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Effect of TBAB on the Chemical Speciation of Complexes of Zinc(II) with L-Glutamine and Succinic Acid

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Abstract

Effect of Tetrabutylammoniumbromide (TBAB) on the chemical speciation of binary complexes of Zinc(II) with L-glutamine and succinic acid has been studied pH metrically in varying concentrations (0.0-3.0 %, w/v) of TBAB-water mixtures. The temperature and the ionic strength maintained in the titrant are 0.16 mol dm^{-3} and 303 K, respectively. The possible models that represent the title systems are refined by using the computer programs SCPHD and MINIQUAD75. The models contain ML and MLH for succinic acid and ML_2 and ML_2H for L-glutamine complexes of Zinc(II). The variation of the stability constants with a dielectric constant is explained based on electrostatic and non-electrostatic forces. The species distribution with pH at different solvent compositions, corresponding chemical equilibria, and structures of the complex species are described.

Keywords: Complex equilibria; Speciation, L-glutamine; Succinic acid; Zinc; TBAB; MINIQUAD75.

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1. Introduction

Most of the metabolic reactions are catalyzed by metalloenzymes or metal-activated enzymes [1]. The activity of these enzymes is believed to be due to the metal-enzyme-substrate complexes [2]. Zinc is essential for all living organisms and is involved in numerous processes of cellular metabolism [3]. It was estimated that about 10% of human proteins potentially bind with zinc. It is required for the catalytic activity of more than 200 enzymes [4,5], and it plays a role in immune function [5,6], wound healing, protein synthesis, DNA synthesis, and cell division [7]. Zinc is required for proper sense of taste and smell [8,9] and supports normal growth and development during pregnancy, childhood, and adolescence [10-13]. It possesses antioxidant properties, which protect against accelerated aging and help speed up the healing process after an injury [14].

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L-Glutamine (Gln) and succinic acid (Suc) are biologically important ligands [15]. Gln is usually considered a non-essential amino acid, but recent studies have shown that glutamine may become "conditionally essential" during inflammatory conditions. Gln can act as a respiratory fuel, enhancing the stimulation of immune cells [16]. Gln in the diet increased survival to bacterial challenge [17]. It is required to support optimal lymphocyte proliferation [18], production of cytokines by lymphocytes and macrophages [19], and it is a highly conserved outer sphere residue in the active site of E. Coli manganese superoxide dismutase [20]. Suc can be used to manufacture medicaments or nutritional supplements effective for treating insulin resistance [21] in mammals. Suc is involved in the citric acid cycle [22] and the Glyoxalate cycle [23]. The concentration of Suc in human blood plasma is 0.1-0.6 mg/dL. Succinate stimulates insulin secretion and pro-insulin biosynthesis [24]. Tetrabutylammoniumbromide (TBAB) is a cationic surfactant, and it is commonly used as a phase transfer catalyst [25]. It is used to prepare many other tetrabutylammonium salts via salt metathesis reactions [25].

Protonation and complexation equilibria of Gln and Suc were studied in urea-water [26], dimethyl formamide-water [26], ethylene glycol-water [27], and acetonitrile-water [15] media to understand the speciation of their complexes. The protonation constants of Gln and Suc were correlated [27] with the dielectric constant of the medium using various solvents. Effects of urea [28] and DMF [29] on cobalt(II) and nickel(II) complexes of Gln and Suc were studied. Similarly, speciation of cobalt(II) and nickel(II) ternary complexes of Gln and Suc in urea-water [30] and DMF-water [31] mixtures was reported. No such studies in TBAB medium are reported in the literature. Hence, the authors present the speciation of zinc(II) complexes with Gln and Suc in TBAB-water mixtures in the present study.

Materials and Methods

Solutions of zinc chloride, L-glutamine, and succinic acid (E. Merck, Germany) were prepared in triple distilled water. A 99.5 % pure TBAB (Sigma-Aldrich) was used without further purification. The data were subjected to ANOVA [32] to assess the errors that might creep into determining the concentrations of the solutions. The strength of NaOH was determined using the Gran plot method [33]. The glass electrode was equilibrated in the TBAB solution.

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-APX 175E/C pH meter. The titrations were carried out in 1:2 and 1:3 metal-ligand ratios for both succinic acid and L-glutamine in the presence of metal ions. Typical titration curves are shown in Fig. 1.

