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Chemical Speciation of Binary Complexes of Ca(II), Mg(II) and Zn(II) with phenylalanine in ethylene glycol-water mixture

T. Sreevaram1, Dr. N. Vijaya Kumar2 and Dr. B.B.V. Sailaja*
1. Lecturer in Chemistry, Govt. Arts College (A), Rajahmundry, E.G.Dt., A.P.
2. Lecturer in Chemistry, DNR College (A), Bhimavaram, W.G.Dt., A.P.
*Corresponding author: Associate Professor, School of Chemistry, Andhra University, Visakhapatnam-530003, INDIA.

Abstract : *Chemical speciation of Ca(II), Mg(II) and Zn(II) complexes of Phenylalanine in 0.0-60.0 % v/v ethylene glycol-water mixtures has been studied pH metrically maintaining an ionic strength of 0.16 M at 303 K. The active forms of ligand are* LH_2^+*, LH and* L^-*. The predominant species detected are ML,* ML_2 *and* ML_2H_2*. Models containing different numbers of species were refined by using the computer program MINIQUAD75. The best-fit chemical models were arrived at based on statistical parameters. The trend in variation of complex stability constants with change in the dielectric constant of the medium is explained on the basis of electrostatic and non-electrostatic forces.*

Key Words: *Complex equilibria, Chemical speciation, Ethylene glycol, Metals, Phenylalanine.*

Introduction

The speciation study of metal ion complexes is useful to understand the role played by the active site cavities in biological molecules and the bonding behavior of protein residues with the metal ion. The species refined and their relative concentrations under the experimental conditions represent the possible forms of amino acids in biofluid.

Calcium is the fifth most abundant metal in the Earth's crust (4.1%). It is not found uncombined in nature, but occurs abundantly as limestone (calcium carbonate), gypsum (calcium sulfate), fluorite (calcium fluoride) and apatite (calcium chloro- or fluoro-phosphate). Calcium metal is prepared commercially by heating lime with aluminium in a vacuum. Calcium compounds are widely used. Gypsum (calcium sulfate) is used by builders as a plaster and by nurses for setting bones, as 'plaster of Paris'.[1-6] All foods of vegetable origin contain small but useful amounts of calcium. Loss of calcium from the body occurs through urine and faeces. The mechanism is thought to be the high phosphate (and perhaps sulphate) content of protein, which complexes calcium in the renal tubule and enhances its excretion.[7-9] Similarly, each gram of increased sodium intake increases urinary calcium excretion by 15 mg/d. Here the mechanism is through direct competition between the two ions for tubular reabsorption.[10, 11] Vitamin D is necessary for intestinal absorption, making Vitamin D–fortified milk a very well-absorbed form of calcium. Vitamin C and lactose (the sugar found in milk) enhance calcium absorption, whereas meals high in fat or protein may decrease absorption. Over the long term, dietary deficiency eventually depletes bone stores, rendering the bones weak and prone for fracture. When calcium levels fall too low, nerve and muscle impairments, abnormal heart beat will result. Other symptoms of hypocalcemia include numbness and tingling in the fingers, muscle cramps, convulsions, lethargy, poor appetite, and abnormal heart rhythms.[12] Calcium supplements (without co-administered vitamin D) are associated with an increased risk of myocardial infarction.[13] If left untreated, calcium deficiency leads to death.

Magnesium is the eighth most abundant element in the Earth's crust, but does not occur uncombined in nature. It is prepared by reducing magnesium oxide with silicon, or by the electrolysis of molten magnesium chloride. Magnesium sulfate is sometimes used as a mordant for dyes. Magnesium hydroxide is added to plastics to make them fire retardant. Magnesium oxide is used to make heat-resistant bricks for fireplaces and furnaces. Magnesium hydroxide (milk of magnesia), sulfate (Epsom salts), chloride and citrate are all used in medicine.[1- 6] It is a free divalent cation and so it is deeply and intrinsically woven into cellular metabolism. Mg^{2+} is used as a signaling ion and much of nucleic acid biochemistry requires Mg^{2+}, including all reactions which require release of energy from ATP [14-17] for mechanical work including muscle contraction and relaxation. In photosynthetic organisms Mg^{2+} has the additional vital role as the coordinating ion in the chlorophyll molecule. In biological systems, only manganese (Mn^{2+}) is readily capable of replacing Mg^{2+}, but only in a limited circumstance as Mn^{2+} is very similar to Mg^{2+} in terms of its chemical properties, including inner and outer shell complexation.[14] Zinc makes up about 75 ppm (0.007%) of the earth's crust, making it the 24th most abundant element. Sphalerite is the most important ore of zinc.[18] The most important application of zinc and the largest use of the metal is as an anticorrosion agent.[19]

