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Speciation of binary complexes of Ca(II), Mg(II) and Zn(II) with L-glutamic acid in DMSO-water Mixtures

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ABSTRACT

Chemical speciation of L-glutamic acid complexes of Ca(II), Mg(II) and Zn(II) in the presence of (0-60% v/v) Dimethylsulfoxide-water mixtures has been studied pH-metrically at 303.0K and at an ionic strength of 0.16 mol L^{-1}*. The active forms of ligand are of* LH_3^+, LH_2, LH^- *and* L^{2-}*. The models containing different number of species were refined by using the computer program MINIQUAD75. The number of species in the models is chosen based on exhaustive modeling. The predominant species detected are* $ML_2H_4^{2+}$, $ML_2H_3^+$, ML_2H_2, ML_2H^- *and* ML_2^{2-}*. The best fit chemical models were arrived at based on statistical parameters. The appropriateness of the models is ascertained by studying the effect of errors in concentrations of ingredients. The trend in variation of stability constants with change in the composition of medium is explained on the basis of electrostatic and non-electrostatic forces. Chemical speciation was discussed based on the distribution diagrams.*

Keywords: Complex equilibria, Chemical Speciation, L-glutamic acid, Stability constants, DMSO.

INTRODUCTION

Speciation study of essential metal ion complexes is useful to understand the composition of active site cavities in biological molecules and the bonding behaviour of protein residues with the metal ions. In biological fluids, metal ions exist in non-exchangeable form as in metalloproteins or loosely bound to some biological ligands as in metal activated proteins. The loosely bound metal ions are in equilibrium with similar metal ions present in the bio-fluids.

Calcium is the most plentiful mineral found in the human body. Calcium plays an important role in cell membranes and as a structural component and also in muscle contraction [1]. It helps in blood clotting, nerve signalling, muscle contraction and relaxation, and the release of certain hormones. The most significant sources of calcium is dairy products. Green leafy vegetables such as broccoli, collards, kale, mustard greens, turnip greens, and Chinese cabbage [2] are also the sources of calcium. Its dietary requirement is 1000 to 1500 mg day $^{-1}$. It can be found naturally in legumes and soy products such as tofu as well as in seeds, nuts, whole grains and most green leafy vegetables [3]. Calcium deficiency for a long period can lead to rickets and osteoporosis, osteonecrosis, hypertension, and other disorders. High calcium intakes or absorption results in the development of kidney stones, but resent research [4, 5] showed that vitamin D is needed to absorb calcium. Calcium prevents muscle cramps, enhances muscle and nerve functioning and helps in improving bone density, etc.

Magnesium is an essential mineral nutrient for life [6, 7] and its ions stabilize all polyphosphate compounds in the cells and are associated with DNA and RNA synthesis. Magnesium is involved in more than 300 metabolic reactions. It helps to regulate blood sugar levels, promotes normal blood pleasure, and is known to be involved in energy metabolism and protein synthesis [8]. Low levels of magnesium in the body indicate human illnesses such as asthma, diabetes, and osteoporosis. Magnesium deficiency can leads to depression. Magnesium is used to prevent muscle cramps, enhance muscle and nerve functioning and helps in improving bone density, etc. Its dietary requirement is about 400 mg day $^{-1}$. The sources of magnesium are spices, nuts, cereals, coffee, cocoa, tea, and vegetables.

Zinc is the second most abundant essential trace metal (2-3g) after iron and it plays vital role in biosystems [9-12]. Its dietary requirement is about 10–20 mg day $^{-1}$. It supports normal growth and development during pregnancy, childhood and adolescence and is required for proper sense of taste and smell [13]. Thousands of proteins contain zinc [14]. The structures of well over 200 zinc-containing enzymes have now been characterized [15]. Its single redox state, the flexibility of its coordination sphere with respect to geometry and number of ligands associated and the kinetic lability of coordinated ligands are responsible for its broad utility within proteins of the body.

L-glutamic acid (Glu) is a non-essential amino acid, and present in many foods either in free form or in peptides and proteins [16]. Adults may ingest 20-35 mg day $^{-1}$ of this amino acid without any apparent ill-effects. It is important in the metabolism of sugars and fats**.** Glu impairs neuronal calcium extrusion while reducing sodium gradient [17, 18]. Glu acts as a neurotransmitter for the central nervous system, the brain and spinal cord, as a precursor of γ-aminobutyric acid 19] and as excitatory transmitter [20]. Because of its capacity to induce depolarizing effects, it is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers 21, 22]. The interaction of the side chain of Glu with metal ions is of importance in metalloenzymes. Dimethylsulphoxide (DMSO) is a dipolar aprotic solvent and it has high polarity combined with a high dielectric constant.

