

Chemical Speciation of ternary Complexes of L - Phenylalanine and Maleic Acid under mimicked physiological condition; Potentiometric studies.

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Abstract

Chemical speciation of ternary complexes of Co (II), Ni (II) and Cu (II) ions with L- Phenylalanine and Maleic acid was studied pH metrically in the concentration range of 0–50 % v/v DMF-water mixture maintaining an ionic strength of 0.16 mol L⁻¹ at 303.0 K. Alkali metric 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 derived 'best fit' chemical speciation models are based on crystallographic R-factors, χ^2 and Skewness and Kurtosis factors. The species detected are MLXH, MLX and ML₂X for Co (II), Ni (II) and Cu (II) in DMF water mixture. The chemical speciation, metal bioavailability and transportation were explained based on the stability constants.

Keywords: Chemical speciation, ternary complexes, phenylalanine, maleic acid, DMF, MINQUAD75.

1 Introduction

The word speciation while referring to the chemical form of an element also refers to the oxidation state in which that element exists in the environment. Recently the International Union of Pure and Applied Chemistry (IUPAC) sums up that “speciation” denotes to the distribution of an element amongst defined chemical species in a system. Generally, speciation studies involve estimating individual physico-chemical or geochemical forms of an element that collectively make up the total concentration of that element in a sample. While the term is being debated for a definite meaning, various authors have been using it to explain one or more of the following: i) the chemical reactions that transform a set of metal compounds in a sample into another set of components, ii) the assembly of compounds containing a particular element that are present in a sample and iii) the process of identifying and quantifying the metal species present in a sample [1, 2].

In biological fluids, the metal ions exist in nonexchangeable form as metalloproteins or loosely bound to some biological ligands as in metal-activated proteins. The loosely bound metal ions are in equilibrium with

similar metal ions present in the bio-fluids. These simultaneous equilibria involving a variety of metal ions and ligands are important in biological fluids [3].

The usefulness of speciation studies has been identified in chelation therapy as it provides information on co-excretion of some essential metals and indicates the need of 'topping up' of essential metals [4]. Recent observations also stress the need to address pollutions and contaminations from the perspective of chemical speciation rather than looking at the total concentration, as is mostly done. The assimilation of essential trace elements by organisms takes place in preferred chemical forms [5]. Speciation studies have found application in quality check for contamination of food products (e.g., oysters and mussels by organotin, fish by methyl mercury, and wine by lead compounds), risk analysis [6], dental care, total parenteral nutrition [7], metallurgical operations and environmental protection [8]. Speciation and bioavailability, are frequently interlinked because the speciation of the metal is often related to the bioavailability of metals [9]. Furthermore, in considering the interaction of trace metal with living organisms, three broad areas of concern can be identified: i) metal speciation in the external environment, ii) metal interaction with the biological membrane and iii) metal partitioning within the organism and the accompanying biological effects [10]. The biological response (BR) of organisms is more or less proportional to the activity of the free metal ion [11]. Exception to this observation is also known. For example, fat-soluble organo mercurial and chloro complexes of mercury have a preferential partitioning to the fat tissues than Hg^{2+} a process of simple extraction. Complexes are formed by some of the essential elements such as cobalt, nickel, copper, zinc, calcium, magnesium and heavier elements like arsenic, selenium, cadmium, indium, antimony, tellurium, mercury, thallium, lead, and bismuth. These elements and their complexes are mobilized and transported in air, surface waters, in sediments and in the soil, thereby significantly perturbing the environment. Moreover, their intakes by humans via food, water, and particulates in the air have caused health concerns [12]. Amino acids can serve as a source of energy, carbon or nitrogen. It can be joined to its neighbor by a specific type of covalent bond. Every protein molecule can be considered as a polymer of amino acids.

L-phenylalanine (F) is non-polar α -amino acid because of the hydrophobic nature of the benzyl side chain. It 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 phenyl ethylamine, Phe commonly used dietary supplement. L-Phenylalanine is an antagonist at higher doses, this may play a role in its analgesic and antidepressant properties. It is the starting compound used in the flavonoid biosynthesis. It is converted to cinnamic acid by the action of enzyme phenylalanine ammonia-lyase [13].

Maleic acid is an organic compound. It is an unsaturated dicarboxylic acid, a molecule with two carboxyl groups. Maleic acid 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. Maleic acid and fumaric acid do not spontaneously interconvert because rotation around a carbon-carbon double bond is not energetically favorable. However, conversion of the cis isomer into the trans isomer is possible by photolysis in the presence of a small amount of bromine [14].

N, N'-Dimethylformamide (DMF) is a polar (hydrophilic) aprotic solvent with high boiling point. It facilitates reactions that follow polar mechanisms. It is able to form the H-bonded network structures with dipolar aprotic solvents [15]. Therefore, DMF-water mixture was selected as media to maintain the dielectric properties of the medium in comparable levels to those of the physiological fluids since the polarity at the active site cavities should generally be applicable when it is possible to compare ligand binding to the metal ion as in protein residues and solvent environment [16]. This study is undergoing to investigate chemical speciation of ternary complexes of Co (II), Ni (II) and Cu (II) with L-phenylalanine and maleic acid in aqueous and DMF-water mixtures.

2. Materials and methods

2.1 Apparatus

Test tubes, 25 mL volumetric pipette, pH meter, burette, glass electrode, funnel, beakers, 125 mL or 250 mL Erlenmeyer flasks, mass balance, measuring cylinder, dropper and stirrers.

