

Computer Application in pH-metric Investigation of Ternary Complexes of Ca(II), Mg(II) and Zn(II) with L-Aspartic Acid and Ethylenediamine in Propylene glycol -Water Mixtures

Chandra Leela Athota¹, Vijaya Kumar Nethala^{2*}, Rama Swamy Guttula³ And Nageswara Rao Gollapalli⁴

¹Department of Chemistry, Hindu College, Machilipatnam-521001, AP, India

^{2*}Department of Chemistry, DNR (A) College, Bhimavaram-534202, AP, India

³Department of Basic Science, Vishnu Institute of Technology, Bhimavaram-534202, AP, India

⁴Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam-530003, AP, India

Abstract: The stability constants of ternary complexes of Ca(II), Mg(II) and Zn(II) with L-Aspartic acid as primary ligands and Ethylenediamine as a secondary ligand were determined pH-metrically. The study was accompanied in varying concentrations (0-60% v/v) of propylene glycol-water mixtures at an ionic strength of 0.16 mol L⁻¹ using NaCl as an electrolyte at 303.0 K. The Value of $\Delta \log K$ which is responsible for the extra stability of ternary complexes was conferred on the basis of statistical parameters and the nature of the species formed. The formation of various ternary species was established by modeling studies using the computer program MINIQUAD75. The formation and distribution of different species with relative concentrations of metal and ligands (M:L:X = 1.0:2.5:2.5, 1.0:2.5:5.0, 1.0:5.0:2.5) with varying pH were represented in the form of distribution diagrams. Influence of the solvent on the speciation was discussed based on the dielectric constant of the medium. The stability of the mixed ligand complexes are discussed in terms of the molecular structure of L - Aspartic acid and En as well as the nature of the metal ion.

Keywords: Ternary complexes, Speciation, pH-metric study, L-Aspartic acid, Ethylenediamine

INTRODUCTION

The specificity and selectivity of Enzyme-substrate reactions can be achieved by manipulating the equivalent solution dielectric constants (ESDC) at the active site.[1] The ESDC at the active sites in bovine carbonic anhydrase and carboxy peptidase were estimated to be 35 and 70, respectively. In carbonic anhydrase the binding of water (substrate) to the zinc ion simply holds it in the correct stereochemical position suitable for attack by CO₂, where as in

carboxy peptidase the binding of the substrate results in polarization, thus, facilitating hydrolysis. This variation is brought out by the changes in the interactions of side chains of the protein moiety among themselves and with those of the solvent molecules. Hence, modeling studies of ternary complexes have gained popularity in different aqua-organic mixtures with different dielectric constants.[2-6]

Aspartic acid is a non-essential amino acid in mammals, being produced from oxaloacetate by transamination. It plays an

important role in Citric acid cycle or Krebs cycle, during which other amino acids and biochemicals, such as asparagine, arginine, lysine, methionine, threonine and isoleucine are synthesized. The conversion of aspartic acid to these amino acids begins with the reduction of aspartic acid to its semi aldehyde.[7] Aspartic acid is used in the treatment for chronic fatigue because it plays crucial role in generating cellular Energy. Aspartic acid moves the coenzyme nicotinamide adenine dinucleotide (NADH) molecules from the main body of the cell to its mitochondria, where it is used to generate adenosine triphosphate (ATP), and the fuel that powers all cellular activity. In addition, this amino acid helps transport minerals needed to form healthy RNA and DNA to the cells, and strengthEns the immune system by promoting increased production of immune globulins and antibodies. Aspartic acid keeps the mind sharp by increasing NADH in the brain, which is thought to boost the production of neurotransmitter.[8]

1, 2-Diaminoethane is more commonly known as Ethylenediamine. Ethylenediamine is a colourless to yellowish hygroscopic liquid with an ammonia-like odour. Ethylenediamine is used as monodentate, bidentate or bridging ligand. It is also used in the manufacture of EDTA, carbamate fungicides, surfactants and dyes. It is involved in the synthesis of seven membered ring components with β -ketoesters resulting secondary amines and β -Enaminoesters.[9] En plays an important role in the synthesis of Schiff base compounds.[10]

PG is a widely used compound with diverse applications. It is one of the most commonly used humectant substances that have a high affinity for water and have a stabilizing action on the water content of a material. It is used to maintain moisture within a narrow range in certain food products, such as coconut and marsh mallows, as well as in tobacco.[11] It has been extensively employed in the pharmaceutical industry as a solvent for drugs, as a stabilizer for vitamins and in pastes for medicinal purposes. It is used as a solvent in the paint and plastic industries, and to make polyester compounds. It is used as a substitute for ethylene glycol, monoalkyl ethers in all purpose cleaners, coatings, inks, nail polish, lacquers, latex paints

and adhesives. It is also used to create artificial smoke or fog used in fire-fighting training and in theatrical productions.[12] PG is used as a chemical intermediate in the production of unsaturated polyester resins, liquid detergents, antifreeze/Engine coolant, paints and coatings.