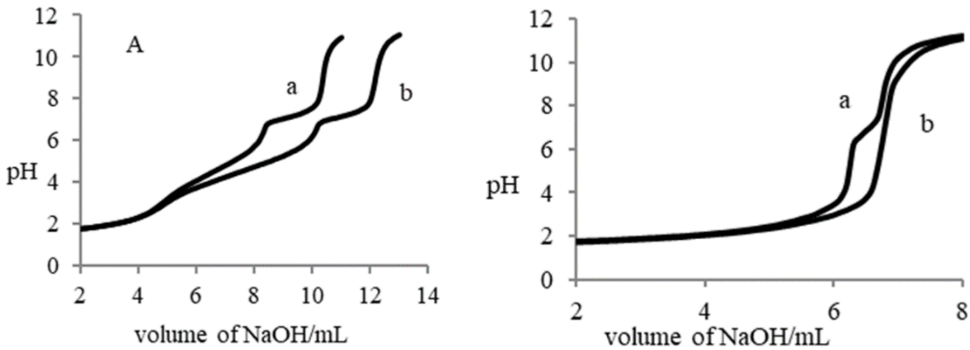


Fig. 1. Alkalimetric titration curves of binary solutions of Zn(II) and (A) Succinic acid, or B) L-glutamine 2.0 %w/v TBAB. M:L = a) 1:2 and b) 1:3.

The computer program SCPHD [34] is used to determine the correction factor, to correct the pH meter dial reading. Other experimental details are given elsewhere [32]. The approximate step-wise stability constants were calculated using SCPHD. By following some heuristics [35] in the refinement of the stability constants and using the statistical parameters of the least-squares residuals, the best-fit chemical models for each system were obtained using the computer program MINIQUAD75 [36].

3. Results and Discussion

Alkalimetric titration curves revealed that the active forms of Gln and Suc in TBAB-water mixtures are in the pH ranges 2.0–10.0 and 2.0-7.0, respectively [37]. Amino and carboxyl groups of Gln and carboxyl groups of Suc are protonated in these pH ranges. Models containing various numbers and combinations of zinc complexes with Gln and Suc were generated using an expert system package CEES [38]. These models were inputted to MINIQUAD75, along with the alkalimetric titration data, to obtain the best-fit models.

Table 1. Best fit models for binary complexes of Zn(II) with Succinic acid in TBAB-water mixtures (pH 2.0 – 7.0).

SL. No	%w/v TBAB	log β_{mlh} (SD)		NP	Skewness	Kurtosis	χ^2	Ucorr×10^6	R- factor
		110	111						
1	0.0	2.58(8)	7.87(8)	156	1.77	6.61	33.9	5.77	0.0103
2	0.5	2.62(1)	7.9(1)	149	1.10	3.52	39.9	3.25	0.0262
3	1.0	-	7.79(1)	135	1.49	5.51	19.6	1.96	0.0194
4	1.5	-	7.71(1)	133	1.47	4.97	13.4	2.74	0.0228
5	2.0	2.58(1)	7.69(1)	134	-1.93	8.87	19.2	3.55	0.0296
6	2.5	2.60(1)	7.65(1)	134	-1.24	6.93	12.6	3.22	0.0282
7	3.0	2.56(1)	7.62(1)	74	-2.22	1.26	13.3	1.72	0.0261

Note: No of titrations in each percentage is 6.

Table 2. Best fit models for the binary complexes of Zn(II) with L-Glutamine in TBAB-water mixtures (pH 2.0 to 6.0).

SL. No.	%w/v TBAB	log β_{mlh}(SD) 120	121	NP	Skewness	Kurtosis	χ^2	Ucorr×10^6	R- factor
1	0.0	21.23(2)	24.05(2)	78	-1.31	2.38	21.45	9.13	0.0240
2	0.5	21.35(4)	24.41(5)	73	-0.37	2.83	7.23	1.37	0.0319
3	1.0	21.27(1)	24.26(1)	63	-0.53	2.45	9.05	1.60	0.0331
4	1.5	21.18(6)	24.07(2)	63	-0.47	2.84	22.17	1.39	0.0301
5	2.0	21.01(1)	23.80(1)	62	-0.69	3.31	12.37	1.64	0.0375
6	2.5	20.81(4)	23.53(4)	64	-0.66	3.21	10.75	1.71	0.0381
7	3.0	20.60(2)	23.38(8)	31	-0.69	3.22	2.05	1.05	0.0413