Galvanization, which is coating of iron or steel as protection against corrosion, is the most familiar form of using zinc in this way. Other applications are in batteries and alloys, such as brass. Its compounds are used as micro fertilizers.[19] Zinc is required for the optimum function of as many as 300 enzymes[20] The role of zinc in these metalloenzymes includes participation in catalytic functions, maintenance of structural stability and regulatory functions.[21] The crystal structure has been identified for 12 zinc enzymes[20] including alcohol dehydrogenase, carboxy peptidases A and B, alkaline phosphatase and carbonic anhydrases I and II. The zinc binding sites vital to catalysis include three amino acid residues and an activated water molecule. The amino acids can include combinations of histidine, glutamine, aspartate or cysteine.[22] The coordination geometry has been found to depend on the type of ligand that is bound to Zn(II) and the solvent accessibility of the metal binding site.[23] Another critical function of zinc in biological systems is the regulation of DNA and RNA synthesis.[24-27] Zinc can also act as an antioxidant by interaction with sulfhydryl groups of macromolecules, there by inhibiting their oxidation and by competing for binding sites on membranes with metals such as copper and iron.[28] Another property of zinc which has been hypothesized to play a role in normal physiological function is its capacity to stabilize membranes.[29, 30]

Hence, the speciation of Ca(II), Mg(II) and Zn(II) complexes with amino acid Phenylalanine (Phe) has been studied. Phenylalanine is found naturally in the breast milk of mammals. It is used in the manufacture of food and drink products and sold as a nutritional supplement for its reputed analgesic and antidepressant effects. It is a direct precursor to the neuromodulator phenylethylamine, a commonly used dietary supplement. The protonation constants of Phe and Mal in EG- and AN- water mixtures were reported earlier.[31, 32]

Experimental

2.1 Materials

Ethylene glycol (EG) (Merck, Mumbai) was used as received. Aqueous solutions of Phenylalanine and sodium chloride (E-Merck, Germany) were prepared. Solutions of Ca(II), Mg(II), Zn(II) chlorides were prepared. To increase the solubility of Phe and to suppress the hydrolysis of the metal salts, mineral acid concentration in the above solutions was maintained at 0.05 M. 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 (ANOVA). The strength of alkali was determined using the Gran plot method.[33, 34]

2.2 Apparatus

The alkalimetric data were obtained with a ELICO (Model L1-120) pH meter (readability 0.01), which was calibrated with 0.05 M potassium hydrogen phthalate in acidic region and 0.01 M borax solution in basic region. The glass electrode was equilibrated in a well-stirred EG-water mixture containing inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of EG (0-60.0 % v/v) maintaining an ionic strength of 0.16 M with sodium chloride at 303.0 ± 0.1 K. Effect of variations in asymmetry potential, 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.[35]

2.3 Procedure

For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with EG-water mixture of equivalent composition as that of titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 mL. Titrations with different ratios (1:2.5, 1:3.5, 1:5) of metal-to-ligand were carried out with 0.4 M sodium hydroxide. Other experimental details are given elsewhere.[36]

2.4 Modeling strategy

The computer program SCPHD[37] was used to calculate the correction factor. By using the pH metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75[38], which exploits the advantage of the constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of binary systems, the correction factor and the protonation constants of phenylalanine[31] were fixed. The variation of stability constants with the dielectric constant of the medium was analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

Results and Discussion

Alkalimetric titration curves in EG-water mixtures revealed that the acido-basic equilibria of Phenylalanine were active in the pH range 2.0-10.2. Based on the active forms of the ligand in this pH range, models containing various numbers and combinations of complex species were fed to MINIQUAD75 along with the alkalimetric titration data. Exhaustive modeling was performed for a typical system (Table 1).