2.2 Chemicals

All the chemicals used in this investigation were of analytical reagents grade purity. L- phenylalanine, maleic acid, HCl, methanol, acetone or diethyl ether, triple distilled water, NaOH pellet, hexamethylenetetramine powder, oxalic acid, potassium hydrogen phthalate, borax, DMF, EDTA, acetic acid, 2-amino ethanol, ZnSO₄, CoCl₂, CuCl₂, NiCl₂, NaCl, Eriochrome-black.T as indicators, xylene orange, murexide and sulphone black-F as indicator.

2.3. Experimental Procedure

2.3.1. Preparation of Solutions

All solutions were prepared in boiled out triple distilled water.

2.3.1.1. L-phenylalanine and Maleic acid Solutions

0.05 moldm⁻³ aqueous solution of L-phenylalanine (GR grade, E-Merck, Germany) and maleic acid (GR grade, E-Merck, Germany) were prepared by dissolving samples in water. To increase the solubility of ligands, 0.05 moldm⁻³ hydrochloric acid concentration was maintained in the solutions. The probable errors that may creep into the concentrations of the stock solutions of the ligands were determined by the computer program COSWT [17]. The pessimistic errors in the preparation of the ligand solutions by weight method did not exceed 0.1%.

2.3.1.2. EDTA Solution

Disodium salt of EDTA was purified by precipitation from aqueous solution using methanol. The precipitate was washed with acetone, diethyl ether and finally air dried. An 0.1 mol dm^{-3} solution was prepared and standardized complex metrically with a 0.1 mol dm^{-3} standard Zn (II) solution using Eriochrome Black-T as indicator.

2.3.1.3. Metal ion Solutions

0.1 mol dm^{-3} aqueous solutions of cobalt (II), nickel (II) and copper (II) chlorides were prepared by dissolving GR grade (E-Merck, India) salts in triple distilled water. The stock solutions were rendered slightly acidic to repress hydrolysis of the metal ions. The concentrations of the metal ions were determined complex metrically by titrating against a standard solution of EDTA using the xylenol orange, murexide and fast sulphon black-F as indicators and hexamethylenetetramine powder as buffer for cobalt to maintain the pH at 5-6. The free hydrogen ion concentration in the stock solution was determined by Gran plot method [18].

2.3.1.4. Sodium Hydroxide

A stock solution of 1 mol dm^{-3} sodium hydroxide was prepared by dissolving GR grade (E Merck, India) sodium hydroxide pellets in triple distilled water. The solution was further diluted to the required concentration. The strength of the alkali was determined by titrating it against a standard solution of oxalic acid and potassium hydrogen phthalate, while the molarity of hydrochloric acid was determined with sodium hydroxide. So as 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) using the computer program COST(concentration of solution by titration). The strength of alkali was determined using the Gran plot method. The errors in the concentration of ligand, metal ions and alkali were subjected to analysis of variance ANOVA [19].

2.3.1.5. Hydrochloric Acid

The concentration of stock hydrochloric acid (GR grade, Merck, India) solution was calculated from its specifications (specific gravity, purity and molecular weight) and diluted to the required concentration by dissolving in triple distilled water. Its strength was determined by titrating with standard sodium hydroxide solution.

2.4. Solvent

GR grade N, N'-Dimethylformamide (Finar, India) of 99.5% purity was used as a solvent.

2.5. Titration procedure

The pH measurements of the proton–ligand systems were carried out in aqueous media containing varying compositions of organic solvent (DMF) in the range of 0 - 50% v/v maintaining an ionic strength of 0.16 mol dm⁻³ with sodium chloride at 303.0 K using a digital pH meter ELICO-LI120 type (readability 0.01). Potassium hydrogen phthalate (0.05 mol dm⁻³) and borax (0.01 mol dm⁻³) solutions were used to calibrate the pH meter. In each titration, the titrand consisted of approximately 1 mmol of nitric acid. The amounts of the L-phenylalanine, maleic acid (ligands) in the titrand are in the range of 0.25–0.50 mmol. The glass electrode was equilibrated in a well stirred DMF–water mixture containing inert electrolyte for several days. At regular intervals, the strong acid was titrated against alkali to check the complete equilibration of the glass electrode. In these titrations, the titrand consisted of mineral acid and ligand, in a total volume of 50 mL. Titrations were performed by adding each time 0.1 cm³ portions of 0.4 mol dm⁻³ sodium hydroxide to the titrand. The pH meter reading was recorded only after a constant value was displayed. Typical duplicate titrations showed that equilibration was fast and titration data did not differ by more than 0.02 units [20].

2.6 Metal-ligand Equilibria

In each of the titrations, the titrand consisted of approximately 1 mmol hydrochloric acid in a total volume of 50 mL and the ionic strength was adjusted to 0.16 mol L⁻¹ with sodium chloride. Solutions containing metal ions and ligands (metal to ligand ratios being in the range of 1:2.5:2.5, 1:2.5:5.0, 1:5.0:2.5) were titrated with 0.4 mol L⁻¹ sodium hydroxide.

2.7 Modeling strategy

The approximate protonation constants of L-phenylalanine and maleic acid were calculated with the computer program SCPHD [21]. The best fit chemical model for each system investigated was arrived at using non-linear least squares computer program, MINQUAD75, which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants (log K) with the mole fraction of the medium was analyzed on electrostatic grounds for the solute–solute and solute–solvent interactions [22].

