

Chemical speciation of Ca^{II} , Mg^{II} and Zn^{II} complexes of L-asparagine in ethylene glycol-water mixtures

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Abstract : Chemical speciation of Ca^{II} , Mg^{II} and Zn^{II} complexes of L-asparagine in 0.0–60.0% v/v ethylene glycol-water mixtures at an ionic strength of 0.16 mol L^{-1} and 303 K has been studied pH metrically. The active forms of the ligand are LH_2 , LH^- and L^{2-} . The predominant species detected are ML_2H_2 , ML_2H^- and ML_2^{2-} . Models containing different numbers of species were refined by using the computer program MINQUAD75. 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.

Keywords : Complex equilibria, chemical speciation, L-asparagine, ethylene glycol, metals.

Introduction

In biological fluids, the metal ions exist in non-exchangeable form as metallo proteins 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. These simultaneous equilibria involving a variety of metal ions and ligands are important in biological fluids¹. Hence the chemical speciation of ligands with metal ions has been studied in this laboratory^{2–6}.

Calcium is the most plentiful metal found in the teeth, bones, nerve cells, body tissues, blood, and other body fluids of the human body⁷. It regulates the growth, maintenance, reproduction, blood clotting, nerve signaling, muscle contraction and relaxation, and the release of certain hormones of the human body. Dairy products are the most significant sources of calcium⁸. Calcium deficiency for a long period leads to osteoporosis, hypertension, and other disorders⁹. Magnesium is an essential metal involved in human nutrition and metabolic reactions. It regulates blood sugar levels, promotes normal blood pressure, prevents muscle cramping, enhance muscle and nerve functioning and helps in improving bone density which is known to be involved in energy metabolism and protein synthesis^{10,11}. It is naturally found in legumes and soy

products¹². Zinc is the second most abundant transition metal found in both plants and animals. It is required for the catalytic activity of the enzymes due to its pronounced Lewis acid characteristics, single redox state of the Zn^{2+} ion^{13,14}.

L-Asparagine is one of the 20 most common natural amino acids on earth. It has carboxamide as the side-chain's group. It participates in the function of the brain and nervous system. It is required by the nervous system to maintain equilibrium and is also required for amino acid transformation from one form to the other in the liver. L-Asparagine has no known toxicity.

EG is a protophilic dipolar protic solvent and acts as a structure former. Having two hydroxyl groups EG is distinctly different from monohydric alcohols. It is more acidic than water^{15,16} due to electron withdrawing effect¹⁷ of CH_2 group. Very few studies have been reported in the literature on effect of dielectric constants in organic solvent-water mixtures^{18,20}. Hence, speciation studies of the title systems have been undertaken based on their involvement in various physiological reactions.

Results and discussion

The results of the best fit models that contain the stoichiometric coefficients of the complex species and their

overall formation constants along with some of the important statistical parameters are given in Table 1. Very low standard deviation in overall stability constants ($\log \beta$) signifies the precision of these constants^{21,22}. 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 EG-water mixtures, as discussed below.

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

Table 1. Parameters of best fit chemical models of M(II)–L-asparagine complexes in EG-water medium

