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# Influence of TBAB on the Chemical Speciation of Copper(II) Binary Complexes with Biologically Important Ligands, L-Glutamine and Succinic Acid

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**Abstract**

Influence of tetrabutylammoniumbromide (TBAB) on the chemical speciation of binary complexes of copper (II) with biologically important ligands, L-glutamine, and succinic acid has been studied in varying concentrations (0.0-3.0 %, w/v) of TBAB-water mixtures using a Control Dynamics-APX 175E/C pH meter at an ionic strength of 0.16 mol $dm^{-3}$ and temperature 303 K. The models for the species of these ligands are refined by using the computer programs SCPHD and MINIQUAD75. Copper(II) active forms are ML, $ML_2$, and $ML_2H$ for succinic acid and $ML_2$ and $ML_2H$ for L-glutamine. The variation of stability constants with % w/v TBAB is explained based on electrostatic and non-electrostatic forces. The formed species distribution with pH at different solvent compositions and equilibria of the species are reported in the present study. The plausible structures for refined species are also presented.

*Keywords*: Chemical speciation; Copper; L-glutamine; Succinic acid; TBAB; MINIQUAD 75.


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## 1. Introduction

Copper is essential for various biological processes, including maintaining the skin's, blood vessels, epithelium, and connective tissue's strength throughout the body [1]. It aids in the formation of hemoglobin, myelin, and melanin and also aids in the normal functioning of the thyroid gland [1]. Copper is an antioxidant as well as a pro-oxidant. It acts as an antioxidant and neutralizes the free radicals, reducing the damage [2]. When copper serves as a pro-oxidant, it contributes to preventing Alzheimer's disease by promoting free radical damage [3,4]. It is critical to maintaining an adequate dietary intake of copper and other minerals such as zinc and manganese [5]. Copper stimulates and is critical for rapid wound healing [6], and it is absorbed in the gastrointestinal tract

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and transported to the liver coupled to albumin. It enters the bloodstream via the plasma protein ceruloplasmin (CP), which is metabolized and eliminated in bile [7].

Additionally, it functions as an enzyme, catalyzing the oxidation of minerals, most notably iron [8]. Copper deficiency causes Wilson's disease [9] and Menkes's illness [10]. Copper proteins play a variety of roles in biological electron and oxygen transport pathways that take advantage of the relatively facile inter-conversion of Cu(I) and Cu(II) [11]. Copper deficiency can express itself in various ways [12], including hernias, aneurysms, blood vessel rupture manifesting as bruising or nosebleeds, loss of color, weakness, weariness, skin rashes, and impaired thyroid function [6].

Recent research indicates that L-glutamine (Gln) is a conditionally necessary amino acid. It can be used as a respiratory fuel and can boost immune cell stimulation [13]. Gln supplementation enhanced resistance to bacterial assault is necessary to promote lymphocyte proliferation optimally [14-16]. It is a highly conserved outer sphere residue in the active site of Escherichia coli (E. Coli) manganese superoxide dismutase [17].

Succinic acid (Suc) is a component in the citric acid and glyoxylate cycles [18]. Succinate is formed when succinic semi-aldehyde is oxidized. Gamma-aminobutyric acid (GABA) is inactivated in neurotransmission by transamination to succinic semi-aldehyde, then oxidized to succinate. Suc is a nutritional supplement that is useful in treating insulin resistance in mammals. Suc concentrations in human blood plasma range between 0.1 and 0.6 mg/dl [19].

Tetrabutylammoniumbromide (TBAB) is a cationic surfactant with a positively charged head group that significantly modifies aqueous media behavior. It is a quaternary ammonium salt containing a bromide counter ion frequently employed as a catalyst for phase transition [20]. It is utilized in the salt metathesis reactions to synthesize various tetra butyl ammonium salts [20].