Hence speciation of complexes of Ca(II), Mg(II) and Zn(II) with Glu in DMSO-water mixtures has been undertaken based on their involvement in various physiological reactions. Protonation equilibria of L-glutamic acid in DMSO-water mixtures have been already reported [23].

MATERIALS AND METHODS

Materials

0.05 mol L^{-1} solution of L-glutamic acid (Merck, Germany) was prepared in triple-distilled deionised water by maintaining 0.05 mol L^{-1} hydrochloric acid to increase the solubility. DMSO (Qualigens, India) was used as received. 0.2 mol L^{-1} Hydrochloric acid (Qualigens, India) was prepared. 2 mol L^{-1} Sodium chloride (Merck, India) was prepared to maintain the ionic strength in the titrand. Solutions of Ca(II), Mg(II) and Zn(II) chlorides (0.05 mol L^{-1}) were prepared by dissolving G.R. Grade (E-Merck, Germany) salts in triple distilled water maintaining 0.05 mol L^{-1} acid (HCl) to suppress the hydrolysis of metal salts. 0.4 mol L^{-1} Sodium hydroxide (Merck, India) was prepared. All the solutions were standardized by standard methods. To assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification [24]. The strengths of alkali and mineral acid were determined using the Gran plot method [25].

Procedure

The titrimetric data were obtained by using calibrated ELICO (Model LI-120) pH meter (readability 0.01). The glass electrode was equilibrated in a well stirred DMSO-water mixture (0-60% v/v) containing inert electrolyte for several days. The effect of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor. For the determination of stability constants of binary species, initially, strong acid was titrated against alkali at regular intervals to check the complete equilibration of the glass electrode. Then, the calomel electrode was refilled with DMSO-water mixture of equivalent composition as that of the titrand. All the titrations were performed in media containing varying concentrations of DMSO-water mixtures (0-60 % v/v) pH metrically at 303.0±0.1 K. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 cm^3. Titrations with different metal-to-ligand ratios (1.0:2.5, 1.0:3.75 and 1.0:5.0) were carried out with 0.4 mol L^{-1} sodium hydroxide.

Modeling Strategy

The computer program SCPHD [26] was used to calculate the correction factor. By using pH metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75 [27] which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and protonation constants of Glu were fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on the basis of electrostatic/non-electrostatic, solute-solute and solute-solvent interactions.

RESULTS AND DISCUSSION

The results of exhaustive modeling for the Mg(II)-Glu in 10% DMSO-water mixture are given in Table 1.

Table 1: Exhaustive modeling of Mg(II)–Glu complexes in 10.0% v/v DMSO-water mixture pH range = 3.0-11.0; Number of points = 66.

Model number	log β_{mlh}(SD)				U_{corr} x10^8	χ^2	Skewness	Kurtosis	R-factor
	121	122	123	124					
1	15.03(20)	----	----	----	14.52	70.44	-1.21	4.69	0.0299
2	----	24.36(14)	----	----	15.54	55.09	-0.92	5.06	0.0309
3	----	----	28.34(75)	----	19.23	71.33	-2.16	7.01	0.0345
4	----	----	----	33.7(30)	18.15	144.14	-2.39	7.86	0.0335
5	14.82(23)	24.23(20)	----	----	13.76	41.84	-0.43	3.01	0.0289
6	15.03(20)	----	28.34(65)	----	14.57	32.87	-1.23	4.76	0.0298
7	15.03(19)	----	----	33.07(26)	13.45	74.40	-1.43	5.54	0.0286
8	----	24.60(12)	29.39(22)	----	13.34	41.19	-0.53	4.82	0.0285
9	----	24.97(8)	----	34.16(10)	6.39	43.70	-1.38	7.75	0.0197
10	----	----	28.85(55)	33.22(33)	18.13	195.86	-2.45	8.18	0.0332
11	14.83(23)	24.60(15)	29.39(21)	----	11.78	27.86	0.22	2.86	0.0265
12	15.22(14)	25.17(9)	----	34.33(10)	3.95	66.57	-0.12	4.67	0.0154
13	15.03(19)	----	28.85(47)	33.22(29)	13.30	118.53	-1.48	-1.48	0.0282
14	----	25.02(6)	29.99(14)	34.27(11)	4.43	78.53	-1.86	13.27	0.0163
15	15.32(9)	25.26(6)	30.21(9)	34.48(7)	1.61	84.18	1.27	9.69	0.0097

U_{corr}= U/ (NP-m); NP = Number of points; m = number of stability constants; SD = Standard deviation

The models indicate better statistics as the number of species was increased, confirming better fit. There was no further improvement in the fit on inclusion of some more species in the model containing MgL_2H, MgL_2H_2, MgL_2H_3, MgL_2H_4. This indicates that the exhaustive model appropriately fit the experimental data. Such exhaustive modeling was performed for all the systems. The results of the best fit models that contain the stoichiometry of the complex species and their overall formation constants along with some of the important statistical parameters are given in Table 2.