EG	log β_{mlh} (SD)			pH-	NP	U _{corr}	χ^2	Skewness	Kurtosis	R-factor
% v/v	ML ₂	ML ₂ H ₁	ML ₂ H ₂	Range		$\times 10^8$				
Ca ^{II}										
0.0	7.69(48)	16.49(46)	21.84(69)	2.5–9.0	12	3.82	8.44	0.01	3.12	0.0238
10.0	5.75(28)	15.62(26)	22.32(23)	2.0–11.0	80	7.15	32.93	0.13	3.68	0.0191
20.0	5.44(21)	14.24(42)	22.78(27)	2.5–10.0	13	1.6	17.00	0.12	9.76	0.0156
30.0	6.52(34)	14.87(87)	22.75(39)	2.0–10.5	27	7.12	14.53	4.44	27.17	0.0221
40.0	5.18(37)	14.80(32)	23.18(28)	2.3–10.5	16	36.57	21.33	−0.23	3.14	0.0211
50.0	6.68(62)	15.96(60)	22.87(55)	2.3–10.0	15	12.54	13.13	−0.25	3.59	0.0407
60.0	6.14(48)	16.52(40)	23.28(59)	2.2–10.5	21	4.56	22.97	0.97	4.57	0.0195
Mg ^{II}										
0.0	6.53(61)	14.66(69)	22.22(59)	2.5–9.5	14	11.08	9.43	3.09	14.81	0.0433
10.0	5.49(18)	15.02(17)	22.73(24)	2.5–9.9	11	0.65	25.91	−0.12	4.23	0.0100
20.0	7.01(11)	15.77(14)	23.09(05)	1.8–11.0	41	1.57	31.60	−1.15	8.67	0.0060
30.0	–	16.76(38)	24.27(33)	1.9–4.6	68	24.84	14.82	0.38	2.71	0.0289
40.0	4.22(73)	14.32(37)	23.58(12)	5.1–11.6	50	34.97	94.27	−0.01	7.10	0.0904
50.0	7.05(42)	17.29(67)	–	1.5–10.5	43	5.17	61.20	0.74	4.10	0.0391
60.0	–	13.95(74)	24.19(15)	4.2–10.0	61	22.54	21.63	0.12	2.85	0.0388
Zn ^{II}										
0.0	9.94(32)	16.20(66)	21.38(70)	2.5–9.1	54	30.38	79.68	3.36	17.47	0.0520
10.0	8.95(35)	16.19(49)	23.02(23)	2.1–9.5	71	18.98	101.25	1.90	15.72	0.0320
20.0	8.73(46)	16.55(66)	24.04(60)	1.8–10.0	40	6.74	18.40	2.02	10.05	0.0126
30.0	9.05(37)	16.64(69)	–	3.0–8.0	70	0.76	17.48	0.07	4.96	0.0412
40.0	8.62(74)	16.52(70)	23.48(41)	2.0–9.5	22	22.75	36.89	−0.84	5.81	0.0476
50.0	–	17.75(51)	24.36(19)	1.8–7.8	56	32.04	29.90	0.18	3.64	0.0314
60.0	9.02(22)	16.56(36)	–	2.8–9.5	13	2.40	12.90	0.01	4.96	0.0214

the table value, the model is accepted.

Crystallographic *R*-test :

Hamilton's *R*-factor ratio test 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 1 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 1 are between -0.23 and 4.4 for Ca^{II} , -1.15 and 3.09 for Mg^{II} and -0.84 and 3.36 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 2). The order of the ingredients that influence the

Table 2. Effect of errors in influential parameters on the stability constants of Zn^{II} -asparagine complexes in 10% v/v EG-water medium

Ingredient	% Error	log β_{mlh} (SD)		
		120	121	122
Acid	0	8.95(32)	16.16(49)	23.02(23)
	-5	Rejected	Rejected	Rejected
	-2	8.73(30)	16.02(45)	22.84(23)
	+2	8.49(22)	15.84(46)	22.66(22)
	+5	7.76(32)	15.41(55)	22.15(39)
Alkali	-5	7.98(34)	15.49(53)	22.30(34)
	-2	8.44(30)	15.84(46)	22.67(23)
	+2	10.52(24)	17.68(48)	24.59(18)
	+5	Rejected	Rejected	Rejected
	-5	8.94(31)	16.12(51)	22.93(23)
Ligand	-2	8.78(21)	15.97(29)	22.03(34)
	+2	8.98(32)	16.22(47)	23.14(23)
	+5	8.49(33)	16.27(48)	23.11(23)
	-5	9.02(33)	16.25(50)	23.11(23)
	-2	9.00(33)	16.26(49)	23.11(23)
Metal	+2	8.99(34)	16.30(51)	23.14(24)
	+5	8.97(32)	16.28(46)	23.12(23)

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 EG-water mixtures are the combination of aprotic solvents with wide range of dielectric constants and with good solubility for polar as well as non-polar solutes. The variation of overall stability constant values with cosolvent content depends upon two factors, viz. electrostatic and non-electrostatic. Born's²³ 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 Asp with $1/D$ (*D* is the dielectric constant of the medium) of EG-water mixture are

