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A study on the formation and confirmation of ternary complexes of phenylalanine and maleic acid with Ca (II), Mg(II) and Zn(II) metal ions in acetonitrile water mixtures

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Abstract

Chemical speciation of ternary complexes of Ca(II), Mg(II) and Zn(II) ions with Phenylalanine and Maleic acid was studied pH metrically in the concentration range of 0.0–60.0% v/v acetonitrile-water mixtures maintaining an ionic strength of 0.16 mol L⁻¹ at 303.0 K. Alkalimetric titrations were carried out in different relative concentrations (M:L:X = 1:2.5:2.5, 1:2.5:5.0, 1:5.0:2.5) of metal (M) to phenylalanine (L) to maleic acid (X). Stability constants of ternary complexes were calculated and various models were refined with MINQUAD75. The best fit chemical models were selected based on statistical parameters and residual analysis. The species detected are MLXH and ML₂XH for Ca(II), Mg(II) and Zn(II) in AN-water mixtures. The chemical speciation, metal bioavailability and transportation were explained based on the stability constants.

Keywords: Chemical speciation, ternary complexes, phenylalanine, maleic acid, metal ions, acetonitrile, MINQUAD75.

1. Introduction

A number of studies have been reported on ternary stability constants of α -amino acids in different media [1]. Acidity and basicity of a molecule is governed by its structure and solvent effects [2, 3]. Phenylalanine (Phe) is a strong α -amino acid [4]. This essential amino acid is classified as non - polar because of the hydrophobic nature of the benzyl side chain [5]. The first description of phenylalanine was made in 1879, when Schulze and Barbieri identified [6, 7]. Phe 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 neuro modulator phenylethylamine, Phe commonly used dietary supplement. Phe is the starting compound used in the flavonoid biosynthesis. Lignan is derived from phenylalanine and from tyrosine. It is converted to cinnamic acid by the enzyme phenylalanine ammonia-lyase. The biological functions of D-amino acids remain unclear, although D-phenylalanine has pharmacological activity at niacin receptor 2 [8].

L-Phenylalanine is an antagonist at higher doses, this may play a role in its analgesic and antidepressant properties [9]. In the brain, L-phenylalanine is a competitive antagonist at the glycine binding site of NMDA receptor [10] and at the glutamate binding site of AMPA receptor [11]. Maleic acid (Mal) is an organic compound this is an unsaturated dicarboxylic acid, a molecule with two carboxyl groups. Mal is the cis-isomer of butenedioic acid, whereas fumaric acid is the trans-isomer. It is mainly used as a precursor to fumaric acid, and relative to its parent maleic anhydride, Mal is more soluble in water. The solvent effects of phenols, amines and carboxylic acids have been examined [12]. A number of studies have been reported on protonation constants of α -amino acids in different media [13-16]. Acidity and basicity of a molecule is governed by its structure and solvent effects [17, 18]. The protonation constants of phenylalanine and maleic acid were reported earlier [19, 20].

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' [21-26]. 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 [21-26]. Zinc plays vital roles in biological systems [27-30]. Zinc plays catalytic and structural roles to maintain the protein configuration. It is a versatile ion as it can bind to different combinations of ligand types resulting in a broad range of stability, reactivity and functions [31].

Binary stability constants of Phe and Mal [32-35] with Ca (II), Mg (II) and Zn (II) have been reported earlier. Hence, chemical speciation of their ternary complexes is reported in present communication.

2. Experimental

Aqueous solutions (0.1 mol L⁻¹) of Ca(II), Mg(II) and Zn(II) chlorides (GR Grade, E-Merck, Germany) were prepared by dissolving them in triple distilled water, 0.05 mol L⁻¹ aqueous solutions of Phenylalanine (GR Grade E-Merck, Germany) and Maleic acid (AR, Qualigen, India) were also prepared. To increase the solubility of the ligands and metal salts, 0.05 mol L⁻¹ hydrochloric acid was maintained in the solutions. Acetonitrile (AR, E-Merck) is used as received.

The strength of alkali was determined using the Gran plot method [36, 37]. Errors in the concentrations of ligand, metal ions and alkali were subjected to analysis of variance (ANOVA) [38].

