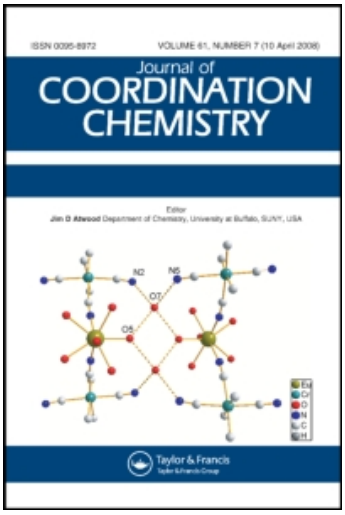

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ZINC(II) COMPLEXES WITH ASPARTATE AND GLUTAMATE

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Aspartate and glutamate have been studied as ligands (L) towards zinc(II) at 25°C and in 1.00 M $NaClO_4$ by measuring the electromotive force of galvanic cells containing glass and zinc amalgam electrodes over a wide concentration range of reagents. The experimental data were explained by assuming the formation of mononuclear species of the type ZnH_pL_r where $p \geq 0$ and $r \geq 1$. For aspartate, evidence for the complexes ZnL, ZnHL, ZnH_2L, ZnL_2, $ZnHL_2$, ZnH_2L_2, ZnL_3, $ZnHL_3$ and ZnH_2L_3 was found, whereas for glutamate, the complexes ZnL, ZnHL, ZnL_2, $ZnHL_2$, ZnL_3, $ZnHL_3$, ZnH_2L_3 were found. Evidence for the species ZnH_2L was found also in the case of glutamate. The relative stability constants have been determined and compared with those of other ligands and cations.

Keywords: Aspartic acid, glutamic acid, zinc(II) complexes, stability constants

INTRODUCTION

The presence of traces of cations in diverse processes of animal and human physiology seems to be of considerable importance.[1] Many cations and aminoacids are present at different concentrations in blood plasma. Zinc(II) is one of the most abundant.[2]

Investigations on equilibria between metal ions and aminoacids are an important field due to the role played by cations in biological systems. In particular, zinc(II) is essential in many enzyme classes[3] and has a role in the stabilization of membranes.[4,5] Many common diseases (for instance liver cirrhosis[6–11] and pancreatic insufficiency[12]) lead to zinc deficiency.

Many analytical methods have been proposed for the determination of zinc(II) in biological fluids,[13] blood serum,[14] plasma, blood and urine[15,16] and in plasma, neutrophils, lymphocytes and erythrocytes.[17] All these methods, generally based on electronic or atomic absorption spectrophotometry, can be used to estimate the total content of zinc(II) in a sample, but they are not able to elucidate the different forms (free or complexed with a ligand) under which zinc(II) can exist. The knowledge of the equilibria occurring in solutions containing aminoacids and zinc(II) can be considered interesting from these different points of view.

In previous papers, glycine[18] α- and β-alanine[19,20] were studied as ligands for zinc(II) and the data showed the presence of protonated species. This paper presents

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the results obtained for aspartic and glutamic acids, which, containing two carboxylic groups and one amino group, can act as terdentate ligands towards zinc(II).

The results of studies carried out until 1973 are collected in "Stability Constants"[21,22] and "Critical Stability Constants".[23] More recently, Barrio Diez-Caballero *et al.*,[24] investigated both aspartate and glutamate as ligands towards zinc(II) and explained their potentiometric data by assuming the formation of ZnL, ZnL_2 and ZnHL for both the ligands. Happe[25] and Ishizuka *et al.*,[26] (only for aspartate) found the presence of ZnHL from nmr measurements, whereas Scheidegger *et al.*,[27] proposed the existence of ZnL_3, only for glutamate. On the basis of these results and by taking into account the limited concentration ranges studied, we decided to investigate the behaviour of aspartate and glutamate as ligands towards zinc(II) over a wide concentration range of reagents in order to evaluate the possibility of the existence of the species of general formula $Zn_qH_pL_r$, where $q \geq 1$, $p \geq 0$ and $r \geq 1$, with stability constants $\beta_{q,p,r}$. For this purpose it was planned to study the systems Zn^{II}-aspartate and Zn^{II}-glutamate at 25°C and in 1 M $NaClO_4$ as constant ionic medium, by measuring the electromotive force (e.m.f.) of galvanic cells containing glass and zinc amalgam electrodes. The results are compared with those in the literature and those obtained for glycine[18] and α- and β-alanine.[19,20]

