SPECIATION OF BINARY COMPLEXES OF Cd(II) WITH L-ASPARAGINE IN ETHYLENE GLYCOL MEDIUM

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Abstract- The formation constants of binary complexes of Cd(II) with L-Asparagine in (0-50% v/v) Ethylene Glycol-water mixtures were determined pH metrically at 303.0 K and an ionic strength of 0.16 molL⁻¹. The existence of various binary complexes was established from modeling studies using the computer program MINIQUAD75. The best fit chemical models were arrived at based on the statistical parameters like crystallographic R factor and sum of squares of residuals in mass-balance equations. The models for the binary species contained ML₂, ML₂H and ML₂H₂ in Ethylene glycol – water mixtures. The trend in the variation of stabilities of binary complexes with change in the dielectric constant of the medium was explained based on electrostatic and non-electrostatic forces. The distribution diagrams of the complex species are also presented.

Keywords- Binary Complexes, L-Asparagine, Ethylene Glycol, Stability Constants.

I. INTRODUCTION

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

In biological fluids, the metal ions exist in non-exchangeable 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[1]. Hence, the chemical speciation of ligands with Metal ions has been studied in this laboratory[2-6].

Heavy metals and metalloids can be involved in a series of complex chemical and biological interactions[7-8]. The most important factors which affect their mobility are pH, sorbent nature, presence and concentration of organic and inorganic ligands, including humic and fulvic acids, root exudates and nutrients[9].

Cadmium is an abundant nonessential element present in food, water and the environment and it accumulates in human tissues, particularly in the lungs, liver, kidneys, brain, heart and testes[10]. Cd(II) can induce oxidative stress and cause oxidative disorders in both animal cells[11] and plants[12].

Ethylene glycol (EG) is a protophilic dipolar protic solvent and acts as a structure former. Ethylene glycol, having two hydroxyl groups, is distinctly different from monohydric alcohols. EG is more acidic (less basic) than water[13] due to the electron withdrawing effect[14] of the CH₂ group. EG offers several advantages as solvent in titration of weak bases[15]. Ethylene glycol plays an important role in protein conformation studies[16-17] because it is a weak protein denaturant compared to urea or other organic solvents such as ethanol, dioxane etc.

L-asparagine is one of the natural amino acids on earth. 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.

II. EXPERIMENTAL

2.1 Materials
L-Asparagine (Qualigens, India) solution (0.05 molL\(^{-1}\)) was prepared in triple-distilled deionised water by maintaining 0.05 molL\(^{-1}\) nitric acid concentration to increase the solubility. Ethylene Glycol (Merck, India) was used as received. 2 molL\(^{-1}\) sodium nitrate (Qualigens, India) was prepared to maintain the ionic strength in the titrand. 0.05 molL\(^{-1}\) aqueous solutions of Cd(II) nitrate were prepared by dissolving G.R. Grade (E-Merck, Germany) salts in triple-distilled water maintaining 0.05 molL\(^{-1}\) nitric acid 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 classification[18]. The strengths of alkali and mineral acid were determined using the Gran plot method[19-20].

2.1.1 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 Ethylene glycol-water mixture containing the inert electrolyte. All the titrations were carried out in the medium containing varying concentrations of Ethylene glycol-water mixture (0-50% v/v) by maintaining an ionic strength of 0.16molL\(^{-1}\) with sodium nitrate at 303.0 ± 0.1 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[21-22].

2.3 Procedure
For the determination of stability constants of metal-ligand binary species, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with Ethylene glycol-water mixture of equivalent composition as that of titrand. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 ml. Titrations with different ratios 1:2.5, 1:3.75 and 1:5.0 in the case of Cd(II) to L-Asparagine were carried out with 0.4 molL\(^{-1}\) sodium hydroxide. Other experimental details are given elsewhere[23].

III. MODELLING STRATEGY
The computer program SCPHD[24] was used to calculate the correction factor. The binary stability constants were calculated with the pH-metric titration data using the computer program MINIQUAD75[25], which exploits the advantage of a constrained least-squares method in the initial refinement and reliable convergence of the Marquardt algorithm. During the refinement of the binary systems, the correction factor and the protonation constants of L-Asparagine were fixed. The variation of stability constants with the mole fraction of the medium was analysed on electrostatic grounds based on solute-solute and solute-solvent interactions.

IV. RESULTS AND DISCUSSION
The results of the best-fit models 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. A very low standard deviation in log β values indicates the precision of these parameters. The small values of U\(_{\text{corr}}\) (the sum of squares of deviations in concentrations of reactants at all experimental points) corrected for degrees of freedom, indicate that the model can 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. Kurtosis 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)[26-27]. If the kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic as well as platykurtic patterns and very few form mesokurtic patterns. The values of skewness recorded in Table are between -0.43 and 0.73. These data suggest 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. These statistical parameters thus show that the best fit models portray the metal – ligand species in Ethylene Glycol media.