3 Result and discussion

The results of the final ‘best-fit’ models that reveal the stoichiometry of the complex species and their overall formation constants along with some of the associated statistical parameters are given in table 1. Very low-standard deviation in overall stability constants (log β) signifies the precision of these data. The small values of Ucorr (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 studied are validated by the residual analysis [23, 24].

Table 1: parameters of best fit chemical model of F-M (II)-MA complexes in DMF-water mixtures. Temperature = 303K, Ionic strength = 0.16mol⁻³.

| DMF % v/v | log $\beta_{\text{mlh}}(\text{SD})$ | | | pH- Range | NP | U_{corr} *10 ⁸ | χ^2 | Skew -ness | Kurt- osis | R- factor |
|--------------|-------------------------------------|----------|-------------------|--------------|----|---------------------------------------|----------|---------------|---------------|--------------|
| | MLXH | MLX | ML ₂ X | | | | | | | |
| Co(II) | | | | | | | | | | |
| 0.0 | 19.60(7) | 12.97(2) | 17.07(7) | 1.5-8.7 | 77 | 5.38 | 5.92 | 0.02 | 2.74 | 0.0083 |
| 10 | 20.17(1) | 13.01(3) | 17.38(6) | 1.5-8.7 | 88 | 14.19 | 7.20 | 0.02 | 2.87 | 0.0043 |
| 20 | 21.13(7) | 14.67(6) | 18.49(8) | 1.5-8.8 | 90 | 8.09 | 3.11 | -0.04 | 2.86 | 0.0008 |
| 30 | 21.14(6) | 15.17(1) | 19.45(3) | 1.5-8.8 | 59 | 5.19 | 6.74 | 0.13 | 3.09 | 0.0047 |
| 40 | 22.12(1) | 14.98(5) | 19.07(5) | 1.5-8.9 | 70 | 19.08 | 6.16 | -0.16 | 2.81 | 0.0066 |
| 50 | 22.18(1) | 15.63(3) | 20.27(9) | 1.5-8.9 | 80 | 12.03 | 8.73 | -0.13 | 2.63 | 0.0030 |
| Ni(II) | | | | | | | | | | |
| 0.0 | 19.13(9) | 12.56(5) | 17.78(9) | 1.5-7.5 | 54 | 1.50 | 1.93 | -0.05 | 2.15 | 0.0018 |
| 10 | 20.35(9) | 13.84(4) | 19.26(7) | 1.5-7.6 | 54 | 9.34 | 25.71 | -0.03 | 3.28 | 0.0016 |
| 20 | 21.03(8) | 14.93(4) | 19.67(2) | 1.5-7.6 | 53 | 7.45 | 6.15 | -0.86 | 2.27 | 0.0004 |
| 30 | 20.15(1) | 13.89(2) | 19.83(2) | 1.9-8.6 | 76 | 5.34 | 5.12 | 0.65 | 3.80 | 0.0039 |
| 40 | 21.19(3) | 14.43(1) | 19.67(3) | 1.7-8.6 | 46 | 8.328 | 6.86 | -0.15 | 2.80 | 0.0062 |
| 50 | 19.08(2) | 12.70(4) | 18.47(3) | 1.7-8.6 | 49 | 7.11 | 4.55 | -1.06 | 2.50 | 0.0056 |
| Cu(II) | | | | | | | | | | |
| 0.0 | 19.73(1) | 14.83(5) | 21.99(3) | 1.8-6.3 | 90 | 3.24 | 6.24 | 0.07 | 2.60 | 0.0009 |
| 10 | 20.16(9) | 14.96(5) | 21.58(3) | 1.8-6.3 | 99 | 8.73 | 9.33 | -0.06 | 2.80 | 0.0024 |
| 20 | 21.32(9) | 16.34(1) | 23.62(2) | 1.8-6.3 | 66 | 6.91 | 4.67 | -0.14 | 3.06 | 0.0003 |
| 30 | 21.06(7) | 16.12(1) | 23.37(7) | 1.9-6.3 | 80 | 7.46 | 8.04 | 0.33 | 2.33 | 0.0093 |
| 40 | 22.55(7) | 17.28(4) | 23.84(2) | 2.2-6.3 | 90 | 7.32 | 6.80 | -0.37 | 3.44 | 0.0093 |
| 50 | 21.14(1) | 16.90(6) | 24.87(7) | 2.1-6.3 | 77 | 8.15 | 8.25 | 0.07 | 3.11 | 0.0011 |

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

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 platykurtic pattern whose values are less than 3. The values of skewness recorded in table 1 are between -1.06 and 0.65 in DMF-water media show that the residuals form a part of normal distribution and hence a least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R factor values, which indicate the need for inclusion of additional species in the model. χ^2 is a special case of gamma distribution which measures the probability of residuals forming a part of standard normal distribution [25]. All the metal complexes of the form MLX_2H_h is rejected by the program MINQUAD75. This may be because of the instability of these complexes due to the inability of the metal ions to accommodate three bulky ligands. The reasons for the existence of different species are ascribed under the head distribution diagrams.

χ^2 test

χ^2 is a special case of gamma distribution whose probability density function is an unsymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation.

Crystallographic R-test

In crystallography, the R-factor (sometimes called residual factor or reliability factor) is a measure of the agreement between the crystallographic model and the experimental X-ray diffraction data. The minimum possible value is zero, indicating perfect agreement between experimental observations and the structure factors predicted from the model. There is no theoretical maximum, but in practice, values are considerably less than one even for poor models, provided the model includes a suitable scale factor. 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 different values are obtained for models containing different numbers of species, 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 [26].