L-glutamine and succinic acid are important ligands in biology [21]. The protonation and complexation equilibria of Gln and Suc in TBAB-water [22], urea-water, dimethylformamide-water [23], ethylene glycol-water [24], and acetonitrile-water [21] media were investigated in detail in order to gain a better understanding of the speciation of its complexes. Using various solvents, the protonation constants of Gln and Suc are correlated [24] with the dielectric constant of the medium. We investigated the effects of urea [25] and DMF [26] on cobalt (II) and nickel (II) complexes of Gln and Suc. TBAB influence on speciation of cobalt (II) [27] and manganese (II) [28] was studied. Similarly, speciation of cobalt (II) and nickel (II) ternary complexes of L-glutamine and succinic acid in urea-water [29] and DMF-water [30] mixtures were investigated. But, no such investigations have been published in the literature. Thus, the author examined the effect of TBAB on the chemical speciation of copper (II) complexes with Gln and Suc in TBAB-water mixtures containing 0.0-3.0 percent w/v TBAB.

## 2. Material and Methods

Copper chloride, L-glutamine, and succinic acid (E. Merck, Germany) solutions were prepared in triple distilled water. A 99.5 % pure TBAB (Sigma, Aldrich) was used without further purification. To assess the errors that might have crept into determining the concentrations of the above solutions, the data were subjected to ANOVA [31].

The strength of alkali (NaOH) was determined using the Gran plot method [32]. Alkalimetric titrations were carried out in the medium containing 0.0 – 3.0 % w/v of TBAB in water at an ionic strength of 0.16 mol $dm^{-3}$ with NaCl at 303.0±0.1 K using a Control Dynamics-APX175E/C pH meter. Under the specified conditions, the experiments were carried out in 1:2 and 1:3 metal-ligand ratios for succinic acid and L-glutamine with the metal ion and titration curves are shown in Fig. 1.

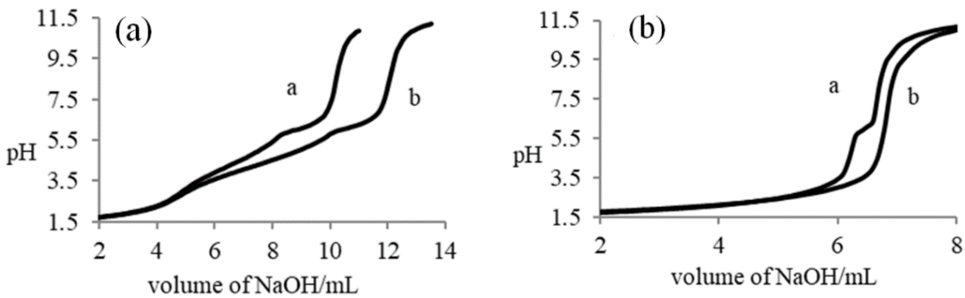


Fig. 1. Alkalimetric titration curves of Cu (II) with Volume of NaOH Vs. 1.0 %w/v TBAB (A) Succinic acid and B) L-glutamine. M: L = a) 1:2 and b) 1:3.

The pH-meter with the glass electrode was equilibrated in the inert electrolyte. The computer program SCPHD [33] was used to determine the correction factor, log F, to correct the pH meter dial reading. Other experimental details are given elsewhere [31]. The approximate protonation constants were calculated using SCPHD. The best-fit chemical models for each system were arrived at using the computer program MINIQUAD75 [34] by following some heuristics [35] to refine the stability constants and use the statistical parameters of the least-squares residuals.

## 3. Results and Discussion

The active forms of Gln are amino and carboxyl groups. Two carboxyl groups of Suc are protonated in the pH ranges 2.0–10.0 and 2.0-7.0, respectively [22] revealed by alkalimetric titration curves in TBAB-water mixtures. An expert system package CEES [36] generates various numbers and combinations of copper (II)complex models with Gln and Suc. These models were inputted to MINIQUAD75 and the alkalimetric titration data, and the best-fit models were obtained. The final model for copper (II) with Suc contains ML, $ML_2$, and $ML_2H$, and $ML_2$ and $ML_2H$ for Gln are given in Tables 1 and 2, along with the statistical parameters.

The skewness between -1.56 to 1.97 for Suc and closeness to zero for Gln indicates that the residuals follow Gaussian distribution to apply the least-squares technique. The low standard deviation in the model parameters (log $\beta$) illustrates the adequacy of the models.