Calcium is essential for living organisms. It is one of the most important elements in the diet because it is a structural component of bones, teeth and soft tissues and is essential in many of the body's metabolic processes. Calcium is used to regulate the permeability and biological membrane (such as cell walls) potentials, which in turn control muscle and blood vessel expansion and contraction, nerve functions, secretion of hormones and Enzymes and transmitting impulses throughout the nervous system.[13] Calcium is also essential for proper blood clotting. 99% of the body's calcium supply is stored in the bones and teeth where it is present as phosphate together with small amounts of CaCO_3 , providing rigidity to the structure.[14] Calcium supplements (without co-administered vitamin D) are associated with an increased risk of myocardial infarction. As calcium supplements are widely used these modest increases in risk of cardiovascular disease might translate into a large burden of disease in the population.[15]

Magnesium is needed for more than 300 biochemical reactions in the body. It helps to maintain normal muscle and nerve function, keeps heart rhythm steady, supports a healthy immune system and keeps bones strong. Magnesium also helps to regulate blood sugar levels, promotes normal blood pressure and is known to be involved in Energy metabolism and protein synthesis. There is an increased interest in the role of magnesium in preventing and managing disorders such as hypertension, cardiovascular disease and diabetes. Increasing magnesium intake may be a valid strategy to Enhance cognitive abilities.[16] Mg^{2+} is also used as a signaling ion and much of nucleic acid biochemistry requires Mg^{2+} , including all reactions which require release of Energy from ATP for mechanical work including muscle contraction and relaxation.[17-19]

Zinc is the second most abundant essential trace metal and is known to play very

important role in the biological systems.[20]It also has useful anti-viral, anti-bacterial, anti-radiation and anti-cancer properties. It is added to toothpaste to protect against the bacterial plaque which is largely responsible for gum diseases. Zinc exerts action against heavy metal poisoning. Zinc also helps to save babies that by giving zinc supplements to infants aged less than six months can reduce episodes of diarrhea and acute respiratory illness.[21] The zinc containing carboxypeptidase is secreted as an inactive zymogen in the pancreatic juice of animals. The Enzyme contains 1 gm atom of zinc per protein of molecular weight of 34,500 and removal of the metal results in a totally inactive apoenzyme. The activity can be restored by re-addition of zinc.[22]

Hence, speciation studies of the title systems have been undertaken based on their involvement in various physiological reactions. The protonation constants[23] of Asp and En and their binary complexes with Ca(II), Mg(II) and Zn(II) in Propylene glycol -water mixtures have already been studied in this laboratory .

EXPERIMENTAL

Chemicals

1,2Propanediol (Merk, India) was used as received. Aqueous solutions of L-Aspartic acid and Ethylenediamine of 0.05 molL^{-1} (Merk, India) and Hydrochloric acid of 0.2 molL^{-1} (Qualigens, India) were prepared in triple-distilled deionized water. Sodium chloride (Qualigens, India) of 2 molL^{-1} was prepared to maintain the ionic strength in the titrand. Solutions of Ca(II), Mg(II) and Zn(II) chlorides were prepared. To increase the solubility of ligands and to suppress the hydrolysis of metal salts, the mineral acid concentration in the above solutions was maintained at 0.05 molL^{-1} . Sodium hydroxide (Qualigens, India) of 0.4 molL^{-1} was prepared. 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).[24] The strengths of alkali and mineral acid were determined using the Gran plot method.[25, 26]

Apparatus

The titrimetric data were obtained using ELICO (Model LI-120) pH meter (readability 0.01), which was calibrated with 0.05 molL^{-1} potassium hydrogen phthalate in acidic region and 0.01 molL^{-1} borax solution in basic region. The glass electrode was equilibrated in a well-stirred PG-water mixture containing the inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of PG-water mixtures (0-60% v/v) by maintaining an ionic strength of 0.16 molL^{-1} with sodium chloride at $303 \pm 0.1 \text{ K}$. The effect of variation in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode was accounted for in the form of correction factor.[27, 28]