EXPERIMENTAL

Methods

To study equilibria between zinc(II) and aspartate (asp^{2-}) and glutamate (glu^{2-}), respectively, in aqueous solutions, the e.m.f. of the following cells was measured at 25°C

$$(-)\ \text{R.E./Solution S/G.E.}\ (+) \qquad (I)$$

$$(-)\ \text{Zn (Hg)/Solution S/R.E.}\ (+) \qquad (II)$$

where R.E., G.E. and Zn(Hg) are reference, glass and zinc amalgam electrodes, respectively.

Solution S had the following general composition

$$B\,\text{M in } Zn^{II},\ H\,\text{M in } H^+,\ A\,\text{M in L},\ (1\text{–}2B - H)\,\text{M in } Na^+,\ (1 - A)\,\text{M in } ClO_4^-$$

where B and A are the total concentrations of zinc(II) and ligand (L), respectively, and H is the analytical excess of hydrogen ions with respect to 1 M $NaClO_4$, H_2O and asp^{2-} or glu^{2-}, respectively.

In a constant ionic medium, according to Biedermann and Sillén,[28] the activity coefficients can be considered to be constant and concentrations can substitute for activities. Thus the e.m.f. of the cells (*I*) and (*II*) at 25°C and in mV units can be expressed as follows

$$E_I = E^\circ_I + 59.16 \log h + E_j$$

$$E_{II} = E^\circ_{II} - 29.58 \log b - E_j$$

where the constants E°_{I} and E°_{II} and E_j, the liquid junction potential, are determined in the first part of each measurement in the absence of ligands, *i.e.*, when $H = h$ and $B = b$. The free concentrations of zinc(II), b, and hydrogen ions, h, were obtained for each point by means of a procedure similar to that described previously.[18] We found that, under the selected experimental conditions $E_j = -62\,h$.

For each set of e.m.f. measurements, B and H were kept constant, while A and $-\log h$ gradually increased. The range $3 \leq -\log h \leq 9$ was studied because under the selected experimental conditions, at $-\log h \leq 3$, zinc amalgam does not work correctly, whereas at $-\log h \geq 9$, the glass electrode shows deviations with respect to the response of a H_2 electrode (assumed as being correct).

Materials and analysis

Zinc(II) perchlorate, perchloric acid, sodium hydroxide and sodium perchlorate were prepared and analysed as previously described.[29] The zinc amalgam (*ca* 5×10^{-3}% weight) was prepared directly in the titration vessel, by reducing coulometrically the calculated amount of zinc(II) on a mercury cathode. The amalgam preparation and behaviour is reported in ref. 29.

L-Aspartic and L-glutamic acid were prepared and analysed as reported.[30] The apparatus is described in a previous paper,[29] where details on e.m.f. measurements are given as well.

RESULTS AND DISCUSSION

Several series of e.m.f. measurements were performed at different H values (0.025, 0.050, 0.070 and 0.100 M) and B values (0.5, 1.0 and 2.0×10^{-3} M). From the E_I and E_{II} values the free concentrations of zinc(II), b and of protons, h could be calculated. It was possible to calculate η ($=\log(B/b)$) and to present the experimental data in the form η $(-\log h)_{H,B}$ for both systems.