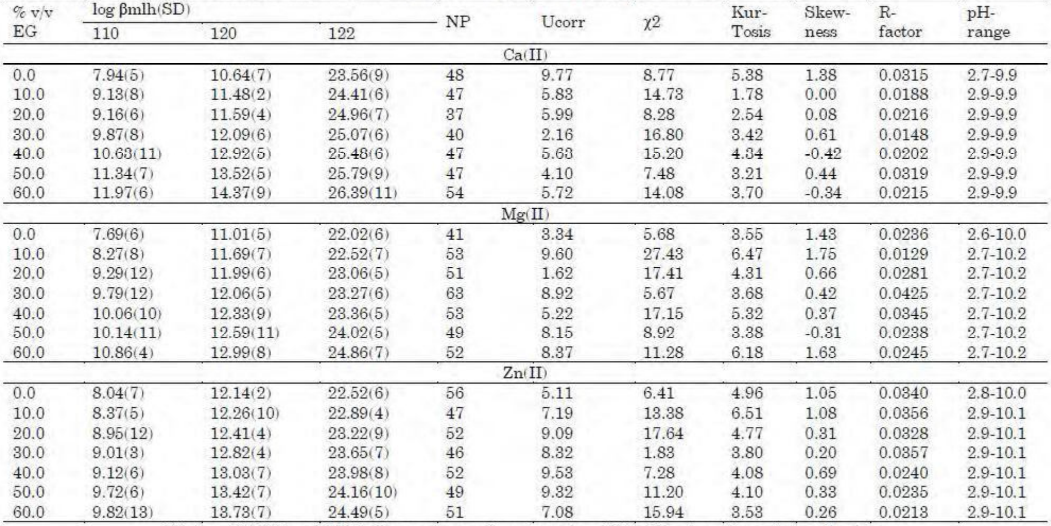
Table 1. Exhaustive modeling of Mg(II)-Phenylalanine complexes in 30% v/v EG-water mixture. pH range = 2.7-10.2; Number of points = 63.

v/v %	log β_{mlh} (SD)			χ^2	U_{corr}	Ske-	Kur-tosis	R-
EG	110	120	122			wness		Factor
1.	9.42(22)	---	---	9.68	5.48	1.3	4.71	0.0360
2.	---	12.31(32)	---	3.38	3.18	0.87	6.10	0.0314
3.	---	---	23.24(42)	9.70	5.80	0.22	4.75	0.0622
4.	---	12.76(30)	23.67(29)	2.69	2.12	2.27	8.04	0.0206
5.	9.71(43)	---	23.71(40)	6.05	9.65	3.98	2.40	0.0262
6.	9.69(39)	12.28(22)	---	3.38	6.24	0.91	5.88	0.0317
7.	9.79(12)	12.06(5)	23.27(6)	5.67	8.92	0.42	3.68	0.0425

U_{corr} = U/(NP-m) x 10^8, where, m = number of species; NP = number of experimental points.

The models indicated 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, MgL_2 and MgL_2H_2. This indicates that the final model appropriately fits the experimental data. Such exhaustive modeling was performed for all the systems. The best-fit model was selected using the statistical parameters [39] of the least squares residuals. The final models along with the statistical parameters are given in Table 2. The results of the best-fit models contain the type of species and overall formation constants. A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in the concentrations of ingredients at all experimental points corrected for degrees of freedom) indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around zero mean with little dispersion.

Table 2: Best fit chemical models of Ca(II), Mg(II) and Zn(II) complexes with Phenylalanine in EG-water mixtures.