Procedure

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 PG-water mixture of equivalent composition as that of the 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, 1:3.75 and 1:5) of metal-to-ligand were carried with 0.4 molL^{-1} sodium hydroxide.[29]

Modeling Strategy

The computer program SCPHD[30] was used to calculate the correction factor. By using pH metric titration data, the binary stability constants were calculated with the computer program MINQUAD75[31] which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. During the refinement of ternary systems, the correction factor and protonation constants of Asp and En and their binary complexes with Ca(II), Mg(II) and Zn(II) in PG-water mixtures were fixed.

RESULTS AND DISCUSSION

The results of the best fit model 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 1. Very low standard deviation in overall stability constants ($\log \beta$) signifies the precision of these constants. The small values of U_{corr} (sum of squares of deviations in concentrations of ligand and hydrogen ion 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.[32]

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 or normal 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 are 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 PG-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 calculated χ^2 is less than 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 there are different numbers of species the models with values 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 -2.36 and -0.31 for Ca(II), -1.44 and -0.47 for Mg(II) and -1.48 and 0.41 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 model 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 and metal (Table 2). The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > primary

ligand >secondary ligand >metal. Some species were even rejected and the standard deviation is high in few cases when errors are introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors conform the

Chart1: Equations for the calculation of $\Delta \log K$

$$\Delta \log K_{1120} = \log \beta_{1120} - \log \beta_{1100} - \log \beta_{1020}$$

$$\Delta \log K_{1121} = \log \beta_{1121} - \log \beta_{1100} - \log \beta_{1021}$$

$$\Delta \log K_{1122} = \log \beta_{1122} - \log \beta_{1100} - \log \beta_{1022}$$

appropriateness of the chosen best fit models. This study also indicates the relative sensitivities of model parameters.

Effect of solvent

PG is an amphiprotic and coordinating solvent. It is a structure former and it enhances the water structures in PG-water mixtures; 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 being a coordinating solvent, it competes with the ligands for coordinating the metals which decreases the stability of the complexes. Hence the stability of the complex is expected either to increase or decrease. The variation of overall stability constant values with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born's [33] 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.

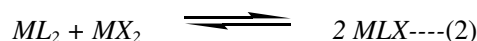
The trends of stability constants ($\log \beta$) values of ternary complexes with $1/D$ (D is the dielectric constant of the medium) of PG-water mixtures are shown in Figure 1. In all cases the trend is almost linear, implies that the dielectric constant or long range interactions are responsible for the stability trend. Linear increase in Figure 1A,B,C indicates the dominance of the structure-forming nature of the solvent over its complexing ability. But the deviations from

linearity are assumed due to some contribution from non-electrostatic forces.

Stability of Ternary Complexes:

The change in the stability of the ternary complexes as compared to their binary analogues was quantified based on the disproportionation constant ($\log X$) given by Equation 1 which corresponds to the equilibrium [34-37] as shown (2).

$$\log X = 2 \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \quad \text{---(1)}$$



$$\Delta \log K = \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \quad \text{---(3)}$$

Under the equilibrium conditions one can expect the formation of 50% ternary complexes and 25% each of the binary complexes statistically and the value of $\log X$ shall be 0.6. [19] A value greater than this, accounts for the extra stability of MLX.

Another approach to quantify the stability of ternary complexes was based on the difference in stability ($\Delta \log K$) for the reactions ML with X and $M_{(aq)}$ with L and X, [35-39] where L is the primary ligand (Asp) and X is the secondary ligand (En). It is compared with that calculated purely on the statistical grounds as given in Equation 3.

The $\Delta \log K$ values are calculated from binary and ternary complexes using the equations given in Chart 1.

Various possible $\log K$ values obtained from these equations are given in Table 3. In the present study, the $\log K$ values range from 0.85 to 2.85 for Ca(II), -1.65 to 4.58 for Mg(II) and -1.68 to 1.65 for Zn(II) and all values are found to be higher than those expected on statistical bases (0.6). These higher values account for the extra stability of the ternary complexes. The reason [40, 41] for the extra stability of these ternary

complexes may be due to interactions outside the coordination sphere such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect and stacking interactions. The log X values could not be calculated for the system due to the absence of relevant binary species.