In Figures 1 and 2 the dependence of η on $-\log h$ is shown for aspartate and glutamate, respectively. For both systems, points obtained at different B, but at the same H, fall on the same curve and, therefore, it can be assumed that polynuclear complexes in zinc(II) are negligible ($q = 1$) so that the prevailing species are of the kind ZnH_pL_r, with the relative stability constants $\beta_{1,p,r}$ (defined by the relationship $[ZnH_pL_r] = \beta_{1,p,r} b h^p a^r$), where $p \geq 0$ and $r \geq 1$.

To evaluate the predominant values assumed by p and r and then the $\beta_{1,p,r}$ values, it is necessary to consider the material balance relative to zinc(II), which, by taking into account the mass action law, can be written as follows:

$$\eta = \log (B/b) = \log (1 + \Sigma_p \Sigma_r \beta_{1,p,r}\, h^p\, a^r) \qquad (1)$$

For the elaboration of the data, it is necessary to calculate the free concentration of the ligand, a. It can be obtained from the material balance H, written as follows:

$$H = h + k_1 h\, a + 2k_1 k_2\, h^2 a + 3k_1 k_2\, k_3\, h^3 a + \Sigma_p \Sigma_r p \beta_{1,p,r} h^p a^r \qquad (2)$$

In (*1*) and (*2*) and in equations that follow, hydrolyzed species of zinc(II) are neglected on the basis of the values assumed by b and h and the results of Biedermann.[31] To calculate a from (*2*), it is necessary to know the protonation

constants k_1, k_2 and k_3 for aspartate and glutamate, respectively. Their values, determined under the same experimental conditions, are for aspartate[30] $\log k_1 = 9.625 \pm 0.01$; $\log k_1k_2 = 13.31 \pm 0.02$; $\log k_1k_2k_3 = 15.42 \pm 0.03$ and for glutamate[32] $\log k_1 = 9.52 \pm 0.01$; $\log k_1k_2 = 13.69 \pm 0.02$; $\log k_1k_2k_3 = 16.00 \pm 0.03$.

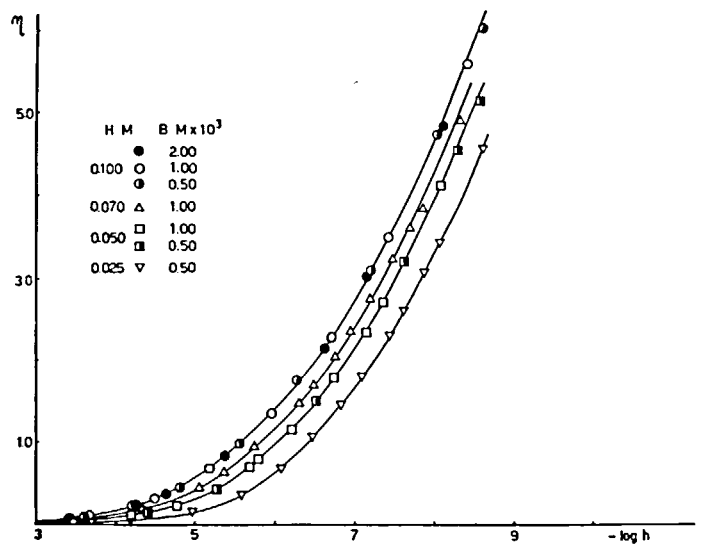

FIGURE 1 Experimental data for the zinc(II)-aspartate system plotted in the form $\eta(=\log B/b)$ *versus* $-\log h$. Curves are calculated by means of the values collected in Table I.

