Thermodynamic Considerations in Co-ordination. Part XXV.[1] Formation of Ternary Complexes containing Two Dissimilar Metal Ions and the Implication for Metal–Metal Stimulation Phenomena *in vivo*

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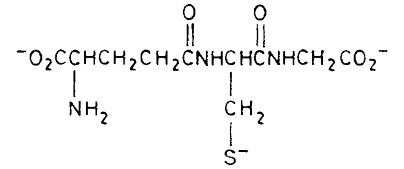
Potentiometric studies are reported for calcium(II), lanthanum(III), and zinc(II) binary complexes and for calcium(II) and zinc(II) or lanthanum(III) and zinc(II) ternary complexes of glutathionate at 37 °C and 0.15 10^{-3} mol dm^{-3} (Na$^+$) [ClO$_4$]. Formation constants have been computed and used to calculate the concentrations of the complexes at various pH. Both calcium and lanthanum ions encourage the formation of neutral membrane-permeable complexes of zinc ions and the systems are used to discuss the principles of metal–metal stimulation phenomena *in vivo*.

THE effective concentrations of metal ions *in vivo* are interrelated and their mutual influence may be one of antagonism (for example, when like ions challenge each other for the same donor groups) or stimulation.[2] The latter phenomenon may involve ions having similar chemistries (for example, the enhancement of the concentration of zinc histidinate by lead ions in Figure 3 of ref. 3), or alternatively, ions of dissimilar elements. We suggest that unlike ions may stimulate by complexing at separate sites of a fairly large ligand so that they modify the profiles of complex concentration against pH compared to parent binary complexes.

The basic requirements are a ligand possessing two complexing sites of different donor character and two chemically dissimilar metal ions. This study is based on glutathionate (γ-L-glutamylcysteinylglycinate), (I), and zinc(II); our interests in these two ions have already been stated.[1,4,5] Initially we used La^{III}, an ion completely unlike Zn^{II}, and then Ca^{II}, an essential ion *in vivo*.[6] This paper reports formation constants for binary and ternary systems at 37.0 °C and I = 0.15 mol dm^{-3} (Na$^+$)[ClO$_4$].



$^-O_2CCH(NH_2)CH_2CH_2C(=O)NHCH(CH_2S^-)C(=O)NHCH_2CO_2^-$

(I)

GluO(CysO–GlyO)$^{3-}$

EXPERIMENTAL

Glutathione, Glu(Cys-Gly) (Sigma Chemical Co.) (Found: C, 39.1; H, 5.55; N, 13.7. Calc for $C_{10}H_{17}N_3O_6S$: C, 39.1; H, 5.60; N, 13.7%), was used without further purification. Zinc(II) perchlorate (G. F. Smith Chemical Co.) solution was prepared as described in ref. 7. Calcium(II) perchlorate solution was prepared by dissolving the hydrate (G. F. Smith Chemical Co.) in perchloric acid and lanthanum(III) perchlorate solution was prepared by dissolving the oxide

TABLE 1

Log β_{pqr} for the protonation of glutathionate(3−) at 37 °C and I = 0.15 mol dm^{-3} (Na$^+$)[ClO$_4$]. 175 Data points were used in these calculations

pqr	101	102	103	104
log β_{pqr}	9.235	17.604	21.079	23.170
± standard deviation	±0.019	±0.020	±0.042	±0.048
Literature values:				
ref. *	9.65	18.40	21.99	
16	9.42	17.96	21.50	23.54
4	9.88	19.04	22.86	25.46

* N. C. Li, O. Gawron, and G. Bascuas, *J. Amer. Chem. Soc.*, 1954, **76**, 225.

TABLE 2

Log β_{pqr} for metal ion–glutathionate systems at 37 °C and I = 0.15 mol dm^{-3} (Na$^+$)[ClO$_4$], together with the standard deviations and the number of experimental points, n

Binary systems	log β_{pqr}		
	Ca^{II}	La^{III}	Zn^{II}
pqr			
110	3.84 ± 0.12		7.98 ± 0.03
210			12.48 ± 0.10
111	12.89 ± 0.12	12.79 ± 0.08	14.11 ± 0.10
211			21.36 ± 0.09
212			28.08 ± 0.11
112	20.68 ± 0.10	19.95 ± 0.10	
11−1	−6.46 ± 0.21	−3.59 ± 0.06	−0.71 ± 0.20
21−1			3.12 ± 0.09
01−1	−9.40 ± 0.15	−8.61 ± 0.17	
n	135	199	185
Ternary systems			
*pq*Zn*r*			
111−1	4.03 ± 0.20	4.77 ± 0.12	
111−2		−2.94 ± 0.15	
n	306	157	