Skewness

Skewness is a measure of the symmetry in a distribution. Conceptually, skewness describes which side of a distribution has a longer tail. If the long tail is on the right, then the skewness is rightward or positive; if the long tail is on the left, then the skewness is leftward or negative. In otherwise, if the skewness is greater than zero, the peak of the error distribution curve is to the left of 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 -0.16 and 0.13 for Co (II), -1.06 and 0.65 for Ni (II) & -0.37 and 0.32 for Cu (II) in DMF-water medium. This data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data [27].

Kurtosis

Kurtosis is a measure of whether the data are heavy-tailed or light-tailed relative to a normal distribution. 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.

3.1 Effect of Systematic Errors

In order to rely upon the 'best-fit' model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was undertaken by introducing pessimistic errors in the influential parameters. The results of effect of pessimistic errors in the concentrations of alkali, mineral acid, ligands, metal and correction factor are given in tables 2 which emphasize that the errors in alkali and acid affect stability constant more than those in the ligands, metal and log F. Some species are even rejected when errors are introduced in the concentrations. This indicates the appropriateness of the experimental conditions and accuracy of the concentrations.

Table 1: Effect of errors in influential parameters on the stability constants of ternary complexes of Co (II) with F and MA in 20% v/v DMF-water mixture.

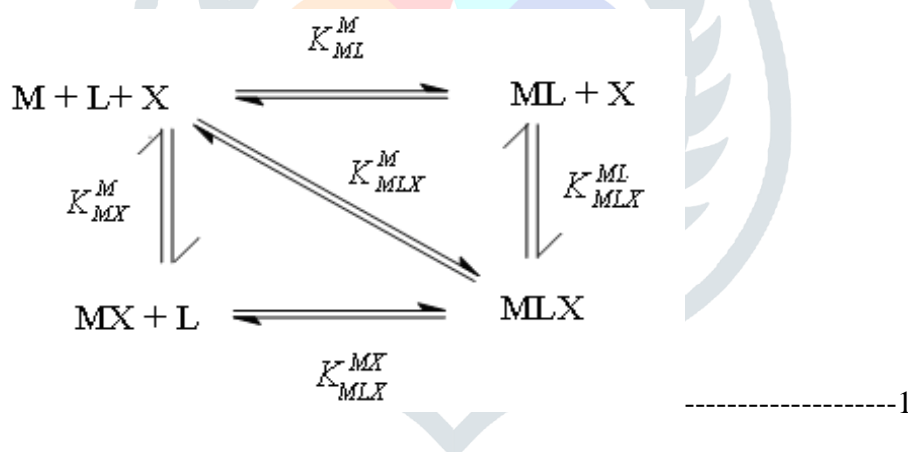
| Ingredient | % Error | log β (SD) | | |
|------------|---------|------------------|-----------|-------------------|
| | | MLXH | MLX | ML ₂ X |
| Alkali | 0 | 21.13(7) | 14.67(6) | 18.49(8) |
| | -5 | 20.87(19) | 12.39(50) | Rejected |
| | -2 | 21.05(21) | 13.33(55) | 16.67(50) |
| | 2 | 22.19(77) | Rejected | 20.16(59) |
| | 5 | Rejected | Rejected | Rejected |
| Acid | -5 | 21.83(36) | 16.39(53) | Rejected |
| | -2 | 21.37(31) | 15.44(39) | 19.83(37) |
| | 2 | 20.84(33) | 13.57(49) | 16.95(59) |
| | 5 | 20.21(46) | 12.25(66) | Rejected |
| F | -5 | 21.07(55) | 14.57(45) | 18.46(49) |
| | -2 | 21.07(77) | 14.64(66) | 18.45(68) |
| | 2 | 21.14(47) | 14.68(65) | 18.44(36) |
| | 5 | 21.43(26) | 14.98(29) | 18.52(38) |
| MA | -5 | 21.17(33) | 15.06(33) | 19.37(39) |
| | -2 | 21.13(17) | 14.81(33) | 18.82(39) |
| | 2 | 21.07(27) | 14.48(39) | 18.08(49) |
| | 5 | 21.05(18) | 14.06(39) | 17.37(52) |
| Metal | -5 | 21.17(16) | 14.82(13) | 18.72(23) |
| | -2 | 21.13(17) | 14.74(29) | 18.53(39) |

| | | | | |
|--|---|-----------|-----------|-----------|
| | 2 | 21.08(17) | 14.61(35) | 18.36(39) |
| | 5 | 21.07(17) | 14.49(29) | 18.18(49) |

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 like concentrations of alkali, mineral acid, ligand and metal. The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > MA > Phe > metal (table 2). Some of the species refined in the absence of errors were even rejected when errors were introduced in the concentrations. One or more of MLXH, MLX and ML₂X species were rejected depending upon the magnitude of error as given in table 2. The rejection of species and increased standard deviations in the stability constants on introduction of errors confirm the appropriateness or correctness of the experimental conditions (concentrations of ingredients) and the choice of the best fit models.

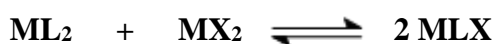
3.2 Stability of Ternary Complexes

The formation of mononuclear unprotonated binary and ternary complexes from a mixture of metal ion (M) and primary (L) and secondary (X) ligands can be shown as the equilibria given in 1.