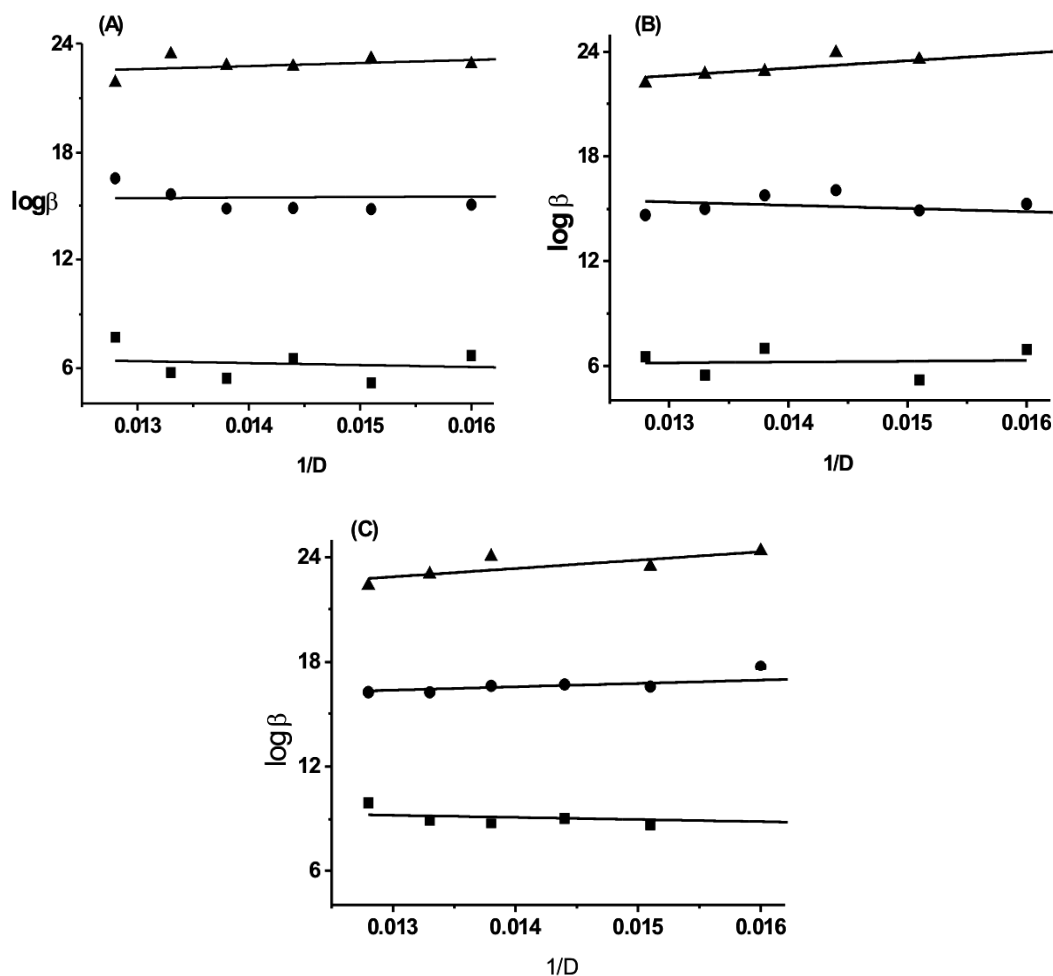


Fig. 1. Variation of overall stability constant values of metal-L-asparagine complexes with EG-water mixtures. (A) Ca^{II} , (B) Mg^{II} and (C) Zn^{II} ; (■) $\log \beta_{ML_2}$; (●) $\log \beta_{ML_2H}$; (▲) $\log \beta_{ML_2H_2}$.

given Fig. 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 electrostatic forces over non-electrostatic forces.

Distribution diagrams :

L-Asparagine is a bidentate ligand that has one dissociable (carboxyl group) and one associable (amino) protons. The different forms of L-asparagine are LH_2^+ , LH , and L^- in the pH range 1.5–5.0, 1.5–11.0, and 5.0–11.0, respectively. Hence, the plausible binary metal-ligand complexes can be predicted from these data. The present investigation reveals the existence of ML_2 , ML_2H , ML_2H_2

for Ca^{II} , Mg^{II} and Zn^{II} . The ML_2 species is the predominant species (Fig. 2) at higher pH and ML_2H_2 is the predominant species at lower pH among all the binary complexes. The formation of various binary complex species is shown in the following equilibria. Some typical distribution diagrams of acetonitrile-water media are shown in Fig. 2. The species ML_2H_2 , ML_2H , ML_2 are formed in the pH range of 2.0–11.0. ML_2H_2 is formed at lower pH. ML_2H and ML_2 formed with the increasing pH. ML_2H and ML_2 species percentage successively increases with increasing pH. Successive deprotonation of ML_2H_2 forms ML_2H beyond a pH 9.0 [Equilibria (1) and (2)]. ML_2 formed at higher pH with high percentage [Equilibria (3), (4), (5) and (6)]. The percentage of the ML_2 species