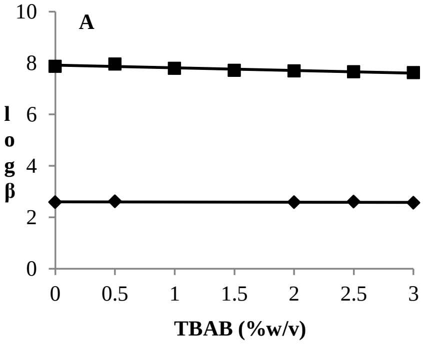
Note: No of titrations in each percentage is 6.

The final model for zinc with Suc contains ML and MLH, and ML_2 and ML_2H for Gln, as given in Tables 1 and 2, along with the statistical parameters. The skewness between -2.22 to 1.77 for Suc and close to zero for Gln indicates that the residuals follow Gaussian distribution, and the least-squares technique can be applied. The low standard deviation in the logβ values illustrates the adequacy of the models.

3.1. *Effect of TBAB on the complex equilibria*

TBAB acts as a structure-breaker of pure water due to a large hydrophobic group and forms cages around itself, with empty spaces in the structure [39, 40]. The critical micellar concentration (CMC)of TBAB is 0.2632 mol/L at 303.16 K in aqueous solutions [41]. The anisotropic water distribution within the micellar structure causes non-uniform micropolarity, microviscosity, and degree of hydration within the micellar media [42]. TBAB is a hydrotrope [43]. The degree of complex 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. 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 [44].

In the present study, the stability constants were found to linearly decrease as the %w/v TBAB increased (Fig. 2). The linear variation indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions. The dielectric constant is one of the most prominent solvent properties that surfactants could alter [45]. The 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. The dielectric constant(ε) of water is 78.4 Debye (D) and for TBAB is 8.93 D [46,47] at 25 °C, much lower than the aqueous medium. Moreover, the destabilization effect of the low dielectric constant is synergized [48] by the cationic TBAB surfactant, which causes the logβ values to decrease linearly.



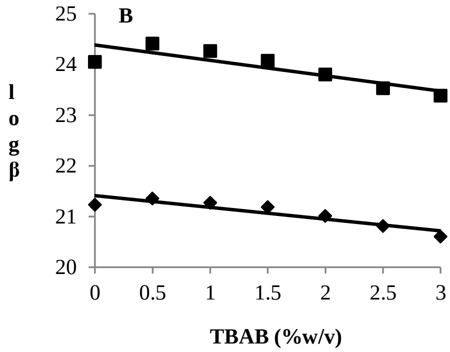


Fig. 2. Variation of log β with TBAB-water mixtures of (A) Zn-Suc: (♦) ML and (■) MLH and (B) Zn-Gln: (♦) ML_2 and (■) ML_2H.

The formation equilibria are represented below based on the above observations. The charges of species are omitted for clarity. The plausible equilibria for zinc with Suc are (a)-(c), and those for Gln are (d) and (e).

$$M(II) + LH_2 \rightleftharpoons MLH + H^+ \quad \text{....(a)}$$
$$M(II) + LH \rightleftharpoons ML + H^+ \quad \text{....(b)}$$
$$MLH \rightleftharpoons ML + H^+ \quad \text{....(c)}$$
$$M(II) + 2LH_2 \rightleftharpoons ML_2H + 3H^+ \quad \text{....(d)}$$
$$ML_2H \rightleftharpoons ML_2 + H^+ \quad \text{....(e)}$$

The ligand's proton accepting ability increases in an acidic environment (in TBAB). The metal ion, protons, and TBAB compete to bind with the ligands. Hence, the stability of complex and magnitude of the stability constants decrease in the TBAB-water mixture. This is in good agreement with the linearity of plots of log β values with % w/v TBAB.

3.2. *Distribution diagrams*

Succinic acid has two carboxyl groups, and both are protonated. L-glutamine has three functional groups (amino, carboxyl, and amido), but only amino and carboxyl groups can associate with protons. The various forms of ligands in the pH range of the study are LH_2^+, LH and L^- for Gln and LH_2, LH^- and L^{2-} for Suc. The zwitterionic form (LH) of Gln is present to the extent of 90 % in the pH range 2.5-8.5, which MINIQUAD75 confirms. A perusal of the models indicates that ML_2H is highly stable at lower pH and ML_2 concentration is constant at higher pH for Gln. For Suc, the ML concentration is almost constant at above pH 6, readily obtained from MLH (Fig. 3).