Reasons for extra stability of ternary complexes

The change in the stability of the ternary complexes as compared to their binary analogues was quantified [28, 29, 30, 31] based on the disproportionation constant (log X) given by Equation 2 corresponding to the equilibrium.



$$\log X = 2 \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M, \quad \dots\dots\dots 2$$

Under these equilibrium conditions one can expect 50% ternary complex and 25% each of the binary complexes to be formed and the value of log X was reported [32] to be 0.6. A value greater than this

accounts for the extra stability of MLX. Another approach [30, 33] 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, where L is primary ligand and X is the secondary ligand. It is compared with that calculated purely on statistical grounds. Equation 5.3 can be formulated based on the properties of the cyclic systems reported earlier [34] from which it is clear that both the ligands in the ternary complex influence mutually to the same extent.

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

The electrostatic theory of binary complex formation and statistical arguments suggest the additional coordination positions of given multivalent hydrated metal ion available for the first ligand than for the second. Hence, the usual order of stability $K_{ML}^M > K_{MLX}^M$ applies. This suggests that $\Delta \log K$ should be negative, although several exceptions [35] have been found. The statistical values of $\Delta \log K$ for bidentate L and X are -0.4, -0.6 and between -0.9 and -0.3 for octahedral, square planar and distorted octahedral complexes, respectively. Negative values of $\Delta \log K$ can be understood as the secondary ligand forms a more stable complex with hydrated metal ion than with ML. 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. $\Delta \log K$ values of ternary complexes containing bipyridyl as the primary ligand are positive [30] for O-donors (malonic acid, pyrocatechol etc.), negative for N-donors (ethylene diamine) and intermediate or negative for amino acids with both N and O co-ordination sites. However, a very high negative value (-2.3) for Cu (en) (iminodiacetic acid) and a positive value (0.82) for Cu (o-F)-(6, 7-dihydroxynaphthalene-2 sulphonate) was also observed [28].

Table 3: $\Delta \log K$ and $\log X$ values of F-M (II)-MA ternary complexes in DMF-water mixtures.

| % v/v DMF | $\Delta \log K_{MLX}$ | $\Delta \log K_{ML_2X}$ | $\log X_{MLX}$ | $\log X_{MLXH}$ |
|---------------|-----------------------|-------------------------|----------------|-----------------|
| Co(II) | | | | |
| 0.0 | 1.112 | 1.232 | 2.226 | 2.584 |
| 10 | 1.114 | 1.239 | 2.228 | 2.609 |
| 20 | 1.166 | 1.267 | 2.332 | 2.649 |
| 30 | 1.180 | 1.289 | 2.361 | 2.650 |
| 40 | 1.176 | 1.280 | 2.351 | 2.689 |
| 50 | 1.194 | 1.307 | 2.388 | 2.692 |
| Ni(II) | | | | |

| | | | | |
|---------------|-------|-------|-------|-------|
| 0.0 | 1.099 | 1.249 | 2.198 | 2.563 |
| 10 | 1.141 | 1.284 | 2.282 | 2.617 |
| 20 | 1.174 | 1.294 | 2.348 | 2.646 |
| 30 | 1.142 | 1.297 | 2.285 | 2.608 |
| 40 | 1.162 | 1.294 | 2.318 | 2.652 |
| 50 | 1.104 | 1.266 | 2.208 | 2.561 |
| Cu(II) | | | | |
| 0.0 | 1.171 | 1.342 | 2.342 | 2.590 |
| 10 | 1.174 | 1.334 | 2.349 | 2.608 |
| 20 | 1.213 | 1.373 | 2.426 | 2.657 |
| 30 | 1.207 | 1.368 | 2.414 | 2.646 |
| 40 | 1.237 | 1.377 | 2.475 | 2.706 |
| 50 | 1.227 | 1.395 | 2.455 | 2.650 |

The logX and $\Delta \log K$ values calculated (Table 3) from binary and ternary complexes are included in tables 4. These values could not be calculated for some systems due to the absence of the relevant binary species. In the present study, the logX values range from 2.198 to 2.706. The higher values account for the extra stability of the ternary complexes. The values of the $\Delta \log K$ ranging from 1.099 to 1.395 for Co (II), Ni (II) and Cu (II). The values are found to be more than -0.4 indicates the ternary complexes of bearing extra stability. 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 [36, 37].

4. Effect of Solvent

When non-polar groups approach each other, there will be a reduction in the number of water molecules in their hydration shells. Thus, the removal of water from the hydration shell (structured water) is accompanied by the relaxation of this water to its bulk state (unstructured water), with a favorable change in the free energy due to entropic effects [38]. These changes, plus the minor contribution of Vander Waal's interactions between atoms in the side chains, stabilize the hydrophobic interaction between the groups. The variations of stability constants as a function of dielectric constant of the medium are shown in Figs.1 a, b and c. The variation of overall stabilities constant values or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic contribution to the free energy change. Hence, the $\log \beta$ values should vary linearly as a function of dielectric constant of the medium, indicates that electrostatic forces and decreasing dielectric constant of the medium [39,40] are dominating the equilibrium

process under present experimental condition. The increase in entropy which is generally observed when the association process is due to electrostatic forces, is caused by the negative temperature coefficient of the dielectric constant of the solvent. The greater the electrostriction around the species associating on account of purely electrostatic forces, the more exothermic the reaction will be. The structural changes exerted by a given metal cation on its solvation shell depend not only on its charge and radius, but also on its individuality.