Very low standard deviation in overall stability constants (log β) signifies the precision of these constants. The small values of U_{corr} (sum of squares of deviations in concentrations of ingredients at all experimental points corrected for degrees of freedom), small values of mean, standard deviation and mean deviation for the systems are validated by the residual analysis.

Residual analysis

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on model parameters) are assumed to follow Gaussian distribution. When the data are fit into the models, the residuals should ideally be equal to zero. If statistical measures of the residuals and the errors assumed in the models are not significantly different from each other, the model is said to be adequate. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random following normal distribution in the least squares analysis, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and R-factor. These statistical parameters show that the best fit models portray the metal-ligand species in DMSO-water mixtures, as discussed below.

Table 2: Parameters of best fit chemical models of Ca(II), Mg(II) and Zn(II)-Glu complexes in DMSO-water mixtures

% v/v DMSO	log β_{mlh} (SD)					pH-range	NP	U_{corr} x10^8	χ^2	Skew-ness	Kurto-sis	R-factor
	120	121	122	123	124							
					Ca(II)							
00.0	----	15.04(12)	25.27(6)	30.01(11)	34.18(9)	3.0-11.0	68	1.73	56.31	0.09	7.31	0.0103
10.0	----	15.89(16)	26.20(11)	31.50(14)	35.90(13)	3.5-11.0	57	1.55	27.88	-0.07	4.17	0.0102
20.0	----	15.82(24)	25.73(20)	30.88(23)	35.44(20)	3.0-11.0	68	3.47	59.53	0.97	6.74	0.0136
30.0	----	15.12(29)	25.11(37)	32.07(28)	36.86(27)	3.5-10.0	43	4.10	15.19	1.22	5.47	0.0138
40.0	----	15.66(26)	25.64(20)	31.25(24)	35.60(22)	2.5-11.0	86	7.29	91.71	-0.70	7.40	0.0162
50.0	----	15.94(23)	25.73(21)	31.54(25)	36.14(32)	3.0-11.0	43	2.56	42.84	-1.26	9.69	0.0130
60.0	----	15.79(29)	25.12(71)	31.79(37)	36.56(84)	4.1-11.0	19	1.05	17.25	0.22	3.68	0.0080
					Mg(II)							
00.0	----	14.26(11)	24.21(5)	28.90(9)	32.57(11)	3.0-11.0	69	1.46	94.49	0.72	8.49	0.0097
10.0	----	15.32(9)	25.26(6)	30.21(9)	34.48(7)	3.0-11.0	66	1.61	84.18	1.27	9.69	0.0097
20.0	----	15.15(10)	24.74(11)	29.97(14)	34.31(12)	3.0-11.0	68	1.86	45.65	0.42	6.52	0.0102
30.0	----	15.02(30)	24.28(70)	30.58(34)	35.36(26)	3.0-11.0	68	9.44	80.24	1.30	6.23	0.0213
40.0	----	15.40(19)	25.11(20)	30.83(21)	35.47(21)	3.0-11.0	69	5.05	67.82	-0.01	5.13	0.0153
50.0	----	16.92(9)	26.10(9)	31.65(10)	36.18(12)	3.0-10.0	63	1.32	36.99	0.35	6.01	0.0073
60.0	----	16.85(10)	26.05(12)	31.98(13)	36.74(17)	3.0-10.0	63	1.85	56.29	0.25	5.07	0.0083
					Zn(II)							
00.0	9.31(13)	17.34(16)	23.67(44)	28.47(31)	32.34(37)	3.0-10.0	58	0.78	36.30	0.28	3.50	0.0065
10.0	9.56(10)	17.56(14)	23.70(58)	28.93(28)	33.14(25)	4.0-9.50	41	0.44	20.67	1.26	5.97	0.0055
20.0	10.31(13)	18.59(15)	25.57(20)	30.75(18)	35.05(18)	4.0-9.50	42	0.20	13.81	0.43	4.20	0.0036
30.0	9.61(28)	18.53(31)	25.58(47)	31.54(34)	36.14(34)	4.0-10.0	47	1.27	39.82	0.35	9.47	0.0088
40.0	10.71(53)	19.42(55)	26.66(61)	32.38(59)	37.38(58)	4.0-10.0	48	0.95	34.22	0.73	6.29	0.0073
50.0	10.66(17)	18.63(22)	25.43(38)	31.12(37)	36.03(48)	4.5-10.0	48	0.79	14.67	0.13	3.31	0.0069
60.0	10.74(15)	18.86(20)	25.87(31)	31.91(29)	37.12(35)	4.5-10.0	48	0.74	24.33	0.30	3.68	0.0065

χ^2 *Test*

χ^2 is a special case of gamma distribution whose probability density function is an asymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the χ^2 calculated is less than the table value, the model is accepted.

