Abstract— Chemical speciation of ternary complexes MLX, ML2X and MLXH formed by Co(II), Ni(II), Cu(II) and Zn(II) with L-arginine as primary ligand (L) and succinic acid as secondary ligand (X) was studied in various concentrations (0.0-2.5% w/v) of cationic surfactant solution maintaining an ionic strength of 0.16 mol dm⁻³ (NaNO₃) at 303K. Titrations were carried out in the presence of different relative concentrations (M: L: X = 1:2:2, 1:2:4, 1:4:2) of metal to L-arginine to succinic acid with sodium hydroxide as titrant. The observed extra stability of ternary complexes compared to their binary complexes was explained based on the electrostatic interactions of the side chains of the ligands, charge neutralization, chelate effect, stacking interactions and hydrogen bonding. The trend in log β values with mole fraction of the surfactant and distribution diagrams were presented. Structures of plausible ternary complexes were also presented.

Keywords— Ternary complexes, L-arginine, succinic acid, essential metals, cationic surfactant.

I. INTRODUCTION

L-arginine (Arg) is an essential amino acid required for polyamine biosynthesis¹ in bacteria, fungi and higher eukaryotes. It also serves as the precursor to nitric oxide (NO) synthesis. Succinic acid (Suc) is involved in citric acid and glyoxylate cycles. For energy production and biosynthesis many plants and bacteria convert acetyl units into succinate units in glyoxylate cycle. It can be used² to manufacture medicaments or nutritional supplements effective for treating insulin resistance in mammals.

Aqueous solution of Cetyltrimethylammonium bromide (CTAB) exhibits complex behaviour with respect to a number of micellar properties, especially when additives like electrolytes and different organic compounds are present.³⁻⁷ The micellar properties are highly specific and depend on the associated counter ions and structure of the additives.

The role of trace metals in biological systems is well recognized.⁸ Trace metal ions like Co(II), Ni(II), Cu(II) and Zn(II) are essential and any variation in their homeostasis leads to metabolic disorders.⁹ Hence, the chemical speciation of title systems has been carried out to examine the speciation behaviour and effect of micelles on ternary complexes with selective bio-ligands.

II. EXPERIMENTAL

Cetyltrimethylammonium bromide (CTAB, AR, Qualigens, India), was used and its purity was checked by determining critical micellar concentration (CMC) conductometrically. The CMC value of CTAB was 9.2 x 10⁻⁴ mol dm⁻³ at 303K. Aqueous solutions of L-arginine, succinic acid, Co(II), Cu(II), Ni(II) and Zn(II) chlorides, nitric acid, sodium hydroxide and sodium nitrate have been prepared by using GR Grade (Merck, India) samples in triple distilled water. All the metal solutions were standardized by usual standard methods.¹¹

To increase the solubility of Arg and Suc and to suppress the hydrolysis of metal salts, nitric acid concentration has been maintained at 0.05 mol dm⁻³. 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). The strengths of alkali and acid were determined using the Gran plot method.¹²
Apparatus

An ELICO (Model LI-120) pH-meter (readability 0.01) was used to monitor the changes in H+ concentration. The pH meter was calibrated as mentioned in our earlier papers. The glass electrode has been equilibrated in a well stirred aqua-surfactant solution containing the inert electrolyte (NaNO3). The effects of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of the correction factor.13

Procedure

The alkalimetric titrations were carried out in the medium containing varying concentrations (0.0-2.5% w/v) of surfactant in water maintaining an ionic strength of 0.16 mol dm−3 with sodium nitrate at 303.0±0.1K. The strong acid was titrated with alkali at regular intervals to check whether complete equilibration was achieved. Free acid titrations have been carried every day prior to the mixed-ligand titrations to calculate the correction factor. In each of the titrations, the titrand consisted of approximately 1 mmol of mineral acid, metal ion, ligands and the inert electrolyte in a total volume of 50 cm³. Titrations with different ratios (M: L: X =1:2:2, 1:2:4, 1:4:2) of metal to primary ligand to secondary ligand were carried out with 0.4 mol dm−3 sodium hydroxide solution. Other experimental details are given elsewhere.14

Modeling strategy

The approximate stability constants of ternary complexes were calculated with the computer program SCPHD.16 Different models containing varied number of ternary species were generated using the expert system CEES.17 The best fit chemical models for each ternary system investigated were arrived at using the computer program MINIQUAD75.18

III. RESULTS AND DISCUSSION

Complex equilibria

\[ \log X = 2 \log K_{MLX}^M - \log K_{MX_2}^M - \log K_{MX_2}^M \quad \text{....(1)} \]

ML₂ + MX₂ ⇌ 2 MLX \quad \text{....(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 reported24 to be 0.6.