The titrations were carried out in the medium containing varying concentrations of AN maintaining an ionic strength of 0.16 mol L⁻¹ with sodium chloride at 303.0 K. The measurements were recorded with an ELICO (Model LI-120) pH meter of 0.01 readability in conjunction with a glass and calomel electrode. The pH meter was calibrated with 0.05 mol L⁻¹ potassium hydrogen phthalate in acidic region and 0.01 mol L⁻¹ borax solution in basic region. The glass electrode was equilibrated in a well stirred AN-water mixtures containing inert electrolyte. The 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 (log F) which was computed from the experimental and simulated acid-base titration data calculated by SCPHD program [38]. A correction was applied to the pH meter dial readings to account for the solvent effect on pH.

Titration of strong acid with alkali was carried out at regular intervals to check whether complete equilibrium was achieved. The calomel electrode was refilled with AN-water mixtures of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of 1 mmol of hydrochloric acid in a total volume of 50 cm³. Titrations were carried out in the presence of different relative concentrations of the metal (M) to Phe (L) and to Mal (X) (M:L:X = 1:2.5:2.5, 1:2.5:5.0, 1:5.0:2.5) with 0.4 mol L⁻¹ NaOH (Table 1).

Table 1: Total initial concentrations of ingredients (in mmol) for mixed ligands (Phe and Mal) titrations in AN-water mixtures. [NaOH] = 0.4 mol L⁻¹; V₀ = 50 cm³; Temperature = 303.0 K; Mineral acid = 1.0 mmol; μ = 0.16 mol L⁻¹.

AN% v/v	TM0			TL0	TX0	TM0:TL0:TX0
	Ca(II)	Mg(II)	Zn(II)	Phe	Mal	
0.0	0.100	0.099	0.102	0.2500	0.2498	1:2.5:2.5
				0.2500	0.4996	1:2.5:5.0
				0.5005	0.2498	1:5.0:2.5
10.0	0.100	0.099	0.102	0.2498	0.2503	1:2.5:2.5
				0.2498	0.5000	1:2.5:5.0
				0.5010	0.2503	1:5.0:2.5
20.0	0.100	0.099	0.102	0.2492	0.2500	1:2.5:2.5
				0.2492	0.5005	1:2.5:5.0
				0.5005	0.2500	1:5.0:2.5
30.0	0.100	0.099	0.102	0.2510	0.2506	1:2.5:2.5
				0.2510	0.4990	1:2.5:5.0
				0.4992	0.2506	1:5.0:2.5
40.0	0.100	0.099	0.102	0.2550	0.2515	1:2.5:2.5
				0.2550	0.5010	1:2.5:5.0
				0.4990	0.2515	1:5.0:2.5
50.0	0.100	0.099	0.102	0.2500	0.2505	1:2.5:2.5
				0.2500	0.4992	1:2.5:5.0
				0.5005	0.2505	1:5.0:2.5
60.0	0.100	0.099	0.102	0.2501	0.2503	1:2.5:2.5
				0.2501	0.4998	1:2.5:5.0
				0.5002	0.2503	1:5.0:2.5

The details of experimental procedure and titration assembly were given elsewhere [39]. The best-fit chemical model for each system investigated was arrived at using a non-linear least squares analysis program MINQUAD75 [40], which

exploits the advantage of constrained least squares method in the initial refinement and reliable convergence of undamped, unconstrained Marquardt algorithm.

3. Results and discussion

3.1. Modeling of chemical speciation

A preliminary investigation of alkalimetric titrations of mixtures containing different mole ratios of Phe and Mal in the presence of hydrochloric acid and inert electrolyte inferred that no condensed species were formed. The protonation constants and the stability constants of the binary metal complexes of these ligands were fixed in refining ternary complexes and in testing various chemical models using MINQUAD75. The best fit models were chosen based on the statistical parameters like χ^2 , R-factor, skewness and kurtosis given in Table 2. The ternary complex species detected are MLXH and ML₂XH for Ca (II), Mg (II) and Zn (II).

A very low standard deviation (SD) overall stability constants (log β) indicates the precision of these parameters. The small values of U_{corr} (sum of squares of deviations in

the concentrations of the metal, the ligands and the hydrogen ion at all experimental points corrected for degrees of freedom) indicate that the models represent the experimental data. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that most of the residuals are very nearer to leptokurtic and a few form mesokurtic patterns. The values of skewness recorded in Table 2 are between -0.38 and 1.87. These data evince that the residuals form a part of normal distribution hence, least-squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values recorded.