% v/v EG	log βmlh(SD) 110	120	122	NP	Ucorr	χ2	Kur-Tosis	Skew-ness	R-factor	pH-range
					Ca(II)					
0.0	7.94(5)	10.64(7)	23.56(9)	48	9.77	8.77	5.38	1.38	0.0315	2.7-9.9
10.0	9.13(8)	11.48(2)	24.41(6)	47	5.83	14.73	1.78	0.00	0.0188	2.9-9.9
20.0	9.16(6)	11.59(4)	24.96(7)	37	5.99	8.28	2.54	0.08	0.0216	2.9-9.9
30.0	9.87(8)	12.09(6)	25.07(6)	40	2.16	16.80	3.42	0.61	0.0148	2.9-9.9
40.0	10.63(11)	12.92(5)	25.48(6)	47	5.63	15.20	4.34	-0.42	0.0202	2.9-9.9
50.0	11.34(7)	13.52(5)	25.79(9)	47	4.10	7.48	3.21	0.44	0.0319	2.9-9.9
60.0	11.97(6)	14.37(9)	26.39(11)	54	5.72	14.08	3.70	-0.34	0.0215	2.9-9.9
					Mg(II)					
0.0	7.69(6)	11.01(5)	22.02(6)	41	3.34	5.68	3.55	1.43	0.0236	2.6-10.0
10.0	8.27(8)	11.69(7)	22.52(7)	53	9.60	27.43	6.47	1.75	0.0129	2.7-10.2
20.0	9.29(12)	11.99(6)	23.06(5)	51	1.62	17.41	4.31	0.66	0.0281	2.7-10.2
30.0	9.79(12)	12.06(5)	23.27(6)	63	8.92	5.67	3.68	0.42	0.0425	2.7-10.2
40.0	10.06(10)	12.33(9)	23.36(5)	53	5.22	17.15	5.32	0.37	0.0345	2.7-10.2
50.0	10.14(11)	12.59(11)	24.02(5)	49	8.15	8.92	3.38	-0.31	0.0238	2.7-10.2
60.0	10.86(4)	12.99(8)	24.86(7)	52	8.37	11.28	6.18	1.63	0.0245	2.7-10.2
					Zn(II)					
0.0	8.04(7)	12.14(2)	22.52(6)	56	5.11	6.41	4.96	1.05	0.0340	2.8-10.0
10.0	8.37(5)	12.26(10)	22.89(4)	47	7.19	13.38	6.51	1.08	0.0356	2.9-10.1
20.0	8.95(12)	12.41(4)	23.22(9)	52	9.09	17.64	4.77	0.31	0.0328	2.9-10.1
30.0	9.01(3)	12.82(4)	23.65(7)	46	8.32	1.83	3.80	0.20	0.0357	2.9-10.1
40.0	9.12(6)	13.03(7)	23.98(8)	52	9.53	7.28	4.08	0.69	0.0240	2.9-10.1
50.0	9.72(6)	13.42(7)	24.16(10)	49	9.32	11.20	4.10	0.33	0.0235	2.9-10.1
60.0	9.82(13)	13.73(7)	24.49(5)	51	7.08	15.94	3.53	0.26	0.0213	2.9-10.1

Ucorr = U/ (NP-m) X 108; where m = number of species; NP = Number of experimental points

For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. Kurtosis is a measure of the peakedness of the error distribution near a modal value. For an ideal normal distribution kurtosis 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 as well as platykurtic patterns and very few are nearer to mesokurtic patterns. The values of skewness recorded in the table are between -0.42 and 1.63. These data evince that the residuals form part of a normal distribution. Hence, the least-squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-value. These statistical parameters thus show that the best-fit models portray the metal-ligand species in EG-water mixture.

Effect of systematic errors on best-fit model

In order to rely upon the best 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[40] like concentrations of alkali, mineral acid, ligand and metal (Table 3). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors was alkali > acid > ligand > metal. Some species were even rejected when errors were introduced in the concentrations. This study confirms the appropriateness of the chosen best-fit models and indicates the relative sensitivities of model parameters.

Effect of solvent

EG is an amphiprotic and coordinating solvent. It is a structure former and it enhances the water structure in EG-water mixtures; hence, it removes water from coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating solvent and competes with the ligands for coordinating the metals. This decreases the stability of the complexes. Hence, the stability of complex is expected to either increase or decrease.

Table 3: Effect of errors in influential parameters on stability constants of Mg(II) complexes with Phenylalanine in 30%v/v EG-water mixture.

	% Error	log β (SD)		
Ingredient		110	120	122
	0	9.79(12)	12.06(5)	23.27(6)
Alkali	-5	Rejected	Rejected	Rejected
	-2	10.32(29)	12.33(23)	Rejected
	+2	10.48(24)	13.66(31)	23.09(71)
	+5	Rejected	14.76(87)	23.12(98)
Acid	-5	Rejected	Rejected	Rejected
	-2	9.51(34)	13.76(43)	24.12(41)
	+2	9.76(42)	13.68(54)	Rejected
	+5	10.28(55)	Rejected	Rejected
Ligand	-5	10.71(72)	13.45(61)	24.10(72)
	-2	9.39(32)	12.73(52)	23.18(22)
	+2	9.42(42)	12.34(45)	23.11(15)
	+5	10.42(82)	13.15(54)	24.11(74)
Metal	-5	9.45(62)	12.32(52)	23.33(28)
	-2	9.48(32)	12.09(9)	23.28(19)
	+2	9.44(21)	12.07(13)	23.25(17)
	+5	9.43(12)	12.42(41)	23.37(27)

The variation of overall stability constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's classical treatment[41] accounts for the electrostatic contribution to the free energy change. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the log β values should vary linearly as a function of reciprocal of the dielectric constant of the medium[40], which is observed in the present study (Figure 1). The linear variation indicates that electrostatic forces dominate the equilibrium process under the present experimental conditions.