Literature log constants for zinc binary complex formation: (*a*) ref. * of Table 1 reports β_{110} = 8.30 at 25 °C and I = 0.15 mol dm^{-3} (K[NO$_3$]); (*b*) ref. 16 reports for β_{110} = 7.94, β_{210} = 12.41, β_{111} = 14.16, β_{211} = 21.56, β_{212} = 28.60, β_{11-1} = −4.8, β_{21-1} = −3.77, and β_{120} = 10.62 at 37 °C and I = 0.15 mol dm^{-3} (K[NO$_3$]); (*c*) ref. 4 reports β_{110} = 8.57, β_{111} = 14.76, β_{11-1} = −0.07, β_{210} = 13.59, β_{211} = 23.27, β_{212} = 30.62, and β_{21-1} = 3.63 at 25 °C and I = 3.00 mol dm^{-3} (Na[ClO$_4$]).

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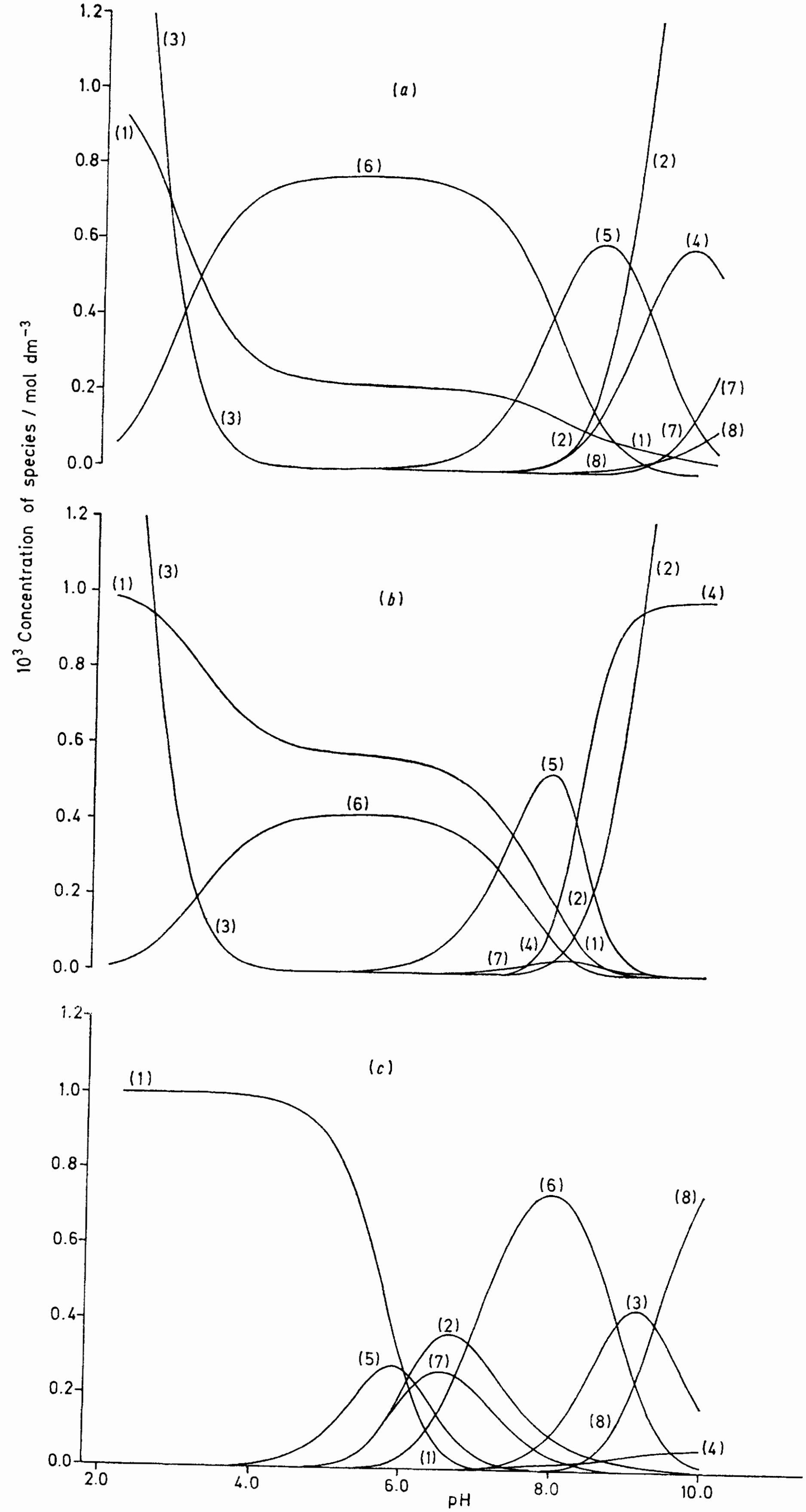
(a)
(b)
(c)
10^3 Concentration of species / mol dm^{-3}
1.2
1.0
0.8
0.6
0.4
0.2
0.0
(1)
(2)
(3)
(4)
(5)
(6)
(7)
(8)
2.0
4.0
6.0
8.0
10.0
pH