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

% v/v AN	log $\beta_{\text{mLXH}}(\text{SD})$		NP	U_{corr}	χ^2	Kur-Tosis	Skew-Ness	R-Factor	pH-range
	1111	1211							
Ca(II)									
0.0	27.79(1)	29.43(1)	92	8.38	10.26	3.23	-0.25	0.0046	1.8-7.7
10.0	27.92(2)	29.78(2)	94	2.35	19.62	3.96	-0.03	0.0125	1.8-7.7
20.0	28.06(2)	29.96(3)	87	7.74	9.93	3.64	-0.15	0.0114	1.8-7.7
30.0	28.42(3)	30.12(1)	118	3.13	22.61	1.94	1.87	0.0026	1.8-7.7
40.0	28.87(1)	30.46(2)	101	7.89	21.09	4.03	0.93	0.0264	1.8-7.7
50.0	29.02(1)	30.88(1)	98	1.97	14.5	5.21	1.02	0.0116	1.8-7.7
60.0	29.32(2)	31.02(2)	96	3.40	30.00	3.42	-0.38	0.0265	1.8-7.7
Mg(II)									
0.0	28.7(1)	30.12(1)	110	1.04	22.76	3.34	-0.28	0.0026	1.8-7.7
10.0	28.92(2)	30.48(2)	87	1.15	6.02	3.34	-0.17	0.0176	1.8-7.7
20.0	29.12(2)	30.86(3)	107	1.73	10.36	3.30	-0.07	0.0180	1.8-7.7
30.0	29.43(3)	31.12(3)	53	8.11	10.42	2.48	-0.10	0.0242	1.8-7.7
40.0	29.86(1)	31.52(2)	69	1.87	15.84	1.48	-0.28	0.0087	1.8-7.7
50.0	30.01(1)	31.96(2)	74	5.34	19.29	1.47	-0.24	0.0487	1.8-7.7
60.0	30.22(1)	32.05(2)	89	2.77	17.71	4.04	-0.40	0.0234	1.8-7.7
Zn(II)									
0.0	29.12(2)	31.75(1)	100	6.62	10.40	3.69	-0.31	0.0036	1.8-7.7
10.0	30.01(4)	32.86(5)	89	1.28	6.52	3.78	-0.18	0.0018	1.8-7.7
20.0	30.85(3)	33.52(3)	113	6.99	14.42	3.09	0.02	0.0137	1.8-7.7
30.0	31.52(3)	34.12(2)	141	2.75	19.74	5.35	0.39	0.0363	1.8-7.7
40.0	32.16(3)	35.02(2)	141	2.79	39.74	5.35	0.39	0.0261	1.8-7.7
50.0	32.77(1)	35.52(1)	117	3.34	21.71	4.02	-0.06	0.0123	1.8-7.7
60.0	33.18(2)	36.15(3)	96	1.32	16.86	4.42	0.64	0.0032	1.8-7.7

$U_{\text{corr}} = U / (\text{NP} - m) \times 10^8$; where m = number of species; NP = Number of experimental points.

3.2. Effect of dielectric constant on stability of ternary complexes

Acetonitrile is a weak base. Therefore cations and especially anions have lower solvation energies in and specific interactions with the solvent. It is a protophilic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. Hence, it removes water from the 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 it competes with the ligands for coordinating the metals. This decreases the stability of the complexes. Hence, the stability of the complexes is expected to either increase or decrease. The variation of overall

stability constants with co-solvent content depends upon electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change^[41]. 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 (1/D) of the medium. The linear variation observed in the present study (Figure 1) indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions. This also indicates the dominance of structure breaking nature of AN over its complexing ability.

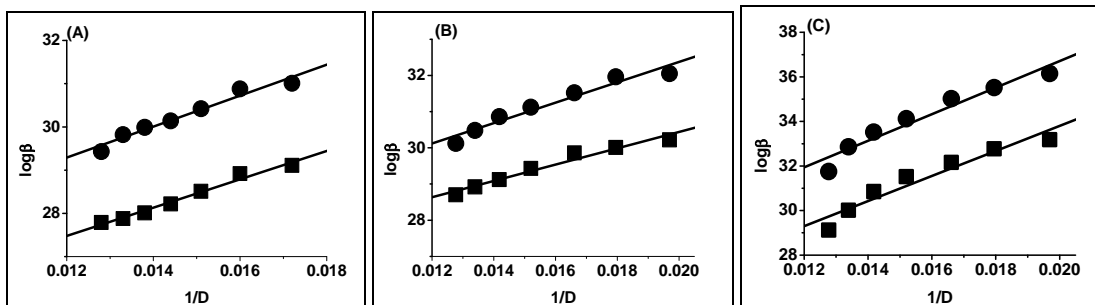


Fig 1: Variation of stability constant values of Phe and Mal complexes with reciprocal of dielectric constant (1/D) of Ca(II) (A); Mg(II) (B) and Zn(II) (C); (■)logβ_{MLXH}; (●)logβ_{ML2XH}; in AN- water mixtures.