Distribution Diagrams

Some typical distribution diagrams in 10% PG-water mixture are drawn using the formation constants of the best fit model are shown in Figure 2 which contain protonated and unprotonated species like MLX₂, MLX₂H and MLX₂H₂ for Ca(II), Mg(II) and Zn(II). The active forms of these ligands are LH₃⁺, LH₂, LH⁻ and L²⁻ for Asp and XH₂²⁺, XH⁺ and X for En. The binary complex species of Asp are ML, ML₂H₂, ML₂H₃ and ML₂H₄ and En are MX₂, MX₂H, MX₂H₂, for Ca(II), Mg(II) and Zn(II). The distribution diagrams indicate the relative abundance of various forms of metal (chemical speciation) at different pH and dielectric conditions. A stable ternary complex shall be responsible for metal ion transportation in biological systems and the weak binary metal complexes make the essential metals bioavailable. The increased concentrations of complexing agents make the essential metal ions unavailable due to the formation of stable binary metal complexes. The formation of the ternary complex species can be represented by the following equilibria. The charges of the species are omitted for clarity.

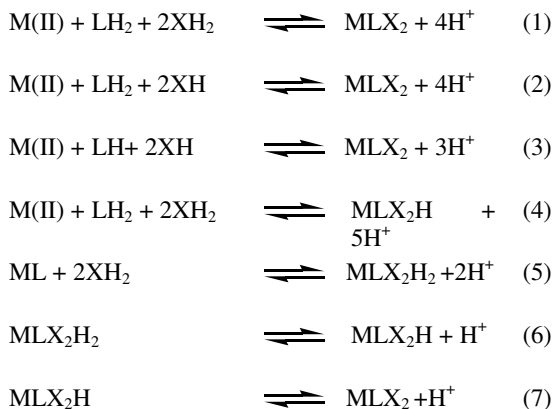


Figure 2A represents the formation of ternary complexes of Asp-Ca-En in the pH range

of 3.0-10.5. The species MLX₂ is formed by the interaction of free metal ion with LH₂, with XH₂ or LH₂ with XH and LH with XH and on deprotonation of MLX₂H (Equilibria 1-3 & 7). MLX₂H species is formed by the interaction of free metal ion with LH₂ and XH₂ or by the deprotonation of MLX₂H₂ (Equilibria 4 and 6). MLX₂H₂ species is formed by the reaction of free metal ion with XH₂ (Equilibria 5).

Figure 2B shows formation of Asp-Mg-En complexes. The concentration of MLX₂H₂ species increased, while the concentration of MLX₂H decreased in the pH range 6.0-10.0 which is formed on deprotonation of MLX₂H₂ (equilibrium 6) beyond 6.0 pH the species MLX₂ formed on deprotonation of MLX₂H (equilibrium 7).

Figure 2C shows the formation of Asp-Zn-En complexes in the pH range 3.0-10.0. The concentration of MLX₂H species is very high when compared with the concentration of MLX₂H₂. MLX₂H₂ is formed from ML and XH₂ (equilibrium 5). The species MLX₂ is formed on deprotonation of higher species (equilibrium 7) in the pH range 7.0-10.0.

Structures of complexes

Based on the protonation and deprotonation equilibrium of Asp and En, depending on the coordinating sites in the ligands and the nature of the metal ions, basic coordination chemistry principles, the possible structures of the ternary complexes are proposed as given in Figure 3. Octahedral structures are proposed for all the metals based on literature reports. [42-45] Asp and En form strong bidentate complexes with transition metals. Asp at higher pH favors the (O, O) coordination and at physiological pH it is bound through the amino acid side chain.

CONCLUSIONS

The following conclusion have been drawn from the modeling studies of the speciation of ternary complexes of Ca(II), Mg(II), and Zn(II)

with L-aspartic acid and ethylenediamine in propylene glycol -water mixtures.

1. The predominant species detected are MLX_2 , MLX_2H and MLX_2H_2 for Ca(II), Mg(II) and Zn(II). Where L = Asp and X = En. The active forms of these ligands are LH_3^+ , LH_2 , LH^- and L^{2-} for Asp and XH_2^{2+} , XH^+ and X^- for En.