FIGURE 1

(American Potash and Chemical Corp.) in perchloric acid. The solutions were analysed for metal by two independent methods, and for mineral acid by Gran titration.[8] Other solutions were prepared as in refs. 7 and 9. Refs. 9 and 10 outline our potentiometric approach.

The protonation curves for glutathionate were superimposable showing that solely mononuclear AH_r species were present, but the metal-ion complexes gave a pattern of $\bar{Z}$ against pa curves as the glutathionate : metal-ion ratio was varied and the protonated, hydroxo-, and polynuclear species were identified using the PSEUDOPLOT,[11] SCOGS,[12] and MINIQUAD [13] computer programs. This investigation necessitated modifying our ZPLOT program so that it calculated the average number of ligands per total metal ion, even when this total is the sum of the concentrations of two dissimilar metal ions. Finally COMPLOT [14,15] computed plots of the concentrations of complex species present under blood-plasma conditions were obtained using the formation constants of Tables 1 and 2.

RESULTS AND DISCUSSION

Binary Systems.—Each system was studied using a pattern of titrations in which both A and B (the total concentrations of ligand and metal ion respectively) were held constant and equal in both titrate and titrant, the sole difference between these two solutions being in their perchloric acid concentrations. For each value of $B = 0$ (for measuring the protonation constants), 1.00×10^{-3}, 1.25×10^{-3}, and 2.00×10^{-3} mol dm^{-3} the $A : B$ ratio was set at 0.5 : 1, 1 : 1, 2 : 1, 4 : 1, and 8 : 1 (for La^{III} a ratio of 16 : 1 was also used). By this means it was possible to obtain formation constants for all the mononuclear binary, and also for the more complicated polynuclear, hydroxo-, and protonated, complexes present. All reasonable combinations of β_{pqr} (actually $p = 0$—3, $q = 0$—2, and $r = -2$ to $+2$) * were tested until a good PSEUDOPLOT fit was obtained. The resulting 'best' log β_{pqr} values are listed in Tables 1 and 2 together with those reported by other workers.

A comparison of the protonation and zinc complex-formation constants with results at 25 °C and $I = 3.00$ mol dm^{-3} previously reported from this laboratory [4] shows that all the constants at 37 °C and $I = 0.15$ mol dm^{-3} are smaller in accordance with the expected Debye–Hückel trend. The 120 complex reported by Perrin and Watt [16] was found to have a negative β value and was rejected in the final MINIQUAD analysis. The calcium and lanthanum complexes show a marked similarity as might be expected as both are hard in the hard–soft acid–base sense (Ahrland derived softness parameters of 0.5 : 0.9 : 3.1 for La^{III} : Ca^{II} : Zn^{II} respectively [17]).

The pK values 9.05 ($\log \beta_{111} - \log \beta_{110}$) and 7.78 ($\log \beta_{112} - \log \beta_{111}$) for calcium may be compared with those of 9.24 and 8.37 (for the sulphydryl and amino-groups) for the parent ligand, suggesting that calcium is not bound to these two groups. Even though an equivalent lanthanum(III) 110 complex could not be detected, it appears likely that this ion also binds to the same donor sites as calcium ions. These sites may be compared to those for zinc ions which have been found to be at least 80% bonded to sulphydryl.[18]

Ternary Systems.—Lanthanum(III) formed a precipitate at pH 8 and elemental analysis using atomic absorption suggested [LaZn{GluO(CysO-GlyO)}(OH)]$^+$ or [LaZn{GluO(CysO-GlyO)}(OH)$_2$]. (COMPLOT models, *e.g.* Figure 3, show that the latter is present in solution at this pH.) Mixed metal precipitates were not found for the calcium–zinc system. The three mixed metal complexes detected in this study were [CaZn{GluO(CysO-GlyO)}(OH)], [LaZn{GluO(CysO-GlyO)}(OH)]$^+$, and [LaZn{GluO(CysO-GlyO)}(OH)$_2$], and from the COMPLOT models shown in Figures 2 and 3 they clearly occur in appreciable quantities.