Table 1: Parameters of best fit chemical models of Cd(II) and L- Asparagine complexes in EG water medium at .pH value 2.0 – 8.0.

| % V/V EG | logβ_{ML2} (SD) | logβ_{ML3H} (SD) | logβ_{ML2H2} (SD) | NP | U_{corr} | Skewness | Z^2 | R- Factor | Kurtosis |
|----------|-----------------|-----------------|-----------------|----|----------|----------|-----|-----------|----------|
| 0        | 8.33(14)        | 16.35(16)       | 23.37(09)       | 75 | 4.82     | 0.14     | 16.10| 0.0153    | 3.93     |
| 10       | 8.42(27)        | 16.29(37)       | 22.85(11)       | 18 | 5.02     | -0.43    | 13.56| 0.0193    | 3.69     |
| 20       | 8.49(19)        | 16.28(23)       | 23.14(10)       | 65 | 9.15     | -0.24    | 13.04| 0.1877    | 2.97     |
| 30       | 8.74(12)        | 16.17(17)       | 23.07(06)       | 74 | 5.39     | -0.13    | 25.64| 0.0145    | 3.79     |
| 40       | 9.20(07)        | 16.11(11)       | 22.73(03)       | 80 | 1.87     | 0.73     | 41.53| 0.0083    | 5.33     |
| 50       | 8.79(13)        | 16.24(16)       | 22.96(06)       | 81 | 5.42     | 0.02     | 14.35| 0.0137    | 3.77     |

Here U_{corr} = U/(NP-m)X10^8, where m = number of species; NP=Number of experimental points;

4.1. Effect of systematic errors on best fit model

In order to obtain the best chemical 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 like concentrations of alkali, mineral acid, ligand and metal. Some species were even rejected when errors are introduced in the concentrations. The rejection of some species and increased standard deviations in the stability constants on introduction of errors confirm the suitability of the experimental conditions (concentrations of reactants) and choice of the best fit models.

4.1.1. Effect of solvent

The variation of protonation constant or change in free energy with co-solvent content depends upon two factors, viz., electrostatic and non-electrostatic. Born’s classical treatment holds good in accounting for the electrostatic contribution to the free energy change[28]. According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the logarithm of overall stability constant (log β) should vary linearly as a function of the reciprocal of the dielectric constant (1/D) of the medium. These plots (Figure 1) in Ethylene Glycol -water mixtures show that the log β values are linearly increasing with decreasing dielectric constant values.

Figure 1: Variation of overall stability constant values of Cd (II) with L-Asparagine complexes with Ethylene Glycol - water mixture.,(■)log β_{ML2};(●)log β_{ML2H};(▲)log β_{ML2H2}. 
4.1.1. 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. The ML_2 species is the predominant species (Figure 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 Ethylene Glycol-water media are shown in Figure 2. The species ML_2H_2, ML_2H, ML_2 are formed in the pH range of 4.0-9.0. 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. The protonation of M(II) with LH_2 forms ML_2H_2 [Equilibria (1)]. The deprotonation of ML_2H_2 and protonation of M(II) with LH_2 forms ML_2H beyond a pH 9.0 [Equilibria (2) and (3)]. ML_2 formed by the deprotonation of ML_2H_2, ML_2H and protonation of M(II) with LH_2 at higher pH with high percentage [Equilibria (4), (5) and (6)]. The percentage of the ML_2 species increases successively with increasing pK 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.

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\begin{align*}
\text{M(II) + 2LH}_2^+ &\rightarrow ML_2\text{H}_2^2+ + 2\text{H}^+ & \text{(1)} \\
\text{ML}_2\text{H}_2^2+ &\rightarrow ML_2\text{H}^+ + \text{H}^+ & \text{(2)} \\
\text{M(II) + 2LH}_2^+ &\rightarrow ML_2\text{H}^+ + 3\text{H}^+ & \text{(3)} \\
\text{ML}_2\text{H}_2 &\rightarrow ML_2\text{H}^2- + 2\text{H}^+ & \text{(4)} \\
\text{ML}_2\text{H}^+ &\rightarrow ML_2 + \text{H}^+ & \text{(5)} \\
\text{M(II) + 2LH}_2^+ &\rightarrow ML_2 + 4\text{H}^+ & \text{(6)}
\end{align*}
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![Figure 2: Distribution diagrams of Cd (II) with L-Asparagine complexes in 50% v/v Ethylene Glycol – water medium.](image-url)

4.1.1.1. 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 (Figure 3). Octahedral structures are proposed to the complexes of all the metal ions. The VSEPR theory suggests that Cd(II) complexes shall be octahedral because there are six outer electron pairs.

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 Figure 3.

Figure 3: Structures of binary complexes of Cd (II) with L- Asparagine. Where S is either solvent or water molecule.

V. CONCLUSIONS

The following conclusions have been drawn from the modeling studies of the L-Asparagine Complexes of Cd(II) in Ethylene Glycol-water mixture.

1. Asparagine forms both protonated and unprotonated complexes in the pH range 1.9-9.0.
2. The common species of ML2H2, ML2H and ML2 formed due to the interaction of L-Asparagine with the toxic metals of Cd(II). These models are validated by statistical treatment of data.
3. The linear variation of stability constants as a function of 1/D of the medium indicates the dominance of electrostatic forces over non-electrostatic forces. The linearly vary indicates the dominance of structure forming nature of co-solvent over its coordinating power.
4. The order of ingredients influencing the magnitudes of stability constants due to incorporation of errors in their concentrations is alkali> acid> ligand> metal.

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