2. The $\Delta \log K$ values indicate that the ternary species have extra stability compared to their binary species, may be due to the interactions outside the coordination sphere, such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect, stacking interactions and the electrostatic interaction between non-coordinated charge groups of the ligands.

3. The linear increase in the stabilities of ternary complexes with decreasing dielectric constants is due to the dominance of electrostatic forces.

4. The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > Asp > En > metal > log F.

5. The study also gives an insight into the metal availability/metal transport in biofluids and toxicity of these metals. The ternary complexes are more amenable for 'metal transport' because of their extra stability and the binary complexes make the 'metal available' in biological systems due to their decreased stability.

Table 1. Parameters of best fit chemical models of Ca(II), Mg(II) and Zn(II)- Asp-En ternary complexes in PG-water mixtures

% v/v PG	log β_{mlxh} (SD)			pH-Range	NP	$U_{corr} \times 10^8$	Skewness	Kurtosis	χ^2	R-factor
	MLX ₂	MLX ₂ H	MLX ₂ H ₂							
Ca(II)										
00.0	10.11(12)	19.51(28)	29.42(15)	3.0-10.0	72	4.79	-0.57	3.08	45.22	0.0113
10.0	10.28(7)	19.77(15)	29.05(19)	3.0-10.0	79	6.62	-0.31	2.83	54.48	0.0131
20.0	12.05(28)	21.59(53)	31.02(46)	2.0-10.5	31	32.89	-1.59	4.39	29.42	0.0332
30.0	11.22(49)	20.60(17)	29.33(41)	2.1-9.5	24	42.43	-2.36	8.14	59.83	0.0370
40.0	11.71(17)	20.71(22)	30.10(21)	3.0-10.0	86	11.33	-1.10	3.98	49.12	0.0181
50.0	11.85(20)	21.03(76)	30.27(50)	3.0-9.5	45	17.62	-1.37	3.50	29.07	0.0222
60.0	12.04(29)	20.65(60)	30.34(31)	3.0-10.0	83	40.13	-1.48	4.72	61.64	0.0345
Mg(II)										
00.0	12.13(23)	21.12(70)	30.38(49)	3.0-9.5	64	85.41	-0.47	5.36	72.13	0.0486
10.0	12.24(31)	22.00(61)	31.61(39)	3.0-10.0	67	97.81	-1.28	3.70	38.63	0.0546
20.0	12.43(26)	21.78(57)	31.33(36)	2.0-10.5	33	37.67	-1.44	4.44	33.82	0.0351
30.0	12.20(17)	20.90(26)	30.37(29)	3.0-10.0	84	14.07	-0.59	4.22	36.29	0.0198
40.0	12.52(45)	21.72(55)	30.90(55)	3.0-9.5	71	40.29	-1.16	3.06	47.21	0.0334
50.0	13.04(24)	21.88(56)	30.95(21)	3.0-9.5	48	17.75	-1.31	3.32	88.33	0.0224
60.0	13.50(25)	21.96(80)	31.11(22)	3.0-9.5	50	17.85	-1.30	3.23	54.72	0.0226
Zn(II)										
00.0	15.62(11)	24.52(7)	31.51(14)	3.0-10.0	80	1.77	0.41	8.81	46.65	0.0070
10.0	15.86(12)	24.95(8)	32.34(9)	2.5-9.5	22	0.51	-0.07	5.16	29.27	0.0043
20.0	16.44(19)	26.09(16)	33.56(13)	3.0-10.0	52	3.41	-1.09	4.20	9.00	0.0099
30.0	16.64(36)	25.04(25)	32.45(16)	3.0-10.0	54	8.90	0.54	6.37	46.74	0.0167
40.0	17.08(19)	25.81(13)	32.99(17)	3.0-10.0	88	4.5	-0.81	4.13	34.41	0.0111
50.0	17.55(27)	26.21(12)	32.78(32)	3.0-9.0	20	1.48	-1.38	3.19	18.20	0.0077
60.0	18.63(58)	26.18(26)	33.01(49)	3.0-9.5	23	4.22	-1.48	3.64	20.35	0.0139