Two different sets of concentrations were used in the COMPLOT computations of species present: (*i*) [Zn^{II}] = [Ca^{II}] = [La^{III}] = 1.000×10^{-3} mol dm^{-3} and [GluO(CysO-GlyO)] = 4.000×10^{-3} mol dm^{-3}, similar to the conditions used in the titrations; (*ii*) concentrations similar to plasma conditions [19] {[Zn^{II}] = 13.46×10^{-6}, [Ca^{II}] = 0.245×10^{-3} mol dm^{-3}, [La^{III}] arbitrarily taken as being the same as [Ca^{II}] even though La does not occur in plasma, and [GluO(CysO-GlyO)] = 1.204×10^{-3} mol dm^{-3}}. Figure 1(*a*)—(*c*) shows the plotted output concentrations of complexes occurring for the parent binary systems and Figures 2 and 3 show the complexes present in the ternary systems. The complexity of these systems is such that a plot showing all the complexes present on the same graph would be very confusing and so Figures 2 and 3 have been divided into two sets of curves, the (*a*) sets referring to zinc binary complexes and the (*b*) sets to

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* The term β_{pqr} refers to the formation constant for the species $A_pB_qH_r$.

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FIGURE 1 COMPLOT plots of the concentration of complexes present as a function of pH for binary metal ion–glutathionate (L^{3-}) systems when A = [L] = 4.0×10^{-3} and $B = 1.0 \times 10^{-3}$ mol dm^{-3} where B = (*a*) [Ca^{II}], (*b*) [La^{III}], and (*c*) [Zn^{II}]. Species: (*a*) Ca^{2+} (1), L^{3-} (2), H$_4$L$^+$ (3), [CaL]$^-$ (4), [Ca(HL)] (5), [Ca(H$_2$L)]$^+$ (6), [CaL(OH)]$^{2-}$ (7), [Ca(OH)]$^+$ (8); (*b*) La^{3+} (1), L^{3-} (2), H$_4$L$^+$ (3), [LaL(OH)]$^-$ (4), [La(HL)]$^+$ (5), [La(H$_2$L)]$^{2+}$ (6), [La(OH)]$^{2+}$ (7); (*c*) Zn^{2+} (1), [ZnL]$^-$ (2), [ZnL$_2$]$^{4-}$ (3), [ZnL(OH)]$^{2-}$ (4), [Zn(HL)] (5), [ZnL(HL)]$^{3-}$ (6), [Zn(HL)$_2$]$^{2-}$ (7), [ZnL$_2$(OH)]$^{5-}$ (8)

binary complexes of the other metals and to the ternary complexes. A complete picture of the variation in complex concentrations with pH requires curves (*a*) and (*b*) to be superimposed. All the curves are shown for the conditions in case (*i*), *i.e.* titration conditions; the corresponding curves for plasma conditions (*ii*) are in Supplementary Publication No. SUP 21749 (10 pp.).*