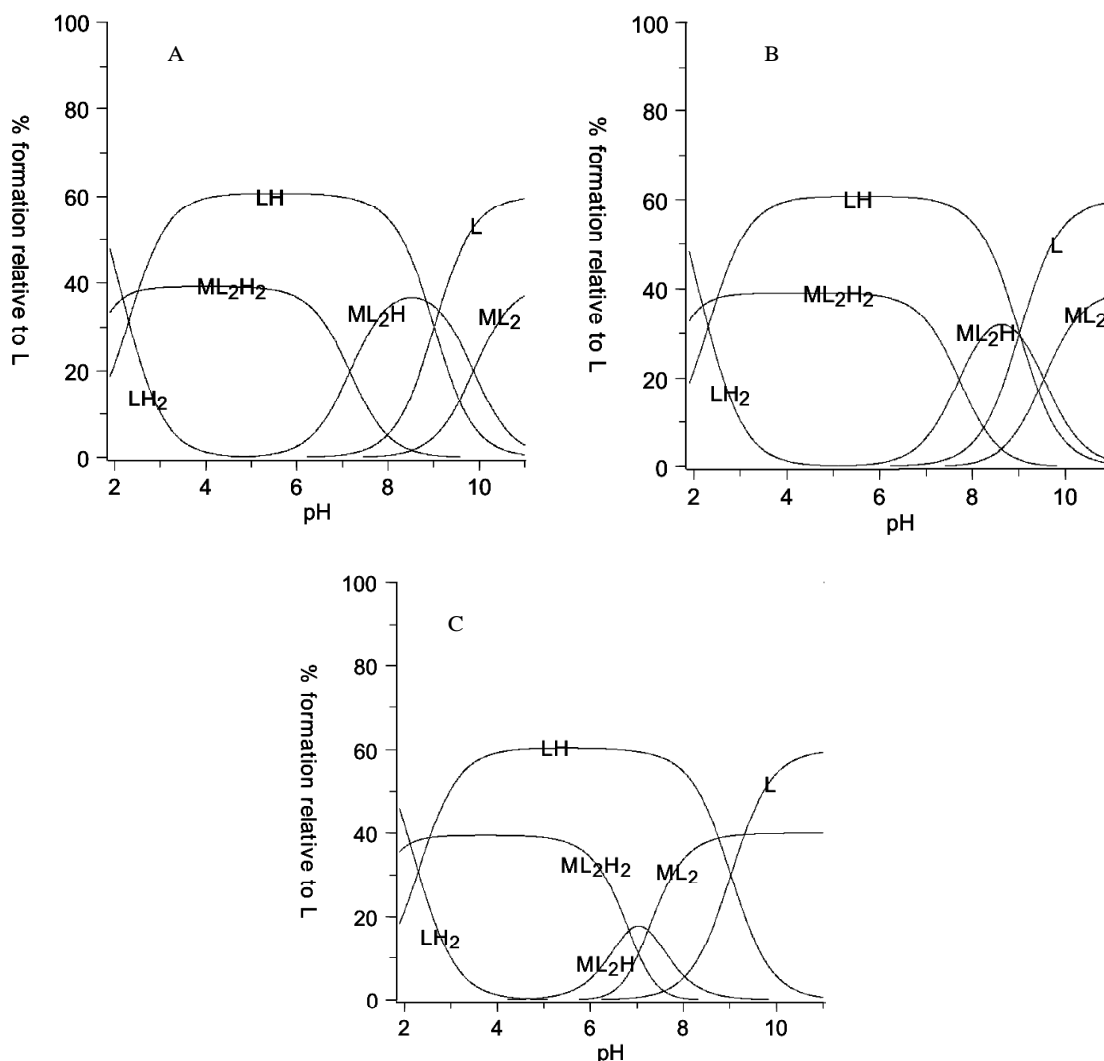
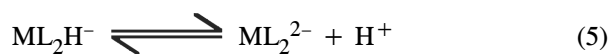
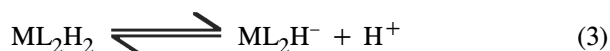
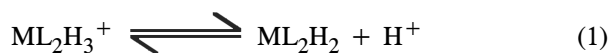


Fig. 2. Distribution diagrams of L-asparagine complexes in 10% v/v EG-water medium. (A) Ca^{II} , (B) Mg^{II} and (C) Zn^{II} .

increases successively with increase in pH up to 9.0. The concentration of ML_2H_2 species decreased, while the concentration of ML_2H and ML_2 increased in the pH range 4.5–9.0.



Structures of complexes :

When the second donor site of L-asparagine is a nitrogen atom, marked bidentate behavior is frequently found, more so when the additional chelation results in a five membered ring (Fig. 3). Octahedral structures are proposed to the complexes of all the metal ions. The VSEPR theory suggests that Pb^{II} and Cd^{II} complexes shall be octahedral because there are six outer electron pairs.