Crystallographic R-test

Hamilton's R-factor ratio test [28] is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method, the readability of pH meter is taken as the R_{limit} which represents the upper boundary of R beyond which the model bears no significance. When these are different numbers of species the models whose values are greater than R-table are rejected. The low crystallographic R-values given in Table 2 indicate the sufficiency of the model.

Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 2 are between -1.26 and 1.22 for Ca(II), -0.01 and 1.30 for Mg(II) and 0.13 and 1.26 for Zn(II). These data evince that the residuals form a part of normal distribution, hence, least-squares method can be applied to the present data.

Kurtosis

It is a measure of the peakedness of the error distribution near a modal value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern.

Effect of systematic errors on best fit model

In order to rely upon the best fit chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the influential parameters like concentrations of alkali, mineral acid, ligand, metal and volume (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal > volume. Some species were even rejected when errors were introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the appropriateness of the experimental conditions and choice of the best fit models.

Effect of solvent

The DMSO-water mixtures are the combination of aprotic and protic solvents with wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The cosolvent induces increased basicity of DMSO-water mixtures and increases the stabilization of protons. At the same time the coordinating solvent (DMSO) competes with the ligand for coordination with the metals. This decreases the stability of the complexes. Hence, the stability of the complex is expected to either increase or decrease. The variation of overall stability constant values with cosolvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's [29] classical treatment holds good in accounting for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constants. The trends of stability constant (log β) values of complexes of Glu with 1/D (D is the dielectric constant of the medium) of DMSO-water mixture are given Figure 1. The trend is almost linear which indicates that the dielectric constant or long range interactions are responsible for the stability trend. This linear increase indicates the dominance of the structure-forming nature of DMSO over the complexing ability.

Table 3: Effect of errors in influential parameters on Mg–Glu complex stability constants 10% v/v DMSO-water mixture

Ingredient	% Error	log β_{mlh}(SD)			
		121	122	123	124
	0	15.32(9)	25.26(6)	30.21(9)	34.48(7)
Alkali	-5	Rejected	27.05(19)	36.49(20)	41.43(26)
	-2	16.02(30)	26.46(20)	31.91(24)	36.40(22)
	2	14.20(17)	Rejected	27.72(92)	Rejected
	5	Rejected	Rejected	Rejected	Rejected
Acid	-5	Rejected	Rejected	28.06(1.86)	Rejected
	-2	14.70(7)	23.96(8)	28.66(12)	Rejected
	2	15.85(24)	26.13(15)	31.44(20)	35.93(18)
	5	Rejected	26.52(14)	35.56(20)	40.36(26)
Ligand	-5	14.28(12)	23.46(25)	28.08(52)	32.81(15)
	-2	14.99(8)	24.79(6)	29.65(10)	33.95(8)
	2	15.59(10)	25.66(7)	30.71(9)	34.96(8)
	5	15.85(16)	26.15(10)	31.37(13)	35.60(12)
Metal	-5	15.37(9)	25.32(6)	30.28(9)	34.55(8)
	-2	15.34(9)	25.28(6)	30.24(9)	34.50(7)
	2	15.30(9)	25.24(6)	30.19(9)	34.45(7)
	5	15.28(9)	25.21(6)	30.15(9)	34.41(7)
Volume	-5	15.22(9)	25.18(6)	30.13(9)	34.40(7)
	-2	15.28(9)	25.23(6)	30.18(9)	34.45(7)
	2	15.36(8)	25.29(6)	30.25(8)	34.51(7)
	5	15.42(8)	25.34(6)	30.29(8)	34.55(7)

Distribution Diagrams

Glu has two dissociable protons and one amino group which can associate with a proton. It exists as LH_3^+ at low pH and gets deprotonated with the formation of LH_2, LH^- and L^{2-} successively with increase in pH, in the pH ranges 2.0-6.0, 3.0-11.0 and above 8.0, respectively[24]. Hence, the plausible binary metal-ligand complexes can be predicted from these data.