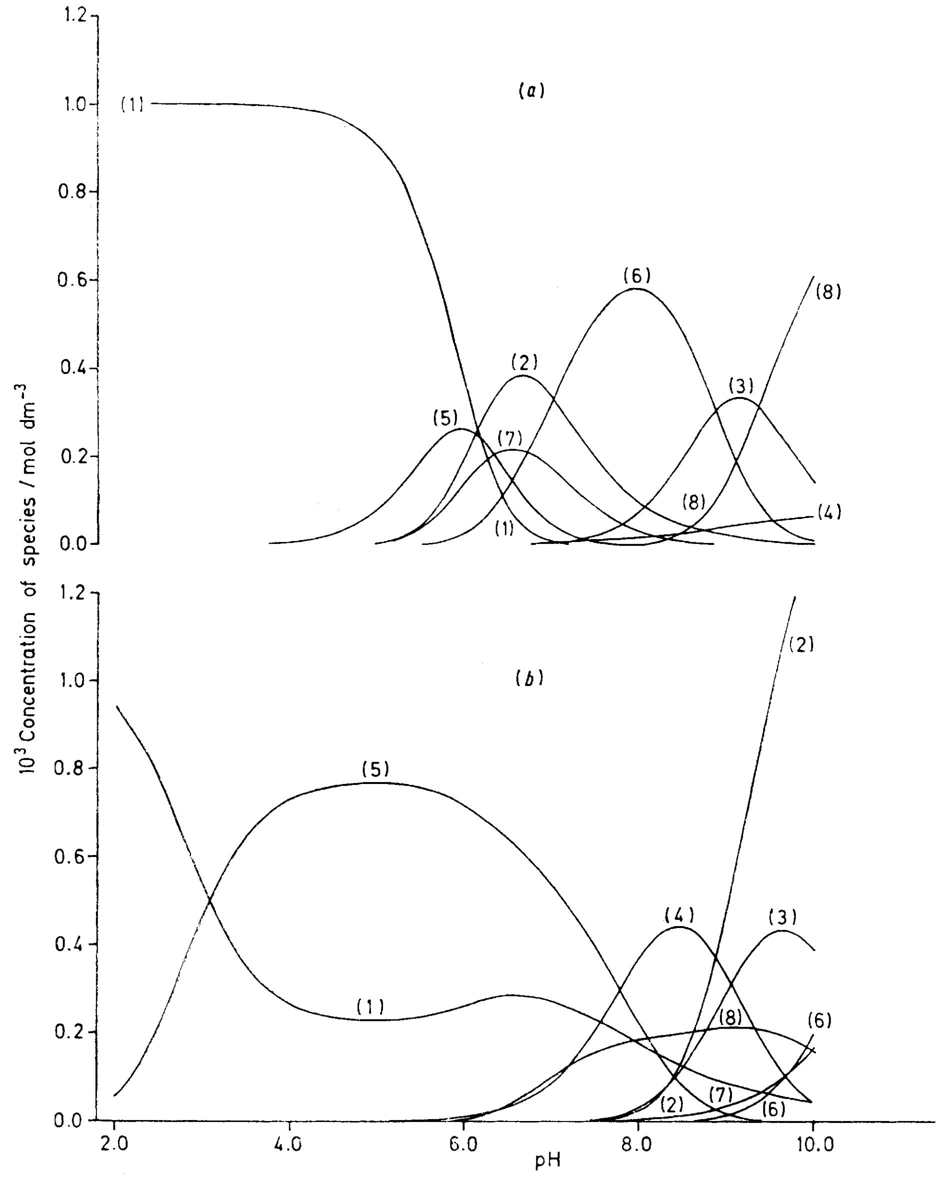

FIGURE 2 COMPLOT plots of the concentration of complexes present as a function of pH for zinc–calcium–glutathionate complexes when $A = 4.0 \times 10^{-3}$ and $[Zn] = [Ca] = 1.0 \times 10^{-3}$ mol dm^{-3}. Plots (*a*) and (*b*) refer to the same complex system. Species: (*a*) as in Figure 1 (*c*); (*b*) Ca^{2+} (1), L^{3-} (2), $[CaL]^-$ (3), $[Ca(HL)]$ (4), $[Ca(H_2L)]^+$ (5), $[CaL(OH)]^{2-}$ (6), $[Ca(OH)]^+$ (7), $[CaZnL(OH)]$ (8)

The following observations may be made. (1) Referring first to the binary systems in Figure 1, calcium is complexed more extensively than lanthanum from pH 2—8 but at pH > 8 the converse is true. (2) In the ternary systems of Figures 2 and 3, all concentrations of binary complexes are at reduced concentrations compared to Figure 1 in order to permit [CaZn{GluO(CysO-GlyO)}(OH)], [LaZn{GluO(CysO-GlyO)}(OH)]$^+$, and [LaZn{GluO(CysO-GlyO}(OH)$_2$] ternary complexes to be formed. (3) As might be expected from the hard–soft acid–base order noted previously, lanthanum–zinc mixed-metal complexes are more prevalent than calcium–zinc ternaries. (4) These ternary complexes are present in significant concentrations, for example the lanthanum–zinc ternaries account for 12.8% of the glutathionate complexed at plasma pH 7.4, and the calcium–zinc ternary complex involves 3.7% of the ligand, the per-

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* For details see Notices to Authors No. 7, *J.C.S. Dalton*, 1975, Index issue.