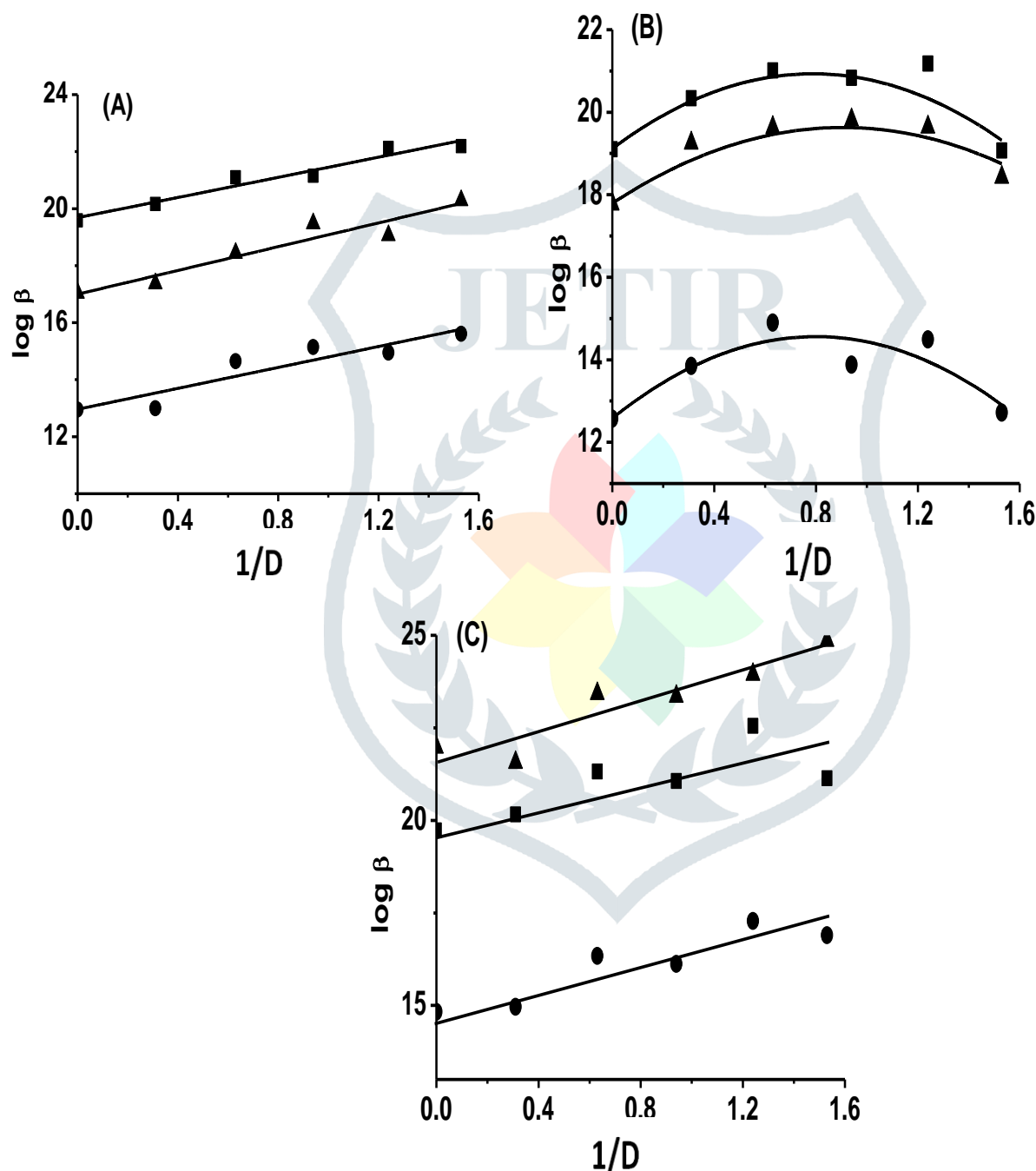


Figure 1: Variation of stability constants of ternary complexes with mole fraction of DMF.

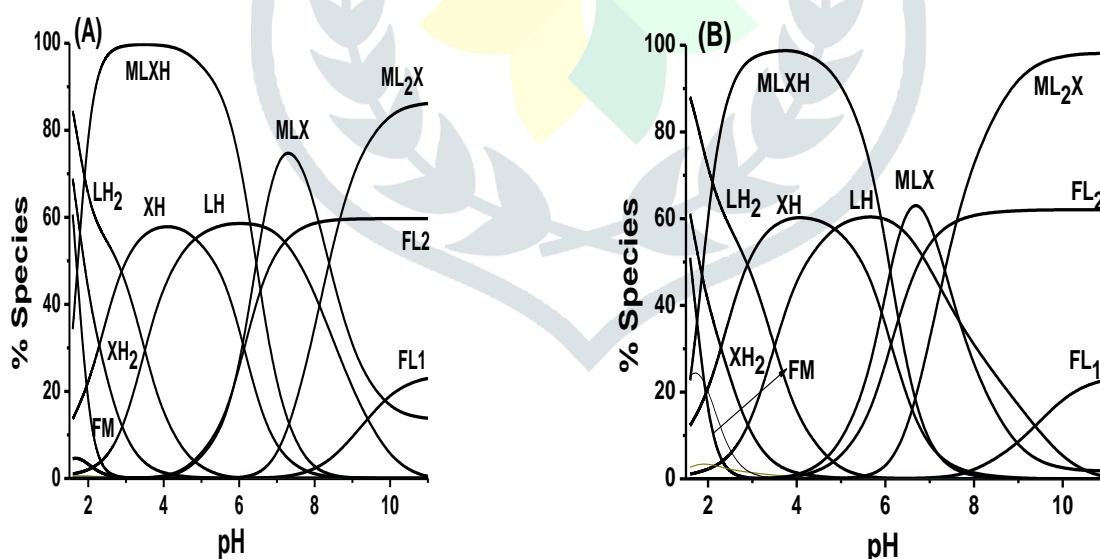
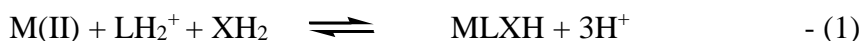
(A) Co (II), B) Ni (II) and C) Cu (II) (■) $\log \beta_{MLXH}$, (●) $\log \beta_{MLX}$, (▲) $\log \beta_{ML2X}$

5. DISTRIBUTION DIAGRAMS

Distribution diagrams were drawn using the formation constants of the best fit model and are shown below.