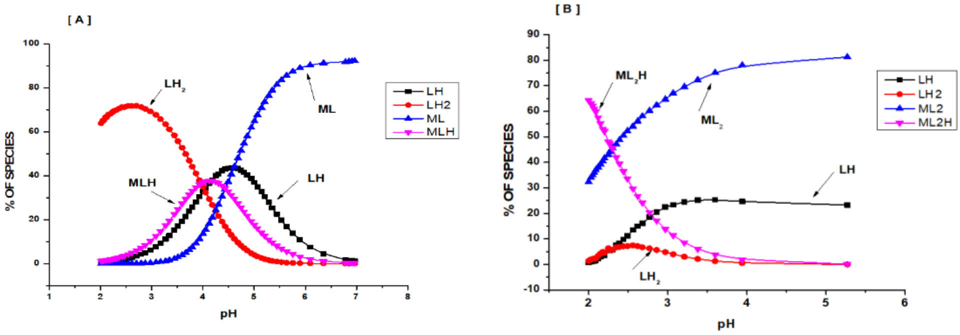


Fig. 3. Distribution diagrams of Zn(II) complexes of (A) Succinic acid and (B) L-glutamine in 0.5 %w/v TBAB-water mixture.

The plausible structures for succinic acid and L-glutamine complexes are proposed based on the best-fit models and equilibria, as given in Fig. 4.

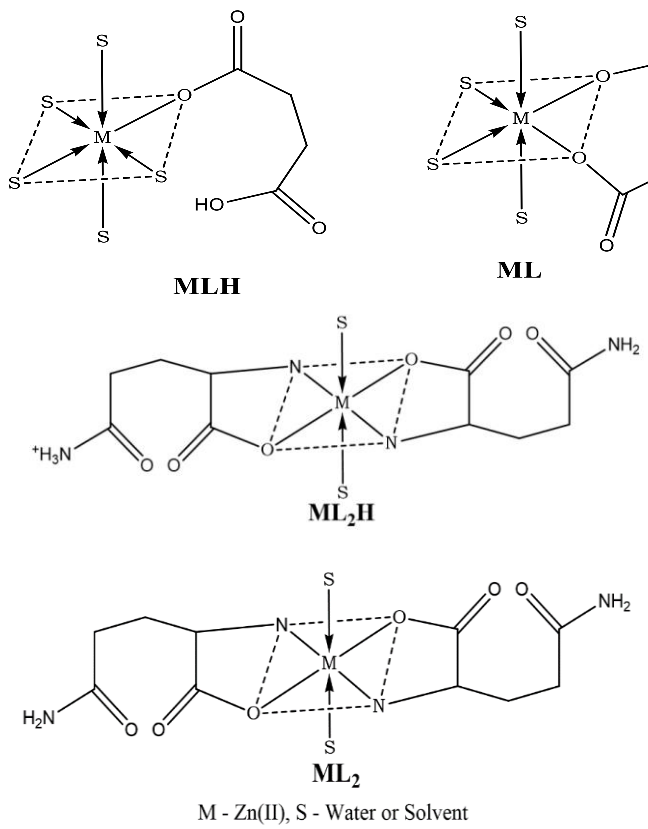


Fig. 4. Structures of binary complexes of succinic acid and L-glutamine with Zn(II).

3.3. *Biological significance of the present study*

The presence of TBAB in an aqueous solution considerably decreases the dielectric constant, and these solutions are expected to mimic the physiological conditions. The present study is helpful to understand the role played by the active site cavities in biological molecules and the bonding behavior of the protein residues with the metal ion in further studies. The refined species and relative concentrations under the present experimental conditions represent the possible forms of glutamine and succinate residues in the proteins.

4. Conclusion

The final models for Zn(II) with Suc are ML and MLH, and for Glu are ML_2 and ML_2H at present experimental conditions. Gln forms more stable complexes than Suc due to the strong complexing nature of Gln. Logβ values decreased with increasing w/vTBAB, 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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