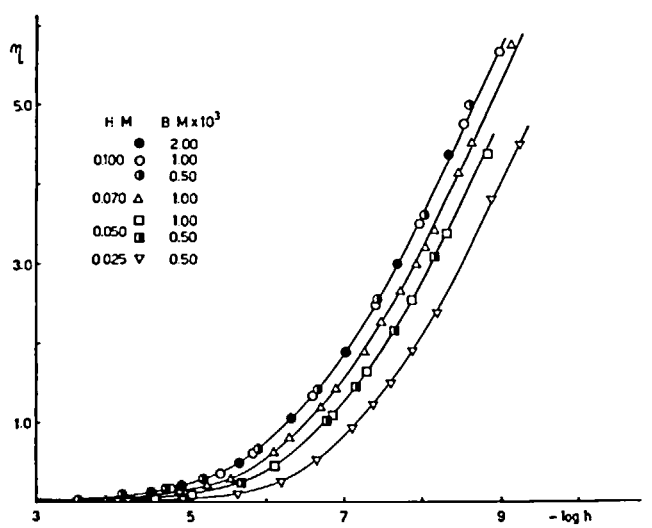

FIGURE 2 Experimental data for the zinc(II)-glutamate system plotted in the form η *versus* $-\log h$. Curves are calculated by means of the values collected in Table I.

Equations for the calculation of a can be derived using the approach indicated by Sillén[33] and applied by Osterberg.[34,35] The values of log a calculated by means of the above procedure coincide within ± 0.02 with those calculated by neglecting the last term of (*2*), because for all the points $B \leq 0.02\,H$. By knowing the log a values, it is possible to plot η as a function of $-\log a$ for aspartate (Fig. 3) and for glutamate (Fig. 4), respectively. It can be seen that for both systems η is an increasing function of H and that protonated species are present, i.e. $p \geq 0$. The procedure followed to obtain the values of p and r and the relative $\beta_{1,p,r}$ values, was similar to that described previously.[30]

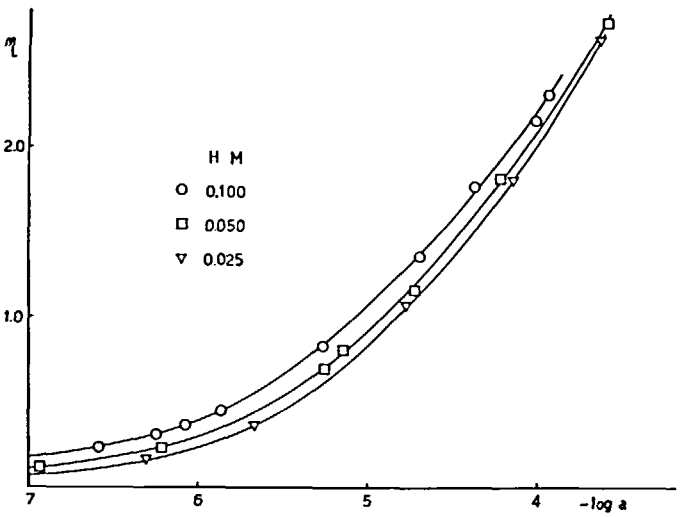

FIGURE 3 The dependence of η on $-\log a$ at different H values for the complexes formed by aspartate. Curves are calculated by means of the constants collected in Table I.

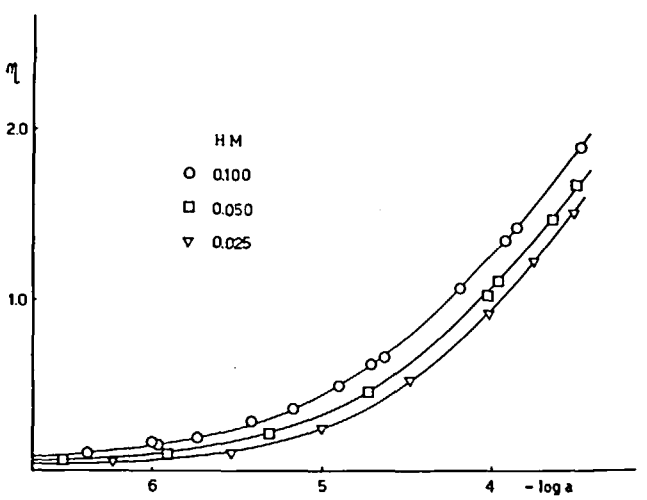

FIGURE 4 The dependence of η on $-\log a$ at different H values, for the complexes formed by glutamate. Curves are calculated by means of the constants collected in Table I.