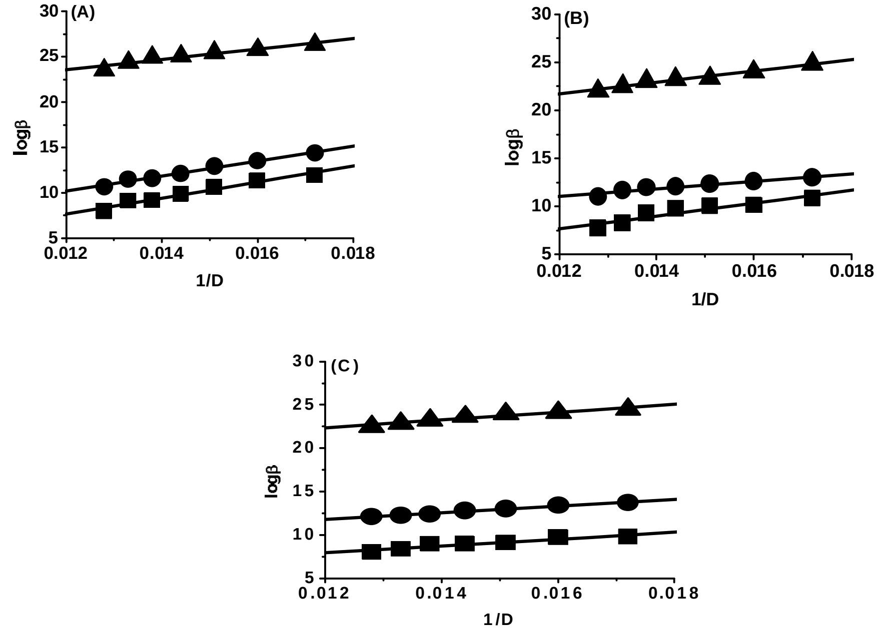


Figure 1. Variation of stability constant values of metal-phenylalanine complexes with reciprocal of dielectric constants (1/D) in EG-water mixtures at 303 K, ionic strength = 0.16 M. (A) Ca(II), (B) Mg(II) and (C) Zn(II); (■) log 110, (•) log 120, (▲) log 122.

The linear increase indicates the dominance of the structure forming nature of EG over its complexing ability. The cation stabilizing nature of co-solvents. specific solvent-water interactions, change dispersion and specific interactions of co-solvents with solute (indicated by the changes in the solubility of different species in the aqua-organic mixtures) account for little deviation from the linear relationship.

Distribution diagrams

Phe has one dissociable carboxyl proton and an amino group that can associate with a proton. The different forms of Phe are LH_2^+, LH and L^- in the pH-regions 2.0-3.0, 3.0-9.0 and 9.0-12.0, respectively. Hence, the plausible species in different systems can be predicted from these data. The species refined and determined are ML, ML_2 and ML_2H_2 for Ca(II), Mg(II) and Zn(II).

