zinc cysteinate. As is usual with zinc complexing, the formation curve is very steep and so points on the horizontal portions of the formation curve were removed from the data treatment, *i.e.* the pA range was 5—13, 135 of an initial 304 points being used as data. Literature reports concerning this system suggest polynuclear species such as $[Zn_3(CysO)_4H_x]$ (CysO = cysteinate).[20-22] In order to encompass such a possibility four levels of searching for constants were used. First, a simple logical model based solely on mononuclear species; secondly, the presence of simple binuclear complexes was permitted by using $[Zn_2(CysO)_3H_x]$. More sophisticated polynuclear complexes of the type $[Zn_3(CysO)_4H_x]$ were then permitted in the first and second models respectively. This involved 44 sets of constants, 12 sets being examined in greater detail using PSEUDOPLOT (see data deposition) and these produced two final choices [Table 1, systems (D) and (E)]. It is noteworthy that a third model [Table 1, system (F)] which included the monocysteinate species ostensibly gave a better ' fit ' on the grounds of attaining the lowest sum of squared residuals. Nevertheless, it was considered inferior because (*i*) the differences in the sum of squared residuals, the MINIQUAD crystallographic *R* factor, the PSEUDOPLOT fit, and the formation constants of the major species are not significant considering the increased degree of freedom conferred by including two additional species, and (*ii*) the standard deviations for the predominant complexes are clearly worse in the case of the larger model.

TABLE 2

Percentage distribution of zinc amongst l.m.w. complexes in human blood plasma as found by computer simulation using formation constants from Table 1 [a]

Complex	Percentage
$[Zn(CysO)_2]^{2-}$	40
$[Zn(CysO)(HisO)]^-$	24
$[Zn(HisO)]^+$	4
$[Zn(CysO)_2H]^-$	3
[Zn(CysO)(HisO)H]	2
$[Zn(HisO)_2]$	2
[Zn(CysO)]	2 [a]
[Zn(CysO)(cystinate)]$^{2-}$	2
[Zn(CysO)(GlnO)]$^-$ [b]	2

[a] See text. [b] GlnO = Glutaminate.

Zinc citrate cysteinate. Although the cysteinate binary constants of Table 1 [system (E)] appear to be the best, there is still the possibility that Table 1 [system (D)] may be applicable and so ternary complexes were computed using both these standpoints. In our experience the SCOGS least-squares program is able to accommodate guesses at log β more widely removed from the ' best ' values than MINIQUAD and so both SCOGS and MINIQUAD computations were employed. The constants of Table 1 [system (D)] gave (E) by SCOGS and (I) by MINIQUAD and Table 1 [system (E)] gave (H) by SCOGS and (J) by MINIQUAD. It is noteworthy that the set containing $[Zn_3(CysO)_4H_x]$ apparently gives the ' best ' fit.

Blood-plasma Models.—The distribution of metal ions amongst nearly 5 000 complexes was computed at $-\log [H^+] = 7.4$ using the ECCLES program as previously described.[1] Simulations were performed substituting in turn each of the possible sets of constants for the zinc cysteinate system from Table 1 [systems (D)—(F)] and combinations thereof. In this way a range of possible zinc cysteinate models was covered. To a precision of 1% the results differed only in respect of the monocysteinate species. When this was included in the simulation using log β 8.60 it accounted for *ca.* 2% of the total l.m.w. zinc. As shown in Table 2, the bis(cysteinate) and ternary cysteinate histidinate complexes are found to be the most predominant. Cysteinate and histidine complexes account for over three quarters of the zinc ions in the l.m.w. fraction of blood plasma.

DISCUSSION

The main objectives of the present researches were to establish the stoicheiometries of ternary complexes formed between zinc, citrate, and cysteinate ions and to determine their respective formation constants. These could then be compared with the results of previous work reported by Ramamoorthy and Manning.[23] However, during the course of this investigation certain difficulties and ambiguities concerning the parent binary complexes became apparent.

Binary Complexes.—(*a*) Computational analyses of the zinc citrate system depend on the choice of NDP; although the alternatives are mathematically equivalent, they offer different advantages. With NDP = 4 the whole range of curves of $\bar{Z}$ against pA can be compared using the PSEUDOPLOT approach (see data deposition). On the other hand, the absolute values of the formation constants corresponding to NDP = 4 vary according to the value of pK_1 which is used (this is too high to be determined exactly by glass-electrode potentiometry) so NDP = 3 is considered more satisfactory from a chemical viewpoint.

Our studies show that a binuclear species, $[Zn_2(citrate)_2{\cdot}(OH)_4]^{4-}$ is important at higher pH. Contrary to previous work,[24] inferior results were obtained from computational models in which (*i*) this species was replaced by $[Zn(citrate)(OH)]^{2-}$ or (*ii*) both these hydroxo-complexes were included simultaneously.