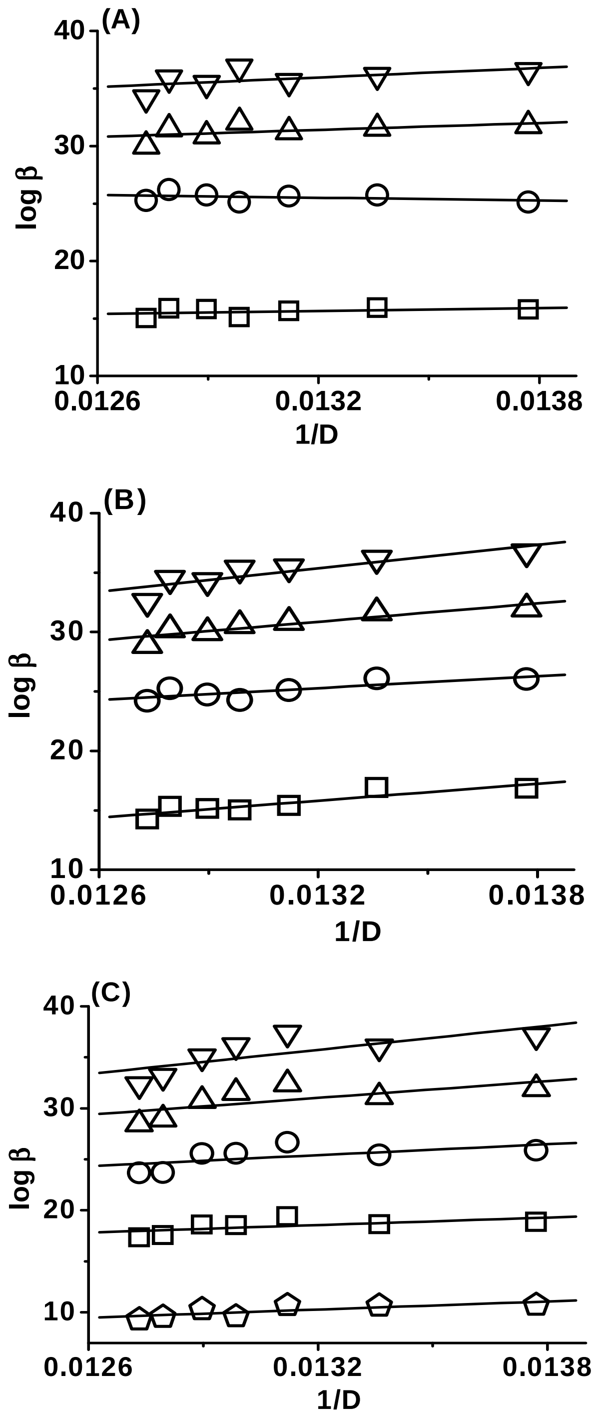
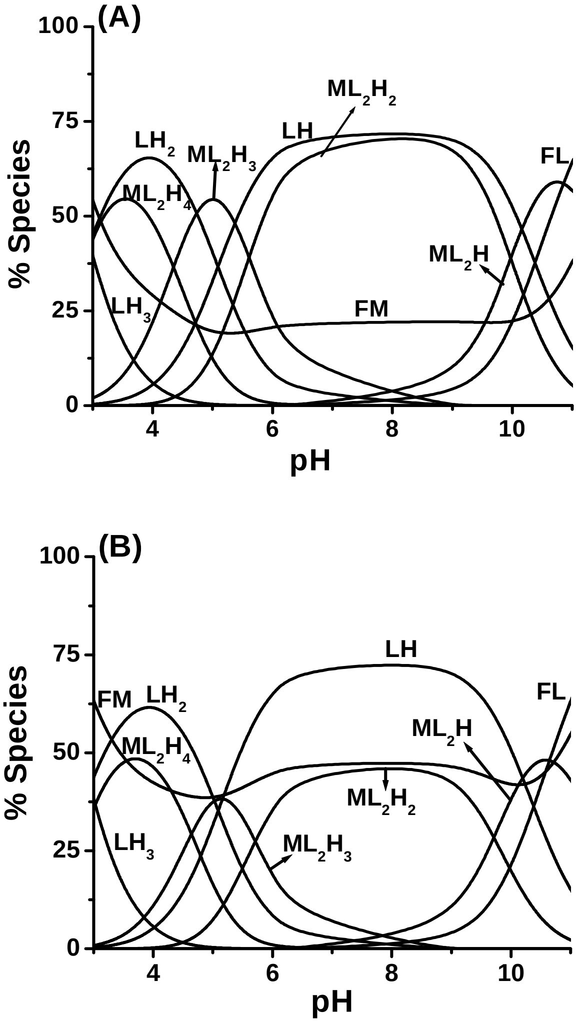


Figure 1: Variation of stability constants of Metal-Glu complexes with reciprocal of dielectric constant (1/D) of DMSO-water mixtures: (A) Ca(II), (B) Mg(II) and (C)Zn(II); (⌂) logβ_{ML2}, (□) logβ_{ML2H}, (○) logβ_{ML2H2}, (Δ) logβ_{ML2H3} and (∇) logβ_{ML2H4}

The present investigation reveals the existence of ML_2H, ML_2H_2, ML_2H_3 and ML_2H_4 for Ca(II) and Mg(II) and ML_2H, ML_2H_2, ML_2H_3, ML_2H_4 and ML_2 for Zn(II). The formation of various complex species is shown in the following equilibria. Typical distribution diagrams in DMSO-water mixtures are shown in Figure 2.