Table 1. Best fit models for binary complexes of Copper (II) with succinic acid in TBAB-water mixtures. (pH 2.0 – 6.0), temp = 303 K, ionic strength = 0.16 mol.dm$^{-3}$.

| Sl. No | % w/v TBAB | Log $\beta_{mlh}$(SD) | | | NP | Skewness | Kurtosis | $\chi^2$ | Ucorr×10$^6$ | R-factor |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 110 | 120 | 111 | | | | | | |
| 1 | 0.0 | 3.13(1) | 5.69(1) | 8.18(1) | 117 | 0.93 | 2.54 | 28.6 | 3.94 | 0.0117 |
| 2 | 0.5 | 3.05(2) | 5.61(2) | 8.06(3) | 125 | 1.97 | 2.07 | 50.6 | 1.26 | 0.0682 |
| 3 | 1.0 | rej | rej | 7.89(1) | 132 | 0.31 | 3.42 | 86.3 | 1.19 | 0.0596 |
| 4 | 1.5 | rej | rej | 7.68(1) | 155 | 0.12 | 2.81 | 52.7 | 1.73 | 0.0569 |
| 5 | 2.0 | 2.85(3) | 5.47(3) | 7.48(2) | 152 | 1.82 | 1.15 | 80.8 | 1.54 | 0.0605 |
| 6 | 2.5 | 2.80(1) | rej | 7.35(1) | 125 | -1.56 | 2.51 | 67.1 | 2.52 | 0.0251 |
| 7 | 3.0 | 2.74(1) | 5.43(1) | 7.22(1) | 58 | -1.22 | 2.16 | 55.7 | 2.51 | 0.0126 |

Note: No of titrations in each percentage are 6.

Table 2. Best fit models for the binary complexes of Copper (II) with L-Glutamine in TBAB-water mixtures. (pH 2.0 to 6.0), temp = 303 K, ionic strength = 0.16 mol.dm$^{-3}$.

| Sl. No | % TBAB | Log $\beta_{mlh}$(SD) | | NP | Skewness | Kurtosis | $\chi^2$ | Ucorr×10$^6$ | R- factor |
|---|---|---|---|---|---|---|---|---|---|
| | | 120 | 121 | | | | | | |
| 1 | 0.0 | 20.66(1) | 24.14(1) | 87 | 1.26 | 2.94 | 10.3 | 7.13 | 0.0063 |
| 2 | 0.5 | 20.57(3) | 24.04(4) | 75 | -0.34 | 2.30 | 20.3 | 1.12 | 0.0278 |
| 3 | 1.0 | 20.48(7) | 23.89(2) | 66 | -0.40 | 2.50 | 16.8 | 1.59 | 0.0326 |
| 4 | 1.5 | 20.38(6) | 23.75(1) | 69 | -0.42 | 2.71 | 33.4 | 1.59 | 0.0312 |
| 5 | 2.0 | 20.31(4) | 23.68(4) | 68 | -0.47 | 3.06 | 11.4 | 1.44 | 0.0338 |
| 6 | 2.5 | 20.24(4) | 23.43(4) | 68 | -0.46 | 3.05 | 10.6 | 1.43 | 0.0337 |
| 7 | 3.0 | 20.22(1) | 23.37(2) | 38 | 0.29 | 1.19 | 11.3 | 1.38 | 0.0421 |

Note: No of titrations in each percentage is 6.

### 3.1. *Influence of TBAB on the complex equilibria*

TBAB is a hydrotrope in the presence of water [37]. The degree complexes' stability could be measured in terms of the magnitude of the overall stability constants of each species formed in metal-ligand dynamic equilibria. The linear and non-linear variations in the magnitude of the stability constants of metal-ligand complexes are due to electrostatic and non-electrostatic opposing factors, respectively. TBAB acts as a structure-breaker of pure water due to a large hydrophobic group of TBAB and thus forming cages around itself, with empty spaces in the structure [38,39]. Critical micellar concentration (CMC) for TBAB is 0.2632 mol/L at 303.16 K in aqueous solutions [40]. The anisotropic water distribution within the micellar structure causes non-uniform micropolarity, microviscosity, and degree of hydration within the micellar media [41]. The viscosity is strongly influenced by the liquid's ability to transport the mass within the liquid, which is immensely responsible for any changes in the chemical reactions. The high viscosity of the TBAB causes the limited mobility of species within, which in turn causes a low conversion of products, especially in enzymatic reactions [42].

In the present study, the stability constants were found to linearly decrease as the percentage of surfactant increased progressively for both Cu-Suc and Cu-Gln complexes (Fig. 2).