3.3. Quantification of change in stability of ternary species

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 [42, 43] which corresponds to the equilibrium $ML_2 + MX_2 \rightleftharpoons 2MLX$. 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. A value greater than this accounts for the extra stability of MLX.

Another approach to quantify the extra 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 [44-47], where L is the primary ligand (Phe) and X is the secondary ligand (Mal). It is compared with that calculated purely on statistical grounds as given in Equation 2.

$$\Delta \log K = \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \dots\dots 2$$

The equations for the calculation of $\Delta \log K$ and log X are given in Chart 1.

Chart 1. Equations for the calculation of $\Delta \log K$ and log X values from the overall stability constants.

$\Delta \log K$				log X		
$\Delta \log K_{1111}$	$=\log \beta_{1111}$	$-\log \beta_{1101}$	$-\log \beta_{1010}$	$\log X_{1111}$	$=2\log \beta_{1111}$	$-\log \beta_{1201}$
	$=\log \beta_{1111}$	$-\log \beta_{1100}$	$-\log \beta_{1011}$		$=2\log \beta_{1111}$	$-\log \beta_{1200}$
	$=\log \beta_{1111}$	$-\log \beta_{1100}$	$-\log \beta_{1010}$		$=2\log \beta_{1111}$	$-\log \beta_{1020}$

The electrostatic theory of binary complex formation and statistical arguments suggest the availability of additional coordination positions of the hydrated metal ion for the first ligand than for the second. Hence, the usual order of stability $K_{ML}^M > K_{ML_2}^M$ applies. This suggests that $\Delta \log K$ should be negative, although several exceptions have been found [48]. The statistical values of $\Delta \log K$ and log X for Phe (L) and Mal (X) are, 14.77 to 18.73 and 21.81 to 31.76

for MLXH species respectively in AN-water mixtures (Table 3). Negative values of $\Delta \log K$ can be understood as the secondary ligand forms a more stable complex with hydrated metal ion than with L. Whenever the experimental values of $\Delta \log K$ exceed the statistical values, it can be inferred that the ternary complex is formed as a result of interaction of ML with X or MX with L.

Table 3: $\Delta \log K$ and log X values of ternary complexes of Ca(II),Mg(II) and Zn(II)-Phe and Mal in AN- water mixtures

% v/v AN	$\Delta \log K$		log X
	1111		1111
Ca(II)			
0.0	18.01		26.20
10.0	17.53		24.53
20.0	16.33		23.64
30.0	14.77		21.81
40.0	15.54		23.21
50.0	15.86		23.31
60.0	15.65		23.23
Mg(II)			
0.0	18.39		29.03
10.0	17.25		27.39
20.0	16.98		27.04
30.0	14.88		26.35
40.0	15.76		26.71
50.0	15.92		27.20
60.0	12.91		24.66
Zn(II)			
0.0	17.93		28.30
10.0	16.67		27.66
20.0	16.67		28.14
30.0	15.95		28.16
40.0	17.72		29.70
50.0	18.73		31.21
60.0	16.83		31.76

The $\Delta \log K$ and $\log X$ values could not be calculated for the ML_2XH system due to the absence of relevant binary species. The $\log X$ values, some of which 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 for the extra stability of these 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 [49, 50]. The extra stability of ternary complexes makes them more amenable for metal transport. The less stable binary complexes make the metals bioavailable.

4. Distribution diagrams

The distribution of metal ions in various complex species (chemical speciation) with pH is represented as distribution diagrams. Some typical distribution diagrams are given in Figure 2.

The mixed-ligand species of Phe (L) and Mal (X)

$M(II) + LH + XH$	\rightleftharpoons	$MLXH + H^+$	1
$M(II) + LH_2 + XH_2$	\rightleftharpoons	$MLXH + 3H^+$	2
$M(II) + LH + XH_2$	\rightleftharpoons	$MLXH + 2H^+$	3
$M(II) + LH_2 + XH$	\rightleftharpoons	$MLXH + 2H^+$	4
$M(II) + 2LH + XH$	\rightleftharpoons	$ML_2XH + 2H^+$	5
$M(II) + 2LH_2 + XH_2$	\rightleftharpoons	$ML_2XH + 5H^+$	6
$M(II) + 2LH + XH_2$	\rightleftharpoons	$ML_2XH + 3H^+$	7
$M(II) + 2LH_2 + XH$	\rightleftharpoons	$ML_2XH + 4H^+$	8