A preliminary investigation of alkalimetric titrations of mixtures containing different mole ratios of Arg and Suc in the presence of mineral acid and inert electrolyte inferred that no condensed species are formed. The binary metal complexes were fixed in the refinement of the ternary complexes in testing various chemical models using MINIQUAD75. The best fit models were chosen as those with low standard deviation in the formation constants and minimum U (sum of squares of deviations in the concentrations of ingredients at all experimental points) corrected for degrees of freedom, which was corroborated by other statistical parameters like \( \chi^2 \), R-factor, skewness and kurtosis given in Table 1. The species detected for all the metal ions (M = Co(II), Ni(II), Cu(II) and Zn(II)) are MLX, ML₂X and MLXH, where L is the primary ligand (Arg) and X is the secondary ligand (Suc).

A very low standard deviation in \( \log \beta \) values indicates the precision of these parameters. The small values of \( U_{corr} \) indicate that the experimental data can be represented by the model. 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 leptokurtic and a few form mesokurtic patterns.19 The values of skewness recorded in Table 1 are between -1.92 to 1.54. 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.

Extra stability of ternary complexes compared to binary complexes

The change in the stability of the ternary complexes as compared to their binary analogues was quantified20-23 based on the disproportionation constant (log X) given by Equation 1, corresponding to the Equilibrium given in Equation 2.
relevant binary species. In the present study, the log $X$ values range from 2.46 to 13.94 which are higher than those expected on statistical basis (0.6). These higher values account for the extra stability of the ternary complexes. 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.\textsuperscript{25}

**Effect of systematic errors on best fit model**

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\textsuperscript{26} like concentrations of alkali, mineral acid, ligands, metal and log F shown in Table 3. The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > Arg > Suc > metal > log F. Some species are even rejected when errors are introduced in the concentrations. This study confirms the appropriateness of the concentrations of the ingredients and the chosen best fit models.

**Effect of micelles**

The CTAB micelles have positive surface charge and negatively charged complexes are stabilized on the micellar surface. The number of micelles increases with the concentration of surfactant anions are concentrated in the Stern layer.\textsuperscript{27} The variation of log $\beta$ values of ternary complexes as a function of the mole fraction of the surfactant is shown in Figure 1. Similar to binary complexes,\textsuperscript{28} the stabilities of ternary complexes also exhibit non-linear trend may be due to considerable contribution from non-electrostatic forces and decreased dielectric constant of the medium with increased surfactant.\textsuperscript{29}

**Distribution diagrams**

A perusal of the distribution diagrams (Figure 2) reveals that the concentrations of the ternary species increases with increase in pH. The protonated ternary species, MLXH\textsuperscript{+} is distributed at lower pH (6.0-10.0) than the unprotonated species, MLX\textsuperscript{2-} and ML\textsubscript{2}X\textsuperscript{2-}. The lower concentrations of binary species than those of the ternary species indicate the existence of more stable ternary complexes. The ternary species exist in the pH ranges 6.0-8.0 and 9.0-10.5 for Co(II) and Ni(II), respectively, whereas in the case of Cu(II) and Zn(II), the complex species are distributed in the pH range 3.0-7.5 and 8.5-10.0. The formation of the complex species can be represented by the following equilibria.

\[
\begin{align*}
\text{M(II) + LH}_1^2+ + XH_2 & \rightarrow \text{MLXH}_2^+ + 3H^+ \quad \text{(Major)} \quad (1) \\
\text{MLXH}_2^+ & \rightarrow \text{MLXH}^+ + H^+ \quad \text{(Major)} \quad (2) \\
\text{M(II) + LH}_1^2+ + XH_2 & \rightarrow \text{MLXH}^+ + 3H^+ \quad \text{(Minor)} \quad (3) \\
\text{MLX}^{-} & \rightarrow \text{MLX}^2- + H^+ \quad \text{(Major)} \quad (4) \\
\text{M(II) + LH} + XH & \rightarrow \text{MLX}^2- + 2H^+ \quad \text{(Major)} \quad (5) \\
\text{MLX}^{-} + LH & \rightarrow \text{ML}_2XH^+ + 2H^+ \quad \text{(Minor)} \quad (6) \\
\text{ML}_2XH^+ & \rightarrow \text{ML}_2X^2- + H^+ \quad \text{(Minor)} \quad (7) \\
\text{M(II) + 2 LH} + XH & \rightarrow \text{ML}_2X^2- + 3H^+ \quad \text{(Major)} \quad (8)
\end{align*}
\]