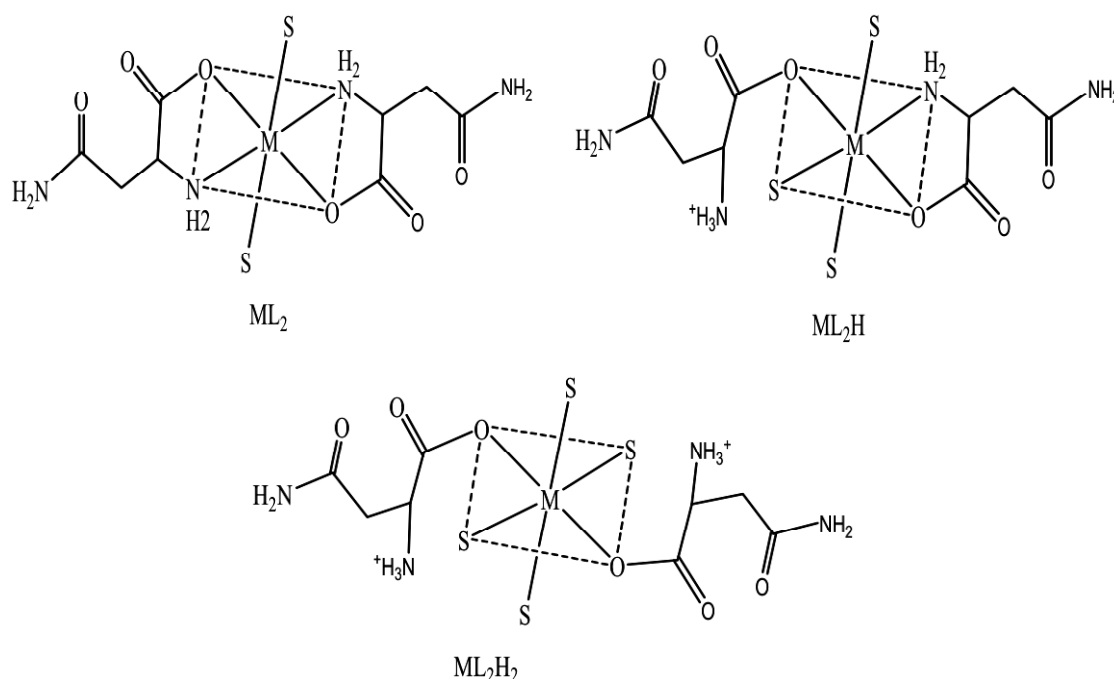


Fig. 3. Structures of binary complexes of L-asparagine with M(II). Where S is either solvent or water molecule.

Amino nitrogen atoms 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. Hence, protonated complex species are detected in the present study. Amino nitrogen and carboxyl oxygen of L-asparagine participate in bonding with metal ions. This argument supports the structures of complexes proposed in Fig. 3.

Materials and methods :

0.05 mol L⁻¹ solution of L-asparagine (GR, E. Merck, Germany) was prepared in triple distilled water by maintaining 0.05 mol L⁻¹ acid (HCl) concentration to increase the solubility. Ethyleneglycol (AR, E. Merck) used as received. Sodium hydroxide of 0.4 mol L⁻¹ was prepared. 0.2 mol L⁻¹ Hydrochloric acid (Qualigens, India) was prepared. 2.0 mol L⁻¹ Sodium chloride (Merck, India) was prepared to maintain 0.16 mol L⁻¹ 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. 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 classification²⁴. The strengths of alkali and mineral acid were determined using the Gran plot method²⁵.

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 EG-water mixtures (0.0–60.0% 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 EG-water mixture of equivalent

composition as that of the titrand. All the titrations were performed in media containing varying concentrations of EG-water mixtures (0.0–60.0% v/v) pH metrically at 303.0 ± 0.1 K. In each of the titrations, the titrand consisted of approximately 1.0 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 SCPHD²⁶ was used to calculate the correction factor. By using pH metric titration data, the binary stability constants were calculated with the computer program MINIQUAD75²⁷ 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 L-asparagine 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.

Conclusions

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 asparagine in AN-water mixture.

- (i) Asparagine formed both protonated and unprotonated complexes ML_2 , ML_2H , and ML_2H_2 for Ca^{II} , Mg^{II} and Zn^{II} in the pH range 2.5–11.0.
- (ii) 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. Some species are stabilized due to electrostatic interactions and some are destabilized due to the decreased dielectric constant.
- (iii) The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali > acid

> ligand > metal > volume.

- (iv) The distribution diagrams infer that the protonated and unprotonated complexes are formed successively and simultaneously from higher protonated species, and from free metal and ligand.

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