Organic water-media

A perusal of the distribution diagrams (Figs 2.A, B, C, D, E & F) reveals that the concentrations of binary species are less compared to ternary species which indicates the existence of more stable ternary complexes. The ternary species exist in the pH range 1.5–8.9 for all the metal ions (Co (II), Ni (II) and Cu (II)). The formation of the complex species can be represented by the following equilibria.



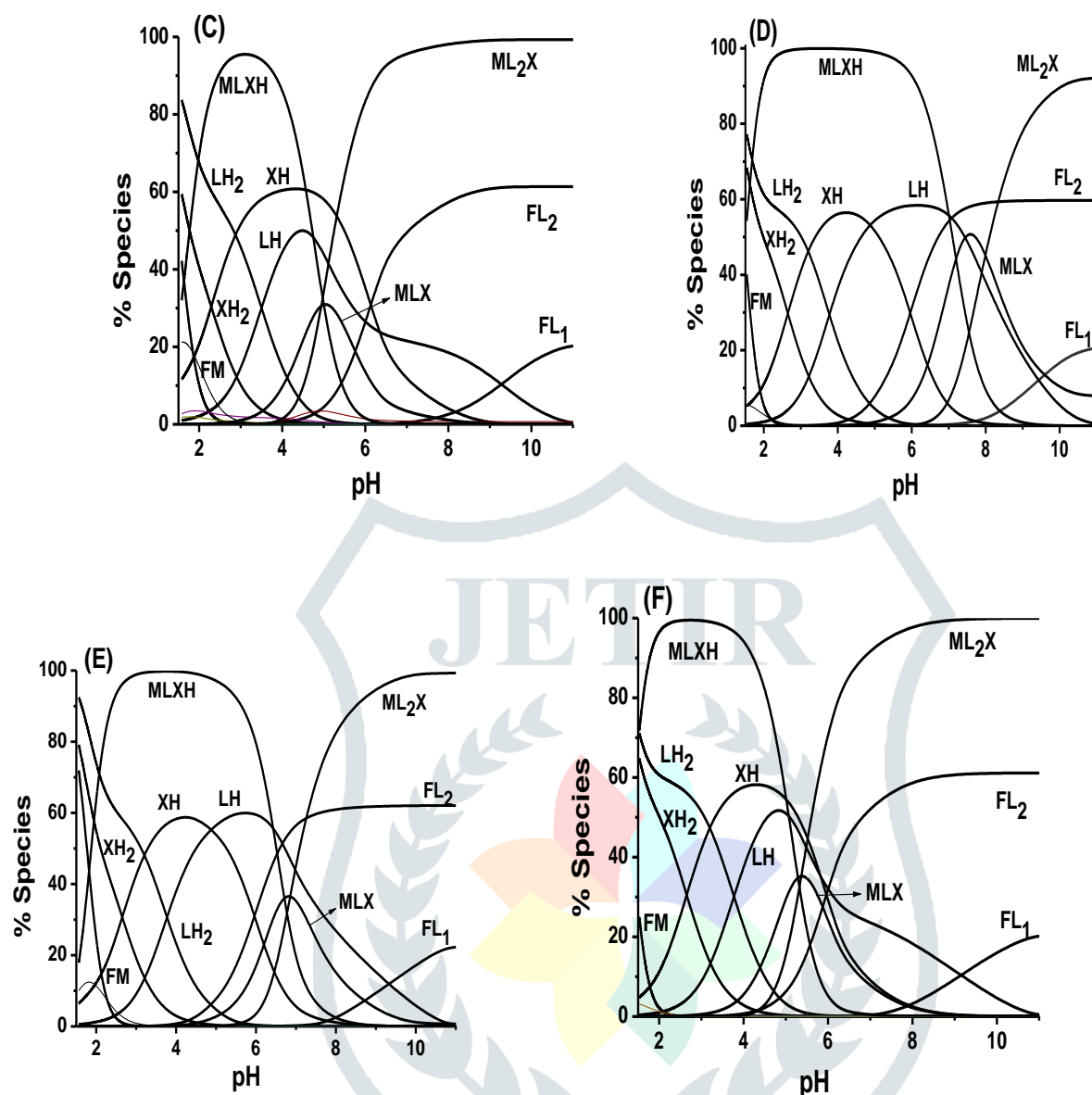


Figure 2: Distribution diagrams for ternary complexes of F and MA with (A) Co (II), (B) Ni (II) and (C) Cu (II) in 1.0% w/v and (D) Co (II), (E) Ni (II) and (F) Cu (II) in 2.0% w/v DMF-water mixtures.