In the pH regions 1.5-11.5 and 1.9-7.5, Arg\textsuperscript{30} and Suc\textsuperscript{31,32} exist as LH\textsubscript{2}2+ and XH\textsubscript{2}, respectively. These protonated ligands interact with the metal ion to form MLXH\textsubscript{2}2+ (Equilibrium 1). The species may successively be deprotonated to form MLXH\textsuperscript{+} and MLX\textsuperscript{2-} (Equilibria 2 and 4). Existence of MLXH species can be explained based on the deprotonation of MLXH\textsubscript{2}2+ species and also due to interaction of the metal ion with ligand species (Equilibrium 3). For the formation of MLXH\textsuperscript{+} Equilibrium 2 is more appropriate because the concentration of MLXH\textsuperscript{+} increases where as that of MLX\textsuperscript{2-} decreases. In the pH region 4.0-9.0 Arg and Suc exists as LH and XH\textsubscript{2}, respectively. These ligands interact with the metal ion to form MLX (Equilibrium 5). ML\textsubscript{2}X\textsuperscript{2-} is formed by the deprotonation of ML\textsubscript{2}XH\textsubscript{2} (Equilibria 6 and 7) and also by the interaction of metal ion with two LH species and one XH\textsubscript{2} species (Equilibrium 8) which is more appropriate than the former because ML\textsubscript{2}XH\textsuperscript{+} and its deprotonated species are not refined and during its formation the concentrations of LH and XH\textsubscript{2} are decreasing. Depending upon the coordinating atoms in the ligands and the nature of the metal ions, structures were proposed for the species detected as shown in Figure 3.
IV. CONCLUSIONS
A study of the chemical speciation of ternary complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Arg and Suc in micellar media reveals the compartmentalization of metabolic reactions. The following conclusions have been drawn from the modeling studies:

- The species detected are $MLX^2$, $MLXH$, $ML_2X^2$ for Co(II), Ni(II), Cu(II) and Zn(II) of L-arginine and succinic acid.
- The change in the stability of the ternary complexes as compared to their binary analogues shows that the ternary complexes are more stable than the binary complexes due to the interactions outside the coordination sphere.
- The existence of $ML_2X$ and absence of $MLX_2$ indicates that LH has more affinity than XH towards the metal ion. This also supports the tridentate behavior of Arg and bidentate nature of Suc.
- The order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > Arg > Suc > metal > logF.
- 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 and the binary complexes make the metal available in biological systems due to their decreased stability.