(*b*) In the zinc cysteinate system some difficulty arises because (*i*) $\log K_{ZnL_2} \geqslant \log K_{ZnL}$ and (*ii*) polynuclear complexes are readily formed. Both phenomena have been partly attributed to the ability of sulphur to accept electrons from the metal by π bonding as this would favour the addition of a second sulphur atom.[20] The size of the sulphur atom also facilitates the

[20] D. D. Perrin and I. G. Sayce, *J. Chem. Soc.* (*A*), 1968, 53.
[21] P. S. Hallman, D. D. Perrin, and A. E. Watt, *Biochem. J.*, 1971, **121**, 549.
[22] H. Shindo and T. L. Brown, *J. Amer. Chem. Soc.*, 1965, **87**, 1904.
[23] S. Ramamoorthy and P. G. Manning, *J. Inorg. Nuclear Chem.*, 1974, **36**, 1671.
[24] J. T. H. Roos and D. R. Williams, *J. Inorg. Nuclear Chem.*, 1977, **39**, 367.

bridging commonly observed for ligands similar to cysteinate with a variety of metal ions.[20,25-27]

The characterization of polynuclear species in solution is most readily achieved by increasing reactant concentrations but this approach was severely limited by precipitation (also noted by Perrin and Sayce [20]). Our work leaves some doubt concerning the set of formation constants which best define the zinc cysteinate system; in our opinion the complexes shown in Table 1 [system (E)] containing $[Zn_3(CysO)_4H_x]$ are the prime choice. This is in essential agreement with Perrin and Sayce except that $Zn(CysO)H^+$ replaces $Zn(CysO)_2H_2$. The above preference is also supported by the isolation of a solid complex which has been assigned the structure $[Zn_3(CysO)_4H_4][ZnCl_4]$.[22] However, in accordance with the ' core + links ' hypothesis,[28] the intermediate set of polynuclear species $[Zn_2(CysO)_3H_x]$ was also considered; it yielded a comparable but nevertheless inferior fit with our experimental data. From Table 1 [systems (D) and (E)] it is clear that the formation constant computed for the most important mononuclear complex, *i.e.* $[Zn(CysO)_2]^{2-}$, differs little whichever polynuclear series is chosen. This is not the case when polynuclear complexes are neglected (see data deposition). Such omissions could have a marked influence on the computation of constants for the ternary zinc cysteinate citrate complexes and may partially account for the discrepancy between the present and previous results.[23]

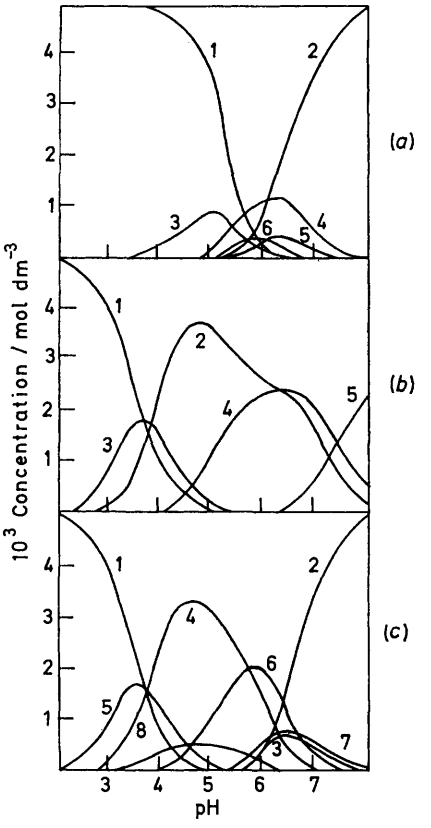
Ternary Complexes.—An estimated value of the log constant for the formation of zinc citrate cysteinate may be obtained by reference to the binary parent complexes using formation constants from Table 1 [systems (C) and (E)] and formula (4).[29] This yields a value of 12.94

$$\log \beta\,[Zn(citrate)(CysO)] = \tfrac{1}{2}\{\log \beta\,[Zn(citrate)_2] + \log \beta\,[Zn(CysO)_2]\} + \log 2 \quad (4)$$

which is almost four orders of magnitude smaller than that reported by Ramamoorthy and Manning.[23] In contrast, the present findings strongly suggest that some destabilization of the complex occurs. This is evidenced by the low formation constant from SCOGS [Table 1, systems (G) and (H)] and the fact that this constant is made negative during refinement by MINIQUAD (see data deposition). It appears that ternary complexes do not exist at significant concentration in solutions of zinc, cysteinate, and citrate ions. Whether or not such a species is present at all is beyond the experimental precision of the potentiometric technique used. These conclusions may be drawn because of (*i*) the difference between the formation constants derived from our experimental data as computed by MINIQUAD and SCOGS [Table 1, systems (G)—(J)] and (*ii*) the large errors obtained using the MINIQUAD computer program (a σ value of 0.3 corresponds to a relative error of *ca.* 70%).