In the pH range 2.1-11.4 and 1.6-10.2, F and MA exist as LH_2^+ , LH , L^- and XH_2 , XH^- , X^{2-} respectively. These protonated ligands interact with the metal ion to form MLXH (Equilibrium 1) which may successfully deprotonated to form MLX^- (Equilibria 4). Formation of MLXH species can be explained based on the protonated ligands interact with the metal ion to form MLXH (Equilibrium 1) and also due to interaction of binary species with ligand species (Equilibrium 2 and 3). MLXH_2 species has not been detected probably because it is less stable or quickly deprotonated to MLXH at lower pH where as for the formation of MLX^- , Equilibria 4 is relevant because the pH at which the concentration of MLXH decreases, MLX^- increases in the pH range of 2.1 -5.2. ML_2X^{2-} is formed by the interaction of metal ion with to XH_2 , LH species and one XH^- species (Equilibrium 5, 6 and 7). The existence of ML_2X and the absence of MLX_2 may be due to the higher affinity of LH than XH towards the metal ion.

Structures

In aqueous solutions, Co (II), Ni (II) and Cu (II) are coordinated by six water molecules. Amino nitrogen can associate with a proton at physiological pH. There is often significant competition between proton and metal ion for this donor site, resulting in the formation of protonated species. Depending upon the nature of the ligands and metal ions and based on the basic chemical knowledge, structures of the complexes are shown in Fig 3. The Cu (II) ion forms distorted octahedral or square planar complexes due to Jahn-Teller effect.

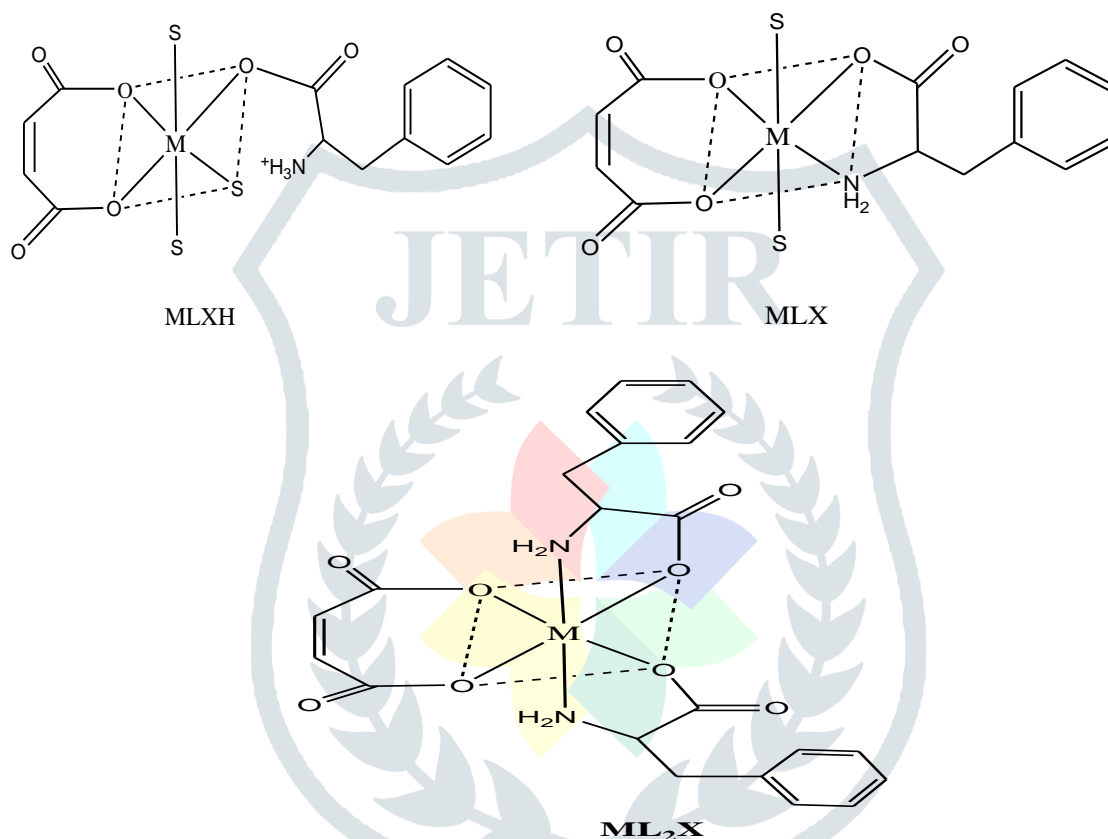


Figure 3: Structures of ternary complexes of F and MA with Co (II), Ni (II) and Cu (II), where S is either solvent or water molecule.

6. Conclusion

- 1) The predominant species detected as MLXH, MLX and ML_2X for Co (II), Ni (II), and Cu (II) where $L=F$ and $X=Ma$. The active forms of these ligands are: LH_2^+ , LH and L^- for F and XH_2 , XH^- , X^{2-} for Ma.
- 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 coordinate ligands, Charge neutralization, chelate effect, stacking interactions and electro static interaction between non-coordinated charge groups of the ligand.
- 3) The linear increase in conditional stabilities of ternary complexes with decreasing dielectric constant is due to the dominance of electrostatic force.

- 4) The order of ingredients that influence the magnitude of conditional stability constants due to incorporation of errors is alkali > acid > Ma > F > metal.
- 5) The study gives an insight in to the metal availability /metal transport in bio fluids and toxicity of these metals. The ternary complexes and more amenable for “metal transport” because of their extra stability and the binary complexes make the “metal available” in biological system due to their decrease conditional stability.
- 6) The pre dominant species detected at physiological PH is MLX for Co (II), Ni (II) and Cu (II) where L=F and X=Ma
- 7) ML_2X is expected antidote for Cu (II) around the biological (physiological) PH =7. Therefore, it recommended to justify the structure of ML_2X .

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