Detected are $MLXH$ and ML_2XH for Ca (II), Mg (II), and Zn(II) in EG - water mixtures. A perusal of the distribution diagrams (Figure 2) reveals that at very low pH the concentrations of the mixed ligand complexes are less than those of protonated ligands. The concentrations of the ternary species increase with pH. The protonated ternary species, $MLXH$ and ML_2XH are distributed at lower pH. The concentrations of binary species are less compared to the ternary species due to more stable ternary complexes. The ternary species of Ca (II), Mg (II) and Zn (II) exist in the pH range 4.0-10.0. The formation of the complex species can be represented by the following equilibria.

Distribution diagrams drawn using the formation constants of the best fit model are shown in Figure 2. Mixed ligand complexes of Ca (II), Mg (II), and Zn(II) are detected in the pH range 4.0-10.0. $MLXH$ is the predominant species formed in the pH range 4.0-8.0 by the interaction of M (II) with protonated ligands (LH, LH_2 , XH and XH_2) (Equilibria 1-4). The ML_2XH species is formed at the pH range 7.0-10.0. ML_2XH species may be formed from the Equilibria 5-8. Suggested structures for ternary complexes of Phe and Mal, where M = Ca (II), Mg(II) and Zn(II) in EG-water are given in Figure 3.

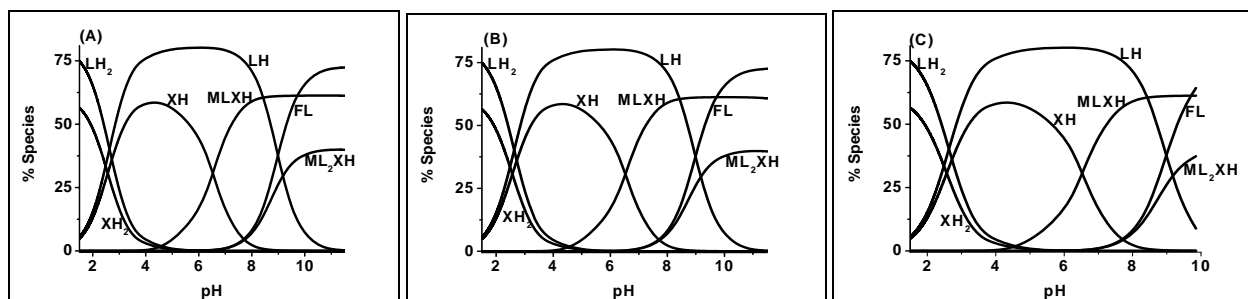


Fig 2: Species distribution diagrams of Phe and Mal ternary complexes in 30% v/v, AN- water mixtures. (A) Ca(II), (B) Mg(II) and (C) Zn(II).

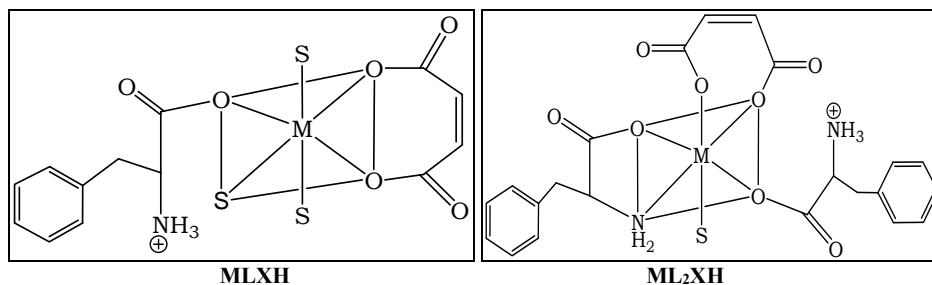


Fig 3: Proposed structures of ternary complexes of Ca (II), Mg(II) and Zn(II), where S is either solvent or water molecules.

5. Conclusions

The following conclusions have been drawn from the modeling studies of the speciation of ternary complexes of Ca(II), Mg(II) and Zn(II) with Phe and Mal in AN-water mixtures.

1. The ternary metal complex species detected are MLXH and ML₂XH for Ca(II), Mg(II) and Zn(II), where L = Phe and X = Mal.
2. The values of $\Delta \log K$ and $\log X$ 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 interactions 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 study also gives an insight into the metal availability/metal transport in biofluids. The ternary complexes are more amenable for "metal transport" because of their extra stability.

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