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| % w/v | Log β (SD) | NP | U corr | Skewness | χ² | R-Factor | Kurtosis | pH Range |
|-------|------------|----|--------|----------|----|----------|----------|----------|
|       | CTAB       | MLX| ML2X   | MLXH     |    |          |          |          |
| Co(II)|            |    |        |          |    |          |          |          |
| 0.0   | 13.97(4)   | 18.70(8) | 20.69(18) | 101 | 5.71 | 0.65 | 34.22 | 0.0822 | 7.32 | 2.0-10.5 |
| 0.5   | 12.62(4)   | 17.10(8) | 19.16(20) | 85  | 5.00 | 0.54 | 28.92 | 0.0741 | 5.42 | 2.0-10.0 |
| 1.0   | 12.54(5)   | 16.53(7) | 18.59(14) | 87  | 5.83 | 0.34 | 21.05 | 0.0452 | 3.82 | 2.0-10.2 |
| 1.5   | 12.74(5)   | 16.75(7) | 18.67(15) | 81  | 8.33 | -0.09 | 33.03 | 0.0624 | 4.97 | 2.0-10.0 |
| 2.0   | 12.95(3)   | 16.79(7) | 18.73(18) | 95  | 1.55 | 0.74 | 31.08 | 0.0714 | 5.77 | 2.0-10.5 |
| 2.5   | 12.99(4)   | 16.87(9) | 18.85(170) | 97  | 5.47 | 0.87 | 35.71 | 0.5250 | 6.47 | 2.0-10.5 |
| Ni(II)|            |    |        |          |    |          |          |          |
| 0.0   | 13.97(3)   | 18.50(10) | 20.90(19) | 99  | 5.74 | -1.27 | 105.02 | 0.0714 | 6.54 | 2.0-10.5 |
| 0.5   | 12.27(3)   | 16.40(12) | 18.88(17) | 88  | 5.52 | -1.22 | 103.21 | 0.0683 | 5.92 | 2.0-10.0 |
| 1.0   | 12.29(4)   | 16.55(12) | 18.87(14) | 92  | 1.23 | -1.33 | 98.390  | 0.0793 | 3.94 | 2.0-10.5 |
| 1.5   | 12.38(4)   | 16.67(10) | 18.98(20) | 97  | 2.02 | -1.48 | 110.42  | 0.0954 | 7.05 | 2.0-10.5 |
| 2.0   | 12.57(2)   | 16.83(9)  | 18.76(21) | 95  | 5.10 | -1.54 | 121.92  | 0.0763 | 6.92 | 2.0-10.5 |
| 2.5   | 12.76(3)   | 16.75(10) | 18.73(20) | 100 | 3.81 | -1.65 | 118.65  | 0.0895 | 5.55 | 2.0-11.0 |
| Cu(II)|            |    |        |          |    |          |          |          |
| 0.0   | 16.73(5)   | 18.50(10) | 21.50(20) | 101 | 3.18 | 1.09 | 54.41 | 0.0931 | 8.07 | 2.0-10.5 |
| 0.5   | 15.82(6)   | 17.67(12) | 20.40(21) | 81  | 0.76 | -1.22 | 39.02  | 0.0720 | 7.03 | 2.0-10.0 |
| 1.0   | 15.92(4)   | 17.82(10) | 20.53(18) | 85  | 2.78 | -1.39 | 49.05  | 0.0825 | 8.64 | 2.0-10.2 |
| 1.5   | 15.49(3)   | 17.59(12) | 20.94(17) | 88  | 7.76 | -1.92 | 59.34  | 0.0734 | 7.94 | 2.0-10.5 |
| 2.0   | 15.56(2)   | 17.87(12) | 20.77(18) | 95  | 8.91 | 1.54 | 39.36  | 0.0847 | 6.74 | 2.0-10.5 |
| 2.5   | 15.83(2)   | 17.57(9)  | 20.96(19) | 91  | 2.61 | 1.30 | 45.08  | 0.0733 | 5.97 | 2.0-10.5 |
| Zn(II)|            |    |        |          |    |          |          |          |
| 0.0   | 12.94(3)   | 18.73(9)  | 20.97(18) | 97  | 5.53 | 0.29 | 39.82  | 0.0744 | 7.04 | 2.0-10.5 |
| 0.5   | 11.35(3)   | 17.33(8)  | 18.87(17) | 81  | 0.38 | 0.07 | 64.06  | 0.0811 | 7.37 | 2.0-10.0 |
| 1.0   | 11.49(2)   | 17.48(10) | 18.80(19) | 88  | 9.29 | -0.21 | 50.51  | 0.0722 | 6.24 | 2.0-10.2 |
| 1.5   | 11.58(3)   | 17.32(12) | 18.98(20) | 83  | 9.00 | -0.70 | 48.21  | 0.0655 | 5.32 | 2.0-10.6 |
| 2.0   | 11.69(4)   | 17.20(14) | 18.77(21) | 95  | 2.93 | 0.79 | 37.03  | 0.0534 | 4.94 | 2.0-10.5 |
| 2.5   | 11.74(4)   | 17.51(11) | 18.89(18) | 97  | 5.36 | 0.69 | 40.05  | 0.0443 | 4.99 | 2.0-10.5 |

U corr = U/(NP-m)X10⁸, where m = number of species; NP=Number of experimental points, SD= standard deviation

Table 2. Variation of stability of ternary complexes of Arg and Suc in CTAB-water mixtures.

| % w/v | CTAB | log XM| log XM₂ | Log XM|XM|XM|XM|XM|XM|
|-------|------|------|--------|------|---|---|---|---|---|
|       |      |      |        |      |   |   |   |   |   |
| Co(II)|      |      |        |      |   |   |   |   |   |
| 0.0   | 10.54| 13.91| 6.93   |      |   |   |   |   |   |
| 0.5   | 8.68 | 11.64| 4.27   |      |   |   |   |   |   |
| 1.0   | 8.43 | 10.45| 2.9    |      |   |   |   |   |   |
| 1.5   | 8.57 | 10.66| 2.69   |      |   |   |   |   |   |
| 2.0   | 8.73 | 10.42| 2.46   |      |   |   |   |   |   |
| 2.5   | 8.73 | 10.47| 3.64   |      |   |   |   |   |   |
| Ni(II)|      |      |        |      |   |   |   |   |   |