$M(II) + 2LH_3^+ \rightleftharpoons ML_2H_4^{2+} + 2H^+$ 1
$M(II) + 2LH_3^+ \rightleftharpoons ML_2H_3^+ + 3H^+$ 2
$M(II) + 2LH_3^+ \rightleftharpoons ML_2H_2 + 4H^+$ 3
$ML_2H_4^{2+} \rightleftharpoons ML_2H_3^+ + H^+$ 4
$ML_2H_3^+ \rightleftharpoons ML_2H_2 + H^+$ 5
$ML_2H_3^+ \rightleftharpoons ML_2H^- + 2H^+$ 6
$M(II) + 2LH_2 \rightleftharpoons ML_2H_2 + 2H^+$ 7
$ML_2H_2 \rightleftharpoons ML_2H^- + H^+$ 8
$ML_2H^- \rightleftharpoons ML_2^{2-} + H^+$ 9
$M(II) + 2LH^- \rightleftharpoons ML_2^{2-} + 2H^+$ 10



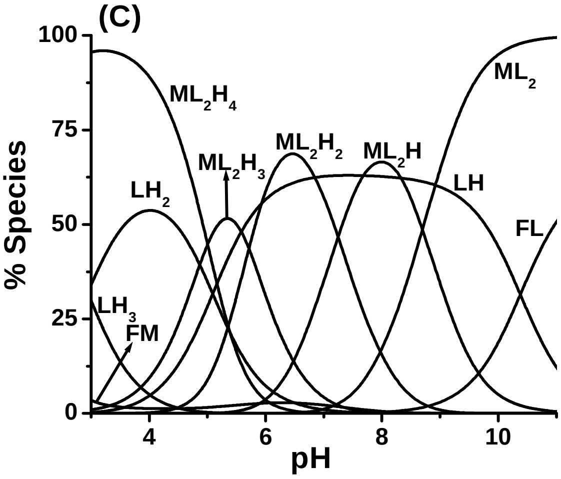


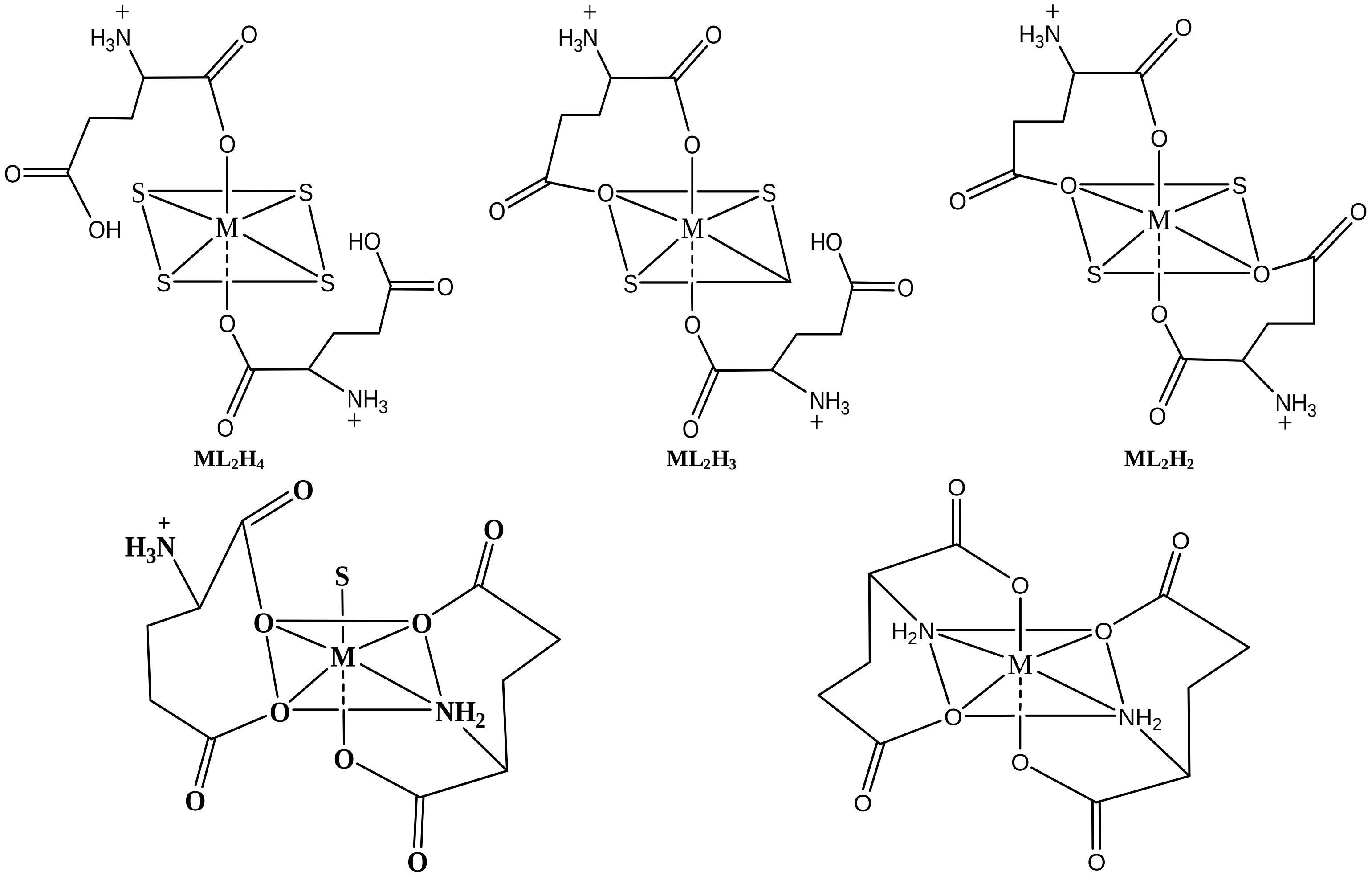
Figure 2: Distribution diagrams of binary complexes of Glu in 40% v/v DMSO-water mixture. (A) Ca(II), (B) Mg(II) and (C) Zn(II)