For the zinc(II)–aspartate system, the experimental data could be accounted for by the assumption of the species ZnL, ZnHL, ZnH_2L, ZnL_2, $ZnHL_2$, ZnH_2L_2, ZnL_3, $ZnHL_3$ and ZnH_2L_3 being present, while for the zinc(II)–glutamate system, the species ZnL, ZnHL, ZnL_2, $ZnHL_2$, ZnL_3, $ZnHL_3$ and ZnH_2L_3 were found. For all species the stability constants $\beta_{1,p,r}$ have been determined and are collected in Table I. In the same Table is reported evidence of the presence of the species ZnH_2L, present at low $-\log h$ values, but not in sufficient amount at $-\log h \geq 3$ to be able to calculate an accurate value of $\beta_{1,2,1}$. The values of the stability constants collected in Table I have been used to calculate the curves drawn in Figures 1–4. The good agreement between points and curve supports the validity of our results.

TABLE I

Proposed values for the stability constants ($\log \beta_{1,p,r}$) for the zinc(II)–aspartate and zinc(II)–glutamate systems.

Species	zinc(II)–aspartate	zinc(II)–glutamate
ZnL	5.64 ± 0.05	4.53 ± 0.05
ZnHL	10.49 ± 0.05	10.25 ± 0.10
ZnH_2L	13.37 ± 0.15	evidence
ZnL_2	9.62 ± 0.06	7.79 ± 0.07
$ZnHL_2$	16.12 ± 0.06	15.44 ± 0.06
ZnH_2L_2	20.55 ± 0.10	
ZnL_3	12.38 ± 0.10	9.41 ± 0.10
$ZnHL_3$	19.87 ± 0.07	18.90 ± 0.06
ZnH_2L_3	26.50 ± 0.07	25.08 ± 0.08

The species and their relative stability constants explain well experimental data obtained by studying equilibria at 25°C and 1.00 M $NaClO_4$ between zinc(II) and aspartate or glutamate. Both ligands are able to form several species with protons. As expected, polynuclear species are not present in any appreciable quantity up to $B = 0.01$ M, because data obtained can be explained with species and constants of Table I. A comparison of the results of this work with those in the literature[21–26] shows that the existence of ZnL and ZnL_2 was suggested by many authors, but only Happe,[25] Ishizuka *et al.*[26] and Barrio Diez-Caballero[24] found ZnHL, both for aspartate and glutamate, and only Scheidegger[27] proposed the existence of $Zn(Glu)_3^{4-}$.

The results of this work show that both aspartate and glutamate are able to form ZnL_3 and ZnHL together with other protonated complexes. The species ZnH_2L found for aspartate, and is present also in the case of glutamate, but it is hard in the latter case to determine the relative $\beta_{1,2,1}$ value. This is probably due to the limited range of its existence ($-\log h \leq 3$) where the response of the zinc amalgam electrode is not ideal. Another kind of measurement is necessary to find a more accurate value of $\beta_{1,2,1}$ and to ascertain if other species (for example ZnH_4L_2) can be present at lower $-\log h$.

In the case of aspartate our results can be compared with those obtained by Barrio Diez-Caballero[24] as regards ZnL and ZnL_2, while the value of $\log \beta_{1,1,1} = 10.49$ is lower than those obtained by other authors,[25,26] probably because of the different experimental conditions. In the case of glutamate (see Table I), it can be seen that the value of $\beta_{1,0,1}$ is higher, while those of $\beta_{1,0,2}$ and $\beta_{1,0,3}$ are lower than the values proposed by Scheidegger *et al.*[27] This effect can be attributed to the limited

concentration range investigated by Scheidegger *et al*;[27] they do not consider the presence of protonated species.