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Calculations:
\[ \log X_{MLX} = 2 \log \beta_{MLX} - \log \beta_{ML} - \log \beta_{MX} \]
\[ \log X_{MLXH} = 2 \log \beta_{MLXH} - \log \beta_{ML,H} - \log \beta_{MX,H} \]
\[ \log X_{ML2X} = 2 \log \beta_{ML2X} - \log \beta_{ML2} - \log \beta_{MXH} \]

**Table 3. Effect of errors in influential parameters on the stability constants of ternary complexes of Cu(II) and Ni(II) with Arg - Suc in 1.0 % (w/v) CTAB-water mixture**

| Ingredient | % Error | Cu(II) | Ni(II) |
|------------|---------|--------|--------|
|            |         | \( \log \beta(\text{SD}) \) | \( \log \beta(\text{SD}) \) |
| MLX        | ML\(_2\)X | MLXH   | MLX    | ML\(_2\)X | MLXH   |
| 0          | 15.92(4) | 17.82(10) | 20.53(19) | 12.29(4) | 16.55(12) | 18.87(14) |
| Alkali     | -5       | 16.20(55) | 18.25(62) | 21.24(31) | 13.12(25) | 17.25(41) | Rejected   |
|            | -2       | Rejected  | 18.11(51) | 21.00(25) | Rejected  | Rejected  | 20.99(36) |
|            | +2       | 16.08(42) | Rejected  | Rejected  | 13.02(33) | 17.18(20) | 20.12(44) |
|            | +5       | Rejected  | Rejected  | Rejected  | 13.13(35) | Rejected  | Rejected   |
| Acid       | -5       | Rejected  | 17.62(42) | 20.58(32) | 11.31(59) | 15.35(25) | 18.42(32) |
|            | -2       | 15.59(61) | Rejected  | Rejected  | Rejected  | Rejected  | 18.25(58) |
|            | +2       | 15.32(59) | 17.51(78) | 20.67(30) | 11.13(24) | 15.26(36) | Rejected   |
|            | +5       | Rejected  | Rejected  | Rejected  | Rejected  | Rejected  | 18.37(53) |
| Arg        | -5       | 15.74(29) | 17.98(20) | 20.89(45) | 12.23(20) | 16.02(38) | 19.36(60) |
|            | -2       | 15.69(61) | 18.02(20) | 20.98(20) | 12.44(39) | 16.43(39) | 20.09(24) |
|            | +2       | 15.34(35) | Rejected  | 20.74(54) | 12.56(35) | 15.02(38) | 18.80(42) |
|            | +5       | Rejected  | 17.91(22) | Rejected  | 12.95(30) | 15.82(38) | Rejected   |
| Suc        | -5       | 16.01(29) | 17.88(45) | 20.87(22) | Rejected  | 16.00(32) | 18.65(60) |
|            | -2       | 15.84(39) | Rejected  | 20.68(49) | 12.32(52) | Rejected  | Rejected   |
| Metal | Log F | $\log \beta_{MLX}$ | $\log \beta_{X_2ML}$ | $\log \beta_{MLXH}$ |
|-------|-------|---------------------|---------------------|---------------------|
|       | -5    | 15.64(29)           | 17.89(25)           | Rejected            |
|       | -2    | 15.94(45)           | 17.99(26)           | 20.92(27)           |
|       | +2    | 15.72(39)           | Rejected            | 20.71(28)           |
|       | +5    | Rejected            | Rejected            | 13.54(46)           |
|       |       | Rejected            | Rejected            | 16.41(34)           |
|       |       | Rejected            | Rejected            | 18.91(29)           |
| Log F | -5    | 16.71(42)           | Rejected            | 20.82(35)           |
|       | -2    | 16.69(22)           | 17.89(24)           | 20.77(28)           |
|       | +2    | 16.54(19)           | 18.06(42)           | 20.65(38)           |
|       | +5    | Rejected            | Rejected            | 16.32(33)           |

**Fig1**: Variation of stability constant of ternary complexes with mole fraction of CTAB. (A) Co(II) B) Ni(II) C) Cu(II) and D) Zn(II) $\log \beta_{MLX}$, $\log \beta_{MLXH}$.
FIG 2: Distribution diagrams of ternary complexes of Arg-Suc complexes in 1.0% v/v CTAB-water mixture. (A) Co(II), (B) Ni(II), (C) Cu(II) and (D) Zn(II).
Fig3: Plausible structures of ternary complexes of Co(II), Ni(II), Cu(II) and Zn(II) ions with Arg (L) and Suc (X).