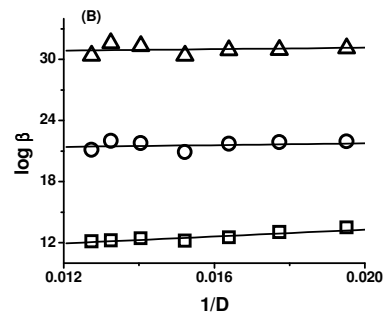
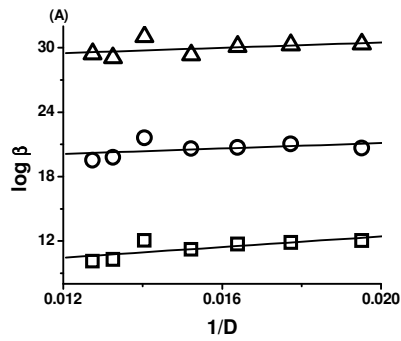
$U_{corr} = U / (NP - m)$, m = number of species; NP=Number of experimental points; SD= standard deviation

Table 2: Effect of errors in influential parameters on stability constants of ternary complexes of Asp - Zn(II)-En in 10% v/v PG-water mixtures

Ingredient	% Error	log β_{mlxh} (SD)		
		MLX ₂	MLX ₂ H	MLX ₂ H ₂
	0	15.62(11)	24.52(7)	31.51(14)
Alkali	-5	Rejected	Rejected	30.66(**)
	-2	14.41(28)	24.11(18)	31.85(17)
	+2	17.21(28)	25.55(18)	32.82(17)
	+5	19.02(49)	25.87(**)	33.63(33)
Acid	-5	19.03(66)	26.11(**)	33.77(42)
	-2	17.15(36)	25.57(22)	32.87(21)
	+2	14.56(32)	24.16(21)	31.79(24)
	+5	Rejected	Rejected	28.74(**)
p(L)	-5	15.42(16)	24.68(11)	32.16(12)
	-2	15.69(11)	24.84(7)	32.27(8)
	+2	16.04(17)	25.05(11)	32.41(12)
	+5	16.31(27)	25.20(16)	32.52(18)
En(X)	-5	16.31(12)	25.15(7)	32.40(9)
	-2	16.04(12)	25.03(8)	32.37(9)
	+2	15.68(13)	24.87(8)	32.32(8)
	+5	15.41(13)	24.73(8)	32.28(8)
	-5	16.19(13)	25.08(8)	32.40(9)
	-2	15.99(13)	25.00(8)	32.36(9)

Metal	+2	15.74(12)	24.90(8)	32.32(9)
	+5	15.56(12)	24.81(8)	32.29(8)
log F	-5	15.83(12)	24.93(8)	32.32(8)
	-2	15.85(12)	24.94(8)	32.33(9)
	+2	15.88(13)	24.96(8)	32.35(9)
	+5	15.89(13)	24.97(8)	32.36(9)

(**) = Standard Deviation is very high



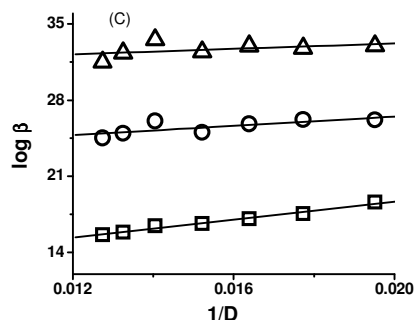


Figure 1: Variation of stability constant ($\log \beta$) values of ternary complexes of (A) Ca(II), (B) Mg(II) and (C) Zn(II) with Asp and En with reciprocal of dielectric constant ($1/D$) of PG - water mixtures: (\square) $\log \beta_{MLX_2}$, (\circ) $\log \beta_{MLX_2H}$ and (Δ) $\log \beta_{MLX_2H_2}$ respectively.

Table 3: The $\Delta \log K$ values of MLX_2 , MLX_2H , MLX_2H_2 of Ca(II), Mg(II) and Zn(II)-Asp and En in PG-water mixtures calculated using various possible equations given below.