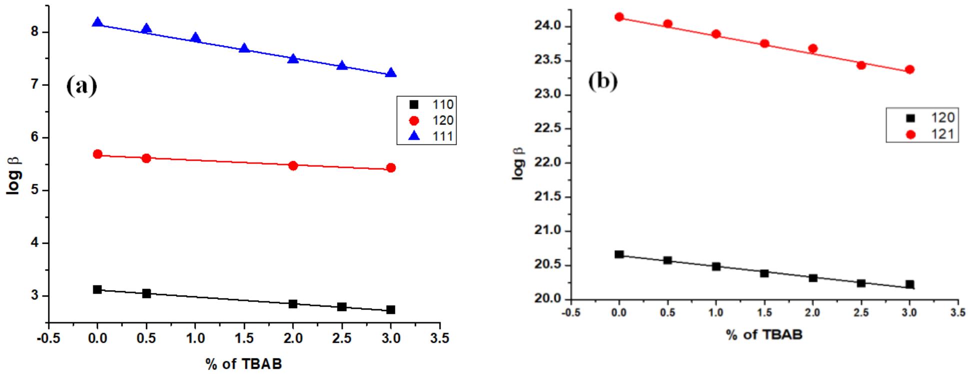


Fig. 2. Variation of log β with 0-3 % w/v TBAB-water mixtures of Cu(II)-(A) succinic acid (■) ML and (●) $ML_2$, (▲) MLH and (B) L-glutamine (■) $ML_2$ and (●) $ML_2H$.

The linear variation of species with increasing % w/v TBAB indicates that electrostatic forces dominate the equilibrium process under the present experimental conditions. The dielectric constant is one of the most prominent solvent properties that surfactants could alter [43]. The destabilization of the metal-ligand complexes could be attributed mainly to the low dielectric constant of the surfactant-mediated solvent compared to the aqueous medium. The dielectric constant ($\varepsilon$) of water is 78.4 Debye (D), and that for TBAB is 8.93 D at 25 °C is much lower than aqueous media [44,45]. The destabilization effect of the low dielectric constant is synergized [46] by the cationic surfactant TBAB, which causes the log $\beta$ values to decrease linearly in the given titration mixtures.

The formation equilibria are represented below based on the above observations. The plausible equilibria for copper with succinic acid

$$M(II) + LH_2 \rightleftharpoons MLH + H^+ \qquad \text{....(a)}$$
$$MLH + LH \rightleftharpoons ML_2 + 2H^+ \qquad \text{....(b)}$$
$$MLH \rightleftharpoons ML + H^+ \qquad \text{....(c)}$$

The plausible equilibria for copper with L-glutamine

$$MLH + LH \rightleftharpoons ML_2H + H^+ \qquad \text{....(d)}$$
$$ML_2H \rightleftharpoons ML_2 + H^+ \qquad \text{....(e)}$$

The charges of species are neglected for clarity. Proton accepting ability of the ligand increases in an acidic environment (in TBAB). Hear, the metal ion, protons, and TBAB are competing for binding with the ligand. Hence, decreasing the availability of the ligand's electron pairs made it difficult to easily donate to the vacant shell of the metal ion in the formation of complexes. As a result of these competing processes, the stability of the complex and values of the stability constant seem to decrease in the TBAB-water mixture. This concept is in good agreement with the linearity of plots of log β values versus % TBAB (low dielectric constant effect of surfactant modified medium).

### 3.2. *Distribution diagrams*

Succinic acid has two carboxyl groups, and both are protonated. The various forms of ligands in the study's pH range (2.0-10.0) are $LH_2^+$, LH, and $L^-$ for Gln and $LH_2$, $LH^-$ and $L^{2-}$ for Suc. L-glutamine has three functional groups (amino, carboxyl, and amido), but only amino and carboxyl groups can associate with protons. The zwitterionic form (LH) of Gln is present to the extent of 90 % in the pH range 2.0-7.0, which is confirmed by MINIQUAD75. A perusal of the models indicates that the species $ML_2H$ is highly stable at pH less than 3, and $ML_2$ concentration is constant at higher pH for Gln and Suc; the species ML and $ML_2$ concentrations are almost constant at pH more than six, which readily obtained from MLH (Fig. 3), equations b and c are simultaneous.