They indicate that the binary complexes of Ca(II), Mg(II) and Zn(II) are formed in the pH range 2.0-11.0. $ML_2H_4^{2+}$ is the predominant species which is formed from M(II) and LH_3^+ form of ligand at a pH below 5.0 (Equilibrium 1). The species $ML_2H_3^+$ may be formed from free metal and LH_3^+ or by the deprotonation of $ML_2H_4^{2+}$. Figure 2 indicates that at lower pH, $ML_2H_3^+$ is formed from free metal and LH_3^+ (Equilibrium 2) and at higher pH, it is formed by the deprotonation of $ML_2H_4^{2+}$ (Equilibrium 4). In the same manner, the species ML_2H_2 may be formed either from FM and LH_3^+ / LH_2 (Equilibria 3 and 7) or deprotonation of $ML_2H_3^+$ (Equilibrium 5). The interaction of free metal ion with LH_3^+ or LH_2 at lower pH (Equilibria 3 and 7) and deprotonation of $ML_2H_3^+$ at higher pH (Equilibrium 5) result in the formation of ML_2H_2 species. Higher concentration of ML_2H_2 than that of $ML_2H_3^+$ indicates that ML_2H_2 is formed from Equilibria 3, 5 and 7. The species ML_2H^- may be formed from deprotonation of either $ML_2H_3^+$ or ML_2H_2 (Equilibria 6 and 8). However the chances of formation of ML_2H^- from deprotonation of ML_2H_2 are more than the deprotonation of $ML_2H_3^+$, since the concentrations of $ML_2H_3^+$ is negligible in the pH range of formation of ML_2H^-. Hence it is concluded that the species ML_2H^- is formed from deprotonation of ML_2H_2 rather than $ML_2H_3^+$.

In the case of Zn(II), the species ML_2^{2-} may be formed from either M(II) and LH^- or deprotonation of ML_2H^- (Equilibria 9 and 10). From concentration considerations with incrasing pH, the deprotonation of ML_2H^- is more prevalent than interaction of M(II) and LH^- form of ligand. Hence ML_2^{2-} is formed from deprotonation of ML_2H^-.

Structures of complexes

The literature suggests that Ca(II), Mg(II) and Zn(II) complexes shall be octahedral. Amino nitrogen atom can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ion for this second donor site. This situation results in the simultaneous existence of a number of equilibria producing an array of successively protonated complexes, which are detected in the present study. Amino nitrogen and carboxyl oxygen of Glu participate in bonding with metal ions. The plausible structures of possible complex species are given in Figure 3.



ML_2H_4 **ML_2H_3** **ML_2H_2**

ML_2H **ML_2**

Figure 3: Structures of Glu complexes of Ca(II), Mg(II) and Zn(II). S is either solvent or water molecule.

CONCLUSION

The following conclusions have been drawn from the modeling studies of the speciation of binary complexes of Ca(II), Mg(II), and Zn(II) with Glu in DMSO-water mixture.