By means of the values of the constants reported in Table I, distribution curves of the complexes found for the zinc(II)–aspartate and zinc(II)–glutamate systems were calculated as a function of $-\log h$ at $A = 0.100$ M and are plotted in Figure 5. It can be observed that both ligands form large amounts of protonated complexes. At $-\log h \sim 7$, species formed with protons reach ~40% of total zinc(II) for aspartate and ~80% for glutamate. Complexes with a high r/p ratio are favoured by high A, while relatively little of ZnH_2L_2 (~8%) is present in the case of aspartate. The same complex is not present in any appreciable quantity in the case of glutamate. ZnH_2L is present for aspartate at low $-\log h$ as expected, and reaches ~7–8% of total zinc(II). Probably, it should exist at higher percentages at lower A and $-\log h$.

The formation of complexes with aspartate starts at lower $-\log h$ than with glutamate. The contribution of the species $ZnHL_3$ is important, especially for glutamate, because in this case, even at $-\log h \sim 8$, it accounts for ~60% of the total zinc(II).

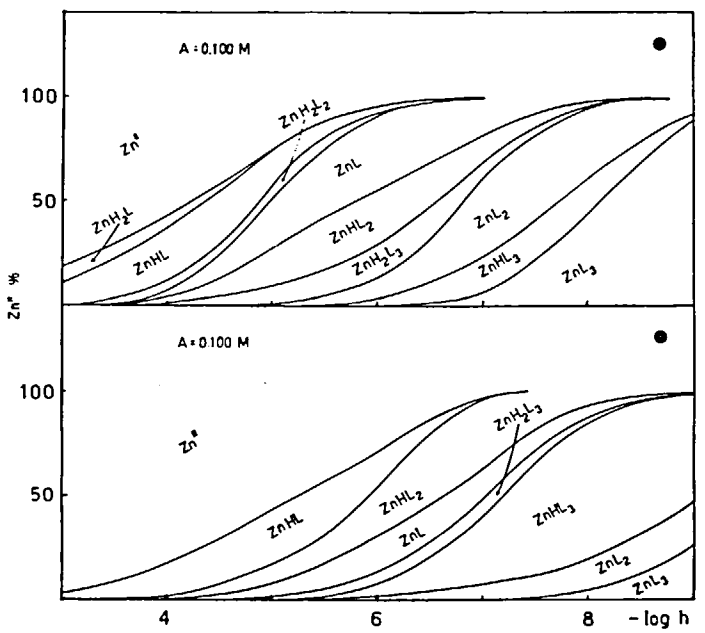

FIGURE 5 Distribution of complexes as a function of $-\log h$ at $A = 0.100$ M and $B = 0.5 \times 10^{-3}$ M for aspartate (a) and glutamate (b). Curves at higher B are practically coincident.

In Table II a comparison of complex formation by aminoacetate, 2-aminopropanoate, 3-aminopropanoate, aspartate and glutamate with zinc(II) is presented. The complexes ZnL, ZnL_2 and ZnL_3 are found for all the ligands, except α- and β-alanine. The species ZnHL is always present. Glycine and α-alanine form only one protonated species (ZnHL), while β-alanine forms ZnHL, $ZnHL_2$, ZnH_2L_2. Aspartate and glutamate are able to form more protonated complexes because of the additional carboxylic group present in the side chain. The formation of species such as $ZnH_{p'}L_{r'}$ with $p' > 2$ at $r' \geq 2$ could be possible, but the high values of H and A

needed, where zinc amalgam does not behave well ($-\log h \leq 3$) and the solubility of both aspartic and glutamic acids did not allow us to test solutions which might contain such species. This subject will be investigated by means of other methods in the near future.

TABLE II

Comparison between the values of log $\beta_{1,p,r}$ for aminoacetate,[18] 2-aminopropanoate,[19] 3-aminopropanoate,[20] aspartate and glutamate as ligands towards zinc(II).