The Figure compares the complex distributions of the binary and ternary systems. It is clear that neither ternary complex is able to compete successfully with the prevailing binary species. At high pH, $[Zn(CysO)_2]^{2-}$ is very strongly formed whilst in the more acid solutions [Zn(citrate)H], $[Zn(citrate)]^-$, and $[Zn(citrate)_2]^{4-}$ predominate.

Much of the difficulty in distinguishing between various sets of complexes when searching for the best constants can be attributed to the relatively low concentration maxima for many of the species illustrated in the Figure. As a consequence, the response of the glass electrode to changes in complex distribution is slight. Use of a zinc-amalgam electrode to improve this position was not considered appropriate in view of the ready



HALTAFALL [17] computed distribution of complex species with pH for the systems (*a*) zinc cysteinate, (*b*) zinc citrate, and (*c*) zinc cysteinate citrate. In each case the total zinc concentration is 5.0×10^{-3} mol dm^{-3} and total ligand concentration(s) is 1.0×10^{-2} mol dm^{-3}. The formation constants were taken from Table 1 [systems (E), (G), and (J)]. Key: (*a*) 1 = Zn^{2+}, 2 = $[Zn(CysO)_2]$, 3 = [Zn(CysO)H], 4 = $[Zn(CysO)_2H]$, 5 = $[Zn_3(CysO)_4]$, 6 = $[Zn_3(CysO)_4H]$; (*b*) 1 = Zn^{2+}, 2 = [Zn(citrate)], 3 = [Zn(citrate) H], 4 = $[Zn(citrate)_2]$, 5 = $[Zn_2(citrate)_2(OH)_2]$; (*c*) 1 = Zn^{2+}, 2 = $[Zn(CysO)_2]$, 3 = $[Zn(CysO)_2H]$, 4 = [Zn(citrate)], 5 = [Zn(citrate)H], 6 = $[Zn(citrate)_2]$, 7 = [Zn(citrate)(CysO)], 8 = [Zn(citrate)(CysO)H] charges omitted for clarity.

[25] D. D. Perrin and I. G. Sayce, *J. Chem. Soc.* (*A*), 1967, 82.
[26] M. Aguilar, S. Alegret, and E. Casassas, *J. Inorg. Nuclear Chem.*, 1977, **39**, 733.
[27] H. F. de Brabander, H. S. Creyf, A. M. Goeminne, and L. C. van Pouke, *Talanta*, 1976, **23**, 405.
[28] L. G. Sillén, *Acta Chem. Scand.*, 1954, **8**, 299, 318.
[29] J. P. Scharff and R. P. Martin in 'An Introduction to Bioinorganic Chemistry,' ed. D. R. Williams, Thomas, Illinois, 1976.

oxidation of cysteine to cystine. It suffices to say that many of the species can be seen to have similar pH profiles, thus increasing the likelihood of compensation between formation constants during the computer-optimization procedure.

Improved Blood-plasma Models.—The considerably diminished importance of zinc citrate cysteinate in blood-plasma computer simulations brought about by the use of the formation constants measured in this study raises several noteworthy considerations.

(*i*) A closer comparison between the current blood-plasma model and Perrin's original computation,[21] which did not include citrate, is possible. Whilst there is broad agreement concerning the identity of the predominant complexes, the greater importance of $[Zn(CysO)_2]^{2-}$ compared with $[Zn(CysO)(HisO)]^-$ (HisO = histidinate) shown in Table 2 is at variance with the earlier work. This is due to the difference in approach adopted towards the question of metal-protein binding: instead of using formation constants for metal-protein complexes which presently cannot be satisfactorily characterized, we have developed methods of a relative nature which are consequently more restricted but considerably more reliable.[1,2] One manifestation of this difference is the higher value we find for the free cysteinate anion concentration (presently calculated to be 9×10^{-9} mol dm^{-3}) that favours the bis complex.

(*ii*) The urinary zinc excretion induced by administered histidine[30,31] is now easily understood in terms of the computer models. More than one third of the l.m.w. zinc in plasma is complexed to this amino-acid (Table 2). This percentage is dramatically increased by higher plasma concentrations of histidinate.

(*iii*) Simulated effects of non-naturally occurring chelating agents in plasma on zinc metabolism[3] will almost certainly be increased by the diminution of the simulated ability of naturally occurring ligands to bind this metal ion *in vivo*.

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[30] E. Giroux and N. J. Prakash, *J. Pharm. Sci.*, 1977, **66**, 391.
[31] D. T. Latto and D. R. Williams, *Inorg. Nuclear Chem. Letters*, 1977, **13**, 73.