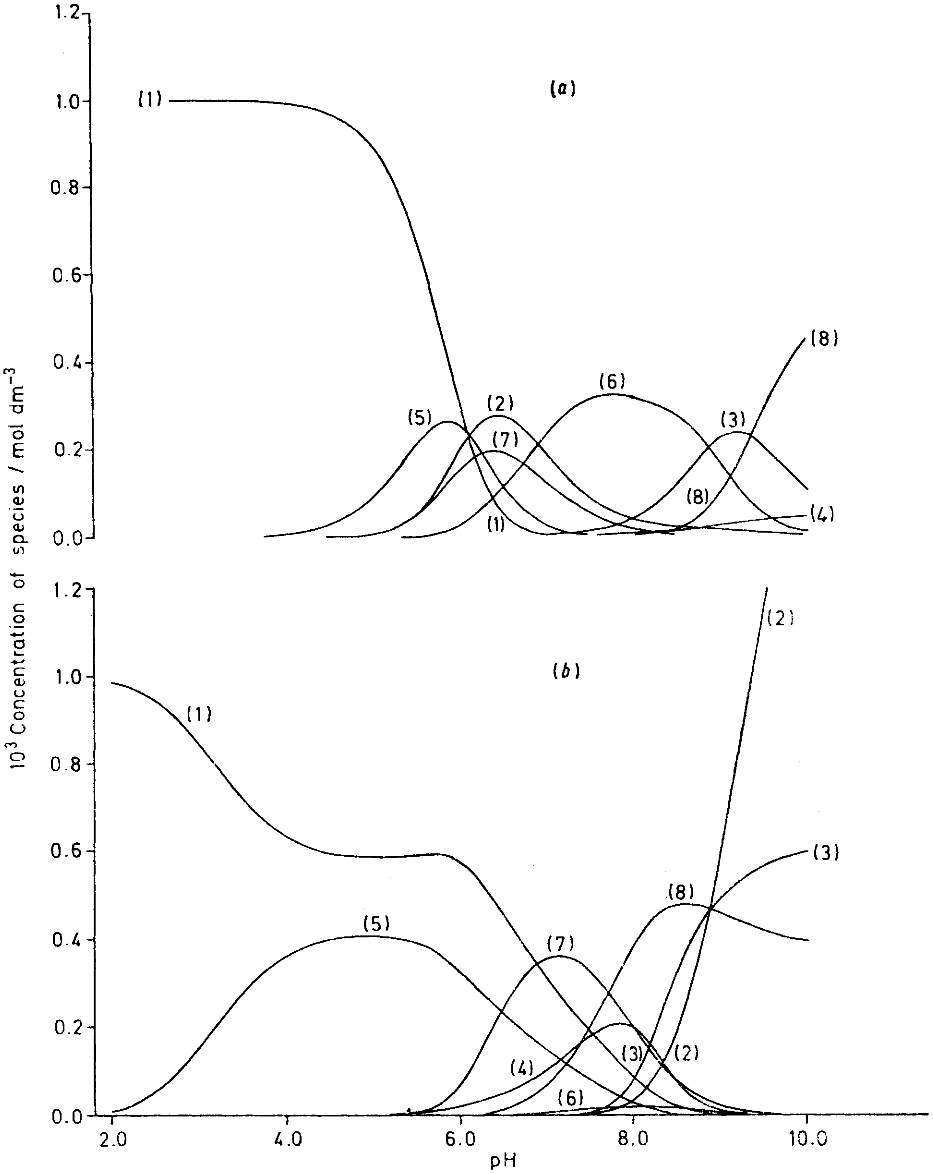

FIGURE 3 COMPLOT plots of the concentration of complexes present as a function of pH for zinc–lanthanum–glutathionate complexes when $A = 4.0 \times 10^{-3}$ and [Zn] = [La] = 1.0×10^{-3} mol dm^{-3}. Plots (*a*) and (*b*) refer to the same complex system. Species: (*a*) as in Figure 1 (*c*); (*b*) La^{3+} (1), L^{3-} (2), $[LaL(OH)]^-$ (3), $[La(HL)]^+$ (4),$[La\ (H_2L)]^{2+}$ (5), $[La(OH)]^{2+}$ (6), $LaZnL(OH)]^+$ (7), $[LaZnL(OH)_2]$ (8)

centages increasing with pH. COMPLOT curves computed for plasma conditions, concentration case (*ii*), have similar properties.

The most important biological conclusion of this study is that ternary-complex formation increases the amount of zinc present in the cell-membrane-permeable, neutral, complex form by 14.9 (Ca–Zn) or 16.1% (La–Zn). Thus we suggest that the phenomenon of dissimilar metal–metal stimulation may be realised in membrane-permeability properties.

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