% v/v PG	$\log K_{MLX_2}$	$\log K_{MLX_2H}$	$\log K_{MLX_2H_2}$
Ca(II)			
00.0	1.91	1.72	1.74
10.0	1.5	1.82	1.54
20.0	2.85	2.31	2.18
30.0	1.48	1.28	1.63
40.0	1.76	1.41	1.43
50.0	0.87	0.85	2.49
60.0	0.98	0.3	1.82
Mg(II)			
00.0	4.38	3.35	3.47
10.0	3.86	4.0	4.58
20.0	2.68	2.19	1.77
30.0	-0.68	-1.65	-0.35
40.0	0.78	0.02	0.68
50.0	1.75	0.8	1.75
60.0	0.67	-0.21	0.98
Zn(II)			
00.0	-1.17	0.39	0.78
10.0	-0.83	0.36	1.4
20.0	-1.68	-0.25	0.44
30.0	-0.09	0.82	1.45
40.0	-1.43	-0.52	0.29
50.0	-1.04	0.42	0.26
60.0	0.89	1.65	1.19

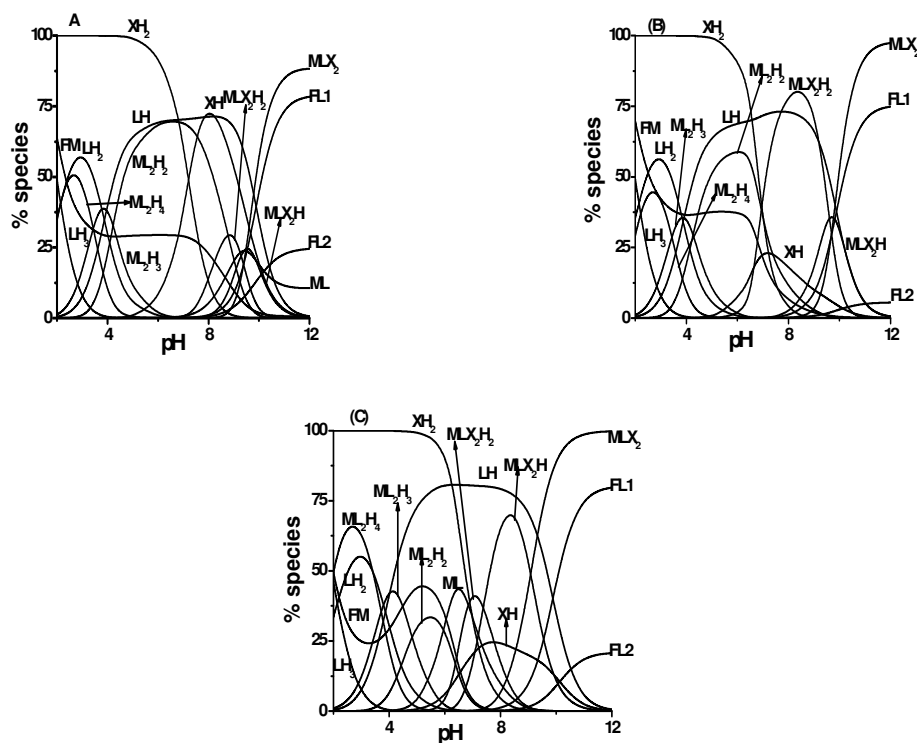
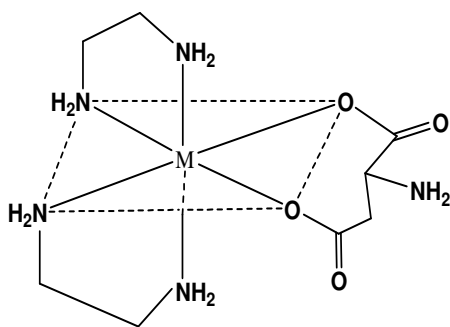
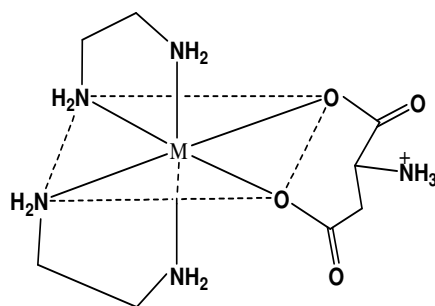


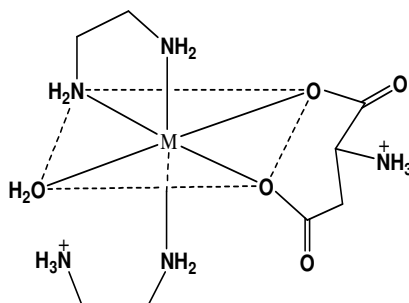
Figure 2: Distribution diagrams of Ternary complexes of Aspand en in 10% v/v PG- water mixture: (A)Ca(II), (B) Mg(II) and (C) Zn(II).



MLX₂



MLX₂H



MLX₂H₂

Figure 3: Possible structures of the ternary complexes

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