Species	Glycine	α-alanine	β-alanine	Aspartate	Glutamate
ZnL	5.05	4.65	4.30	5.64	4.53
ZnHL	9.78	8.75	11.36	10.49	10.25
ZnH_2L				13.37	evidence
ZnL_2	9.41	9.90	8.00	9.62	7.79
$ZnHL_2$			15.6	16.12	15.44
ZnH_2L_2			22.6	20.55	
ZnL_3	12.1			12.38	9.41
$ZnHL_3$				19.87	18.90
ZnH_2L_3				26.50	25.08

From Table II, it is evident that aspartate is the strongest ligand towards zinc(II), whereas β-alanine is the weakest. This can be explained by considering that β-alanine is not able to form a 5-membered ring in its complexes with cations. On the other hand, the long side chain of glutamate can explain the low stability of its complexes with respect to the other ligands. From the comparison of stabilities of ZnL_2 for the four ligands, it can be deduced that glutamate is the weakest ligand.

Tables III and IV report the stability constants of the complexes formed by aspartate and glutamate, respectively, with copper(II),[30] lead(II),[36] cadmium(II)[32] and zinc(II) (this work). As expected, in both cases the order of stability for the complex ML is copper(II) > lead(II) > zinc(II) > cadmium(II). For ML_2 the stability order changes; for aspartate, zinc(II) and cadmium(II) form more stable complexes than lead(II), whereas for glutamate the order becomes copper(II) > zinc(II) > lead(II) > cadmium(II). Copper(II) and lead(II) are not able to form complexes with $r = 3$, while this is possible for cadmium(II) and zinc(II). On the other hand, lead(II) is generally able to form more stable protonated species than the other cations. This can be attributed to the weakness of the aminic nitrogen to lead(II) bond.

It is important to point out that protonated species are present for all four cations of Table III and IV both for aspartate and glutamate even in neutral or in weakly alkaline solutions ($-\log h \sim 8$). Since several species of different stability are present, a high stability of a single complex does not necessarily mean that the considered ligand will be effective under biological competition conditions. Moreover, knowledge of the mechanism of complex formation between zinc(II) and ligands can shed light upon the bioavailability of zinc(II) from different compounds. One of the conclusions of this paper is that bioavalability[37] can depend on experimental conditions. Together with the ratio ligand/zinc(II), the presence of other organic or inorganic ligands, the hydrogen ion concentration, the presence of other cations and their quantity, can promote the formation of one species rather than another.

TABLE III

Stability constants (log $\beta_{1,p,r}$) for the complexes formed by aspartate with copper(II),[29] lead(II),[35] cadmium(II)[31] and zinc(II).

Species	copper(II)	lead(II)	cadmium(II)	zinc(II)
ML	8.40	6.00	4.54	5.64
MHL	12.40	11.50	10.88	10.49
MH_2L	14.25	14.33	13.70	13.37
ML_2	15.90	8.30	7.85	9.62
MHL_2	20.15	16.30	14.71	16.12
MH_2L_2	24.08	22.35	21.45	20.55
MH_3L_2		25.35		
MH_4L_2	28.26	28.30	28.0	
ML_3			11.0	12.38
MHL_3			18.04	19.87
MH_2L_3			25.15	26.50

TABLE IV

Stability constants (log $\beta_{1,p,r}$) for complexes formed by glutamate with copper(II),[29] lead(II),[35] cadmium(II)[31] and zinc(II).

Species	copper(II)	lead(II)	cadmium(II)	zinc(II)
ML	8.20	4.60	4.02	4.53
MHL	12.40	11.49	10.85	10.25
MH_2L	14.67	14.36	14.40	evidence
ML_2	14.93	6.80	6.97	7.79
MHL_2	19.60	15.18	13.59	15.44
MH_2L_2	23.90	22.20	20.27	
MH_3L_2		26.0		
MH_4L_2		29.8	28.83	
ML_3			8.83	9.41
MHL_3			17.14	18.90
MH_2L_3				25.08
MH_3L_3			31.62	

On this basis, one future study will involve the investigation of equilibria between zinc(II) and other aminoacids of different structure and properties (for example histidine) over a wide concentration range in order to try to find a relation between formed species, their stabilities, and bioavailability of zinc (II).

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