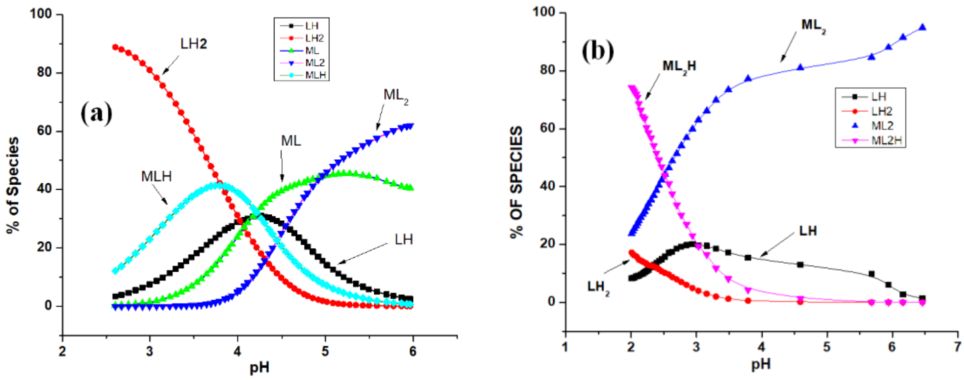


Fig. 3. Distribution diagrams Cu(II) complexes of (A) Succinic acid and (B) L-glutamine in 1.0 % w/v TBAB-water mixtures.

The plausible structures for Suc and Gln complexes with Cu (II) refined and derived from best-fit models and equilibria are given in Fig. 4.

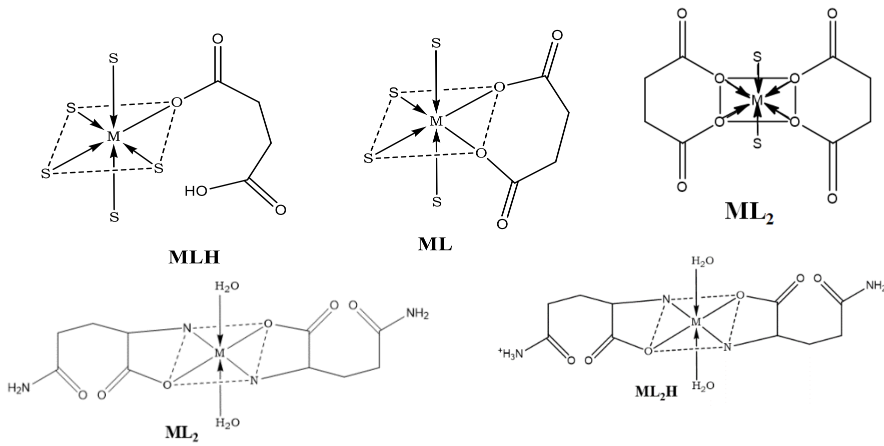


Fig. 4. Structures of MLH, ML, and $ML_2$ species of succinic acid and $ML_2$ and $ML_2H$ species of L-glutamine complexes with Cu(II).

### 3.3. *Biological significance of the present study*

The cationic surfactant TBAB in aqueous solutions considerably decreases the dielectric constant, and these solutions are expected to mimic the physiological conditions. The present study is useful to understand

1. The role played by the active site cavities in biological molecules.
2. The bonding behavior of the protein residues with the metal ions like copper in further studies
3. The refined species and relative concentrations under the present experimental conditions represent the possible forms of glutamine and succinate residues.
4. The biomimetic studies of biologically important ligands Gln and Suc, which forms more stable complexes with copper(II).
5. TBAB has a positively charged head group which plays an important role in modifying the behavior of aqueous media. As a cumulative effect, the stabilities of the species have decreased with TBAB content for Suc and Gln.

## Conclusion

The final model for copper (II) with Suc contains ML, $ML_{2,}$ and $ML_2H$ and $ML_2$ and $ML_2H$ for Gln confirmed by MINIQUAD75. Gln forms more stable complexes than Suc due to the strong complexing nature of Gln. log $\beta$ values decreased with increasing w/v TBAB, destabilization of the metal-ligand complexes could be attributed mainly to the low dielectric constant of the surfactant mediated solvent compared to an aqueous medium.

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