1. L-Glutamic acid forms both protonated and unprotonated complexes in the pH range 2.5-11.0.
2. The binary species detected are CaL_2H, CaL_2H_2, CaL_2H_3, CaL_2H_4, MgL_2H, MgL_2H_2, MgL_2H_3, MgL_2H_4, ZnL_2, ZnL_2H, ZnL_2H_2, ZnL_2H_3 and ZnL_2H_4. These models are validated by statistical treatment of data.
3. The linear variation of stability constants as a function of dielectric constant of the medium indicates the dominance of electrostatic forces over non-electrostatic forces.
4. Some species are stabilized due to electrostatic interactions and some are destabilized due to the decreased dielectric constant.
5. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid > ligand > metal > volume.

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REFERENCES

[1] C.M. Weaver and R.P. Heaney, Calcium. In: M.E. Shils, M. Shike, A.C. Ross, B. Caballero and R. J. Cousins, editors. Modern nutrition in health and disease, 10th Ed, M.D. Baltimore: Lippincott Williams & Wilkins, **2006,** 194-210.
[2] Institute of Medicine, Food and Nutrition Board Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary, **1997.**
[3] U.S. Department of Agriculture. Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 16. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp, **2003**.
[4] G. Bihl, A. Meyers. *Lancet*, **2001**, *358*, 651–656.
[5] W.D. Hall, M. Pettinger, A. Oberman, N.B. Watts, K.C. Johnson, E.D. Paskett, M.C. Limacher and J. Hays, *Am. J. Med. Sci*. **2001**, *322*, 12–18.
[6] J. Leroy, *Cr. Soc. Biol.*, **1926**, *94*, 431-433.
[7] H. Marschner, Mineral Nutrition in Higher Plants. Academic Press, San Diego, **1995**.
[8] N.E.L. Saris, E. Mervaala, H. Karppanen, J.A. Khawaja and A. Lewenstam, *Clin. Chim. Acta* **2000**, *294*, 1-26.
[9] Y. L. Lin, C. Lim, *J. Am. Chem. Soc***. 2004,** *126*, 2602-2612.
[10] E. H. Cox, G. L. McLendon, *Curr. Opin. Chem. Biol.* **2000,** *4*, 162-165.
[11] J. H. Laity, B. M. Lee, P. E. Wright, *Curr. Opin. Struct. Bio.* **2001,** *11***,** 39-46.
[12] T. Dudev, C. J. Lim, *J. Chin. Chem. Soc*. **2003,** *50*, 1093-1102.
[13] A.S. Prasad, F.W. Beck, S.M. Grabowski, J. Kaplan and R.H. Mathog, *Proc. Assoc. Am. Physicians,* **1997**, *109*, 68-77.
[14] B. L. Vallee and K. H. Falchuk, *Physiol. Rev*. **1993**, *73*, 79-118.
[15] D. S. Auld, *Biometals*, **2001**, *14*, 271-313.
[16] H. Tapiero, G. Mathe, P. Couvreur and K. D. Tew, *Biomed. Pharmacother.* **2002**, *56***,** 446–457.

[17] A. V. Fitsamakis and A. Michael, *Toxicol. Appl. Pharm.* **2004**, *204***,** 343–354.
[18] L. Struzynska and G. Sulkowski, *J. Inorg. Biochem.* **2004**, *98***,** 951–958.
[19] V. Sauvinet, S. Parrot, N. Benturquia, E. Bravo-Moraton, B. Renaud and L. Denoroy, *Electrophoresis*, **2003**, *24***,** 3187-3196.
[20] K.Ajito, C. Han and K. Torimitsu, *Anal. Chem*. **2004**, *76***,** 2506-2510.
[21] P. Ody; *The Complete Medicinal Herbal*, Dorling Kindersley, London, **2003**.
[22] D. Colbert; *Toxic Relief*, Siloam Publishing, **2003**.
[23] S. Raju, B. A. Kumar, G. P. Raju, K. B. K. Naik and G. N. Rao, *Curr. Chem. Res. India* (in press) **2011**.
[24] R.S. Rao and G.N. Rao, Computer Applications in Chemistry, Himalaya Publishing House, Mumbai, **2005**, 277-352.
[25] G. Gran, *Analyst*, **1952**, *77*, 661-671.
[26] G. N. Rao, Ph.D. Thesis, Andhra University, Visakhapatnam, India. **1989**
[27] P. Gans, A. Sabatini and A. Vacca, *Inorg. Chim. Acta* **1976**, *18***,** 237-239.
[28] W.C. Hamilton, *Acta Cryst*. **1965,** *18*, 502-510.
[29] M. Born, *Z. Phys.* **1920**, *1*, 45-57.