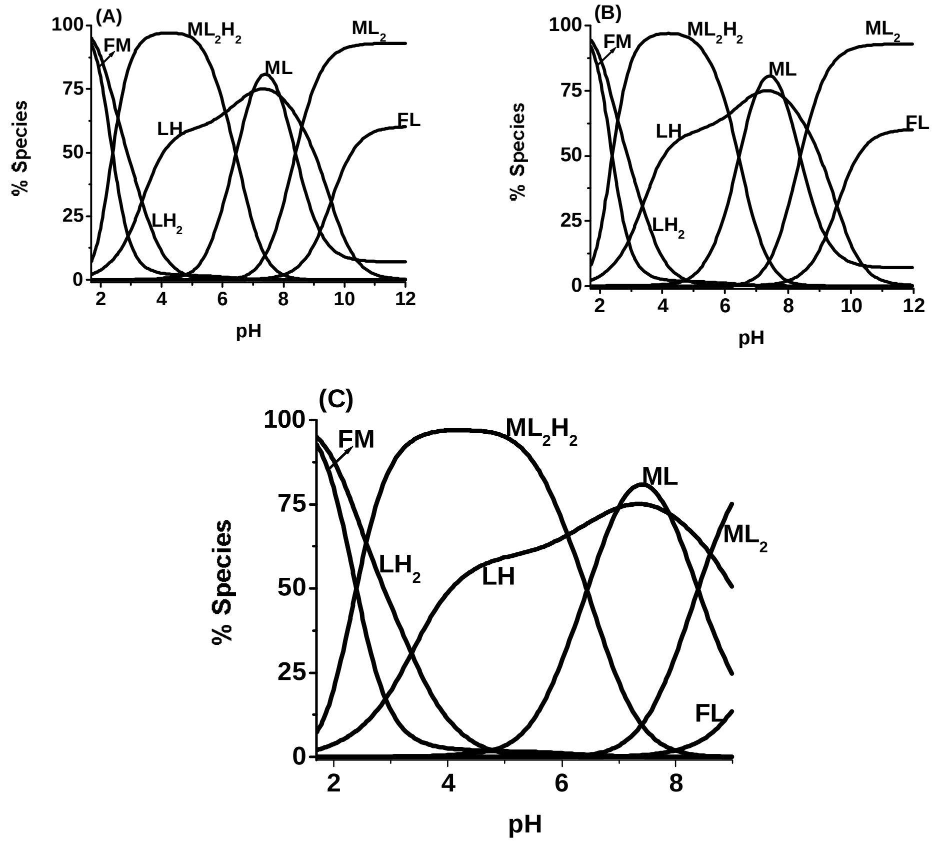


Figure 2. Distribution diagrams of phenylalanine complexes in 20% v/v EG-water mixture at 303 K, ionic strength = 0.16 M. (A) Ca(II), (B) Mg(II) and (C) Zn(II).

The species distribution diagrams of various systems are shown in Figure 2. The formation of various binary complex species is shown in the following equilibria.

(a) $M(II) + LH \rightleftharpoons ML + H^+$

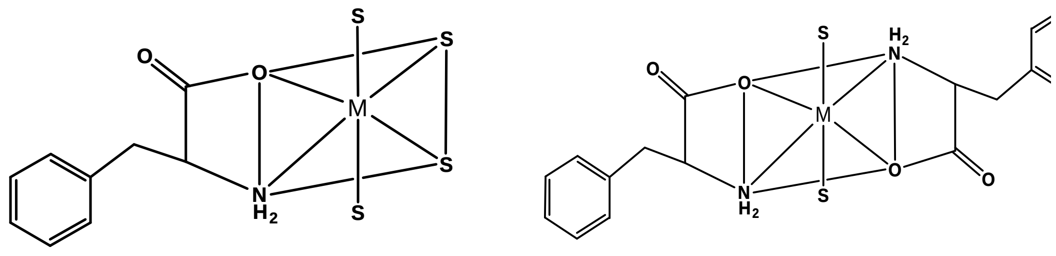
(b) $M(II) + LH_2 \rightleftharpoons ML + 2H^+$

(c) $ML + LH \rightleftharpoons ML_2 + H^+$

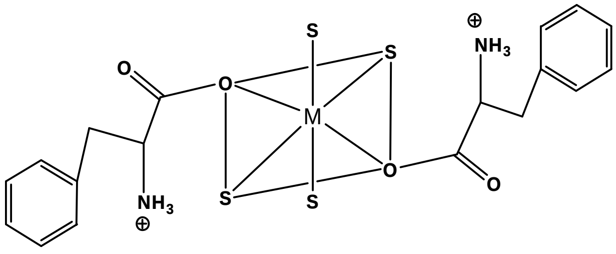
(d) $ML_2H_2 \rightleftharpoons ML_2 + 2H^+$

(e) $M(II) + 2LH_2 \rightleftharpoons ML_2H_2 + 2H^+$

Equilibria (a) and (b) is proposed for ML species. Equilibria (c) and (d) are proposed for the formation of ML_2 species. The (a) and (b) appears to be more appropriate between them because ML is formed with decreasing concentrations of LH and LH_2. Equilibrium (c) is possible for ML_2 species with decreasing the concentration of LH and (d) appears to be more appropriate because the concentration of MH_2 H_2 is decreasing with increasing concentration of ML_2. Equilibrium (e) is proposed the formation of ML_2H_2 with free metal and decreasing the concentration of LH_2. Depending on the coordinating atoms in the ligand and the nature of the metal ions, structures of the complexes are proposed for the species detected as shown in Figure 3.



ML ML$_2$



ML_2H_2

Figure 3. Structures of binary complexes of Ca(II), Mg(II) and Zn(II) with Phe.

Conclusions

(1) The species formed due to the interaction of Phenylalanine Ca(II), Mg(II) and Zn(II) metals are ML, ML_2 and ML_2H_2.

(2) The linear variation of stability constants with dielectric constants of

the medium indicates the dominance of electrostatic forces over non-electrostatic forces. A linear increasing trend with EG content supports the predominance of the structure forming nature of EG over its complexing ability.

(3) The order of the ingredients in influencing the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal.

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