

Speciation of Cobalt(II) complexes with succinic acid and L-glutamine in Tetra Butyl Ammonium Bromide – water mixtures

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Abstract

Speciation of cobalt(II) complexes with L-glutamine (Gln) and succinic acid (Suc) in varying concentrations (0.0-3.0%, w/v) of Tetra-n-butylammonium bromide (TBAB)-water mixtures with pH-meter has been made at an ionic strength of 0.16 mol dm^{-3} and temperature 303 K using a Control Dynamics-APX 175E/C pH meter. Various models for the species of these ligands are refined by using the computer programs SCPHD and MINIQAD75.

The active forms of the Cobalt(II) are ML, ML₂ and MLH for succinic acid and ML and ML₂H for L-glutamine. The trend in the variation of protonation constants with dielectric constant is explained on the basis of electrostatic and non-electrostatic forces. The species distribution with pH at different solvent composition and the plausible equilibria for the formation of the species are also presented.

Keywords: Complex Equilibria, Speciation, L-Glutamine, Succinic Acid, Cobalt, TBAB, MINIQAD 75

1. Introduction

Most of the metabolic reactions are catalysed by metalloenzymes or metal activated enzymes. The activity of these enzymes is believed to be due to the metal-enzyme-substrate complexes. Cobalt is key constituent of cobalamin (vitamin B₁₂) essential for the production of red blood cells. It acts as coenzyme in several biochemical processes. It speeds up Adenosine Triphosphate (ATP) turnover. Cobalt is present as corrin coenzyme in glutamate mutase, dialdehydase, methionine synthase and arginase.

It is present in non-corrin form in dipeptidase. In excess cobalt(II) is led to cardiomyopathi[1]. L-Glutamine(Gln) and succinic acid (Suc) are biologically important ligands[2]. Gln is normally considered to be a nonessential amino acid, recent studies have provided evidence that glutamine may become "conditionally essential" during inflammatory conditions such as infection and injury under appropriate conditions. Glutamine can act as a respiratory fuel and it can enhance the stimulation of immune cells[3]. Gln in the diet increased survival to bacterial challenge[4] is required to support optimal lymphocyte proliferation[5], production of cytokines by lymphocytes and macrop hages[6] and it is highly conserved outer sphere residue in the active site of Escherichia coli (E.Coli) manganese superoxide dismutase[7].

Hence, the protonation equilibria of Gln and Suc in urea-water[8], dimethylformamide-water[9], acetonitrile-water[2] mixtures were studied. Suc can be used for manufacture of medicaments or nutritional supplements effective for treating of insulin resistance[9] in mammals. Suc is involved in citric acid or Tricarboxylic acid (TCA) cycle[10] and Glyoxalate cycle. Succinate is obtained by the oxidation of succinic semialdehyde. In neurotransmission, Gamma aminobutyric acid (GABA) is inactivated by transamination to succinic semi-aldehyde, which is then oxidised to succinate. The concentration of succinic acid in human blood plasma is 0.1-0.6 mg/dl. Succinate stimulates insulin secretion and pro-insulin biosynthesis[11].



Tetra butyl ammonium bromide (TBAB) is a cationic surfactant and has a positively charged head group which plays important role in modifying the behavior of aqueous media. It is a quaternary ammonium salt with a bromide counter ion commonly used as a phase transfer catalyst. It is used to prepare many other tetra butyl ammonium salts via salt metathesis reactions[12]. Protonation and complexation equilibria of Gln and Suc in urea-water[8], dimethylformamide-water[8], ethylene glycol-water[2] and acetonitrile-water[2] media have been studied to thoroughly understand the speciation of its complexes.

The protonation constants of Gln and Suc are correlated[8] with the dielectric constant of the medium using various solvents. Similarly, effect of urea on Cobalt(II) and Nickel(II) complexes of Gln and Suc have studied[13]. In the present study the speciation of cobalt(II) complexes with Gln and Suc in tetra butyl ammonium bromide (TBAB)-water mixtures are presented.

2. Material and Methodology

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

The strength of alkali (NaOH) was determined using the Gran plot method[14]. 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 $303.0 \pm 0.1 \text{ K}$ using a Control Dynamics-APX 175E/C pH meter. The glass electrode was equilibrated in inert electrolyte. The correction factor, $\log F$ to correct the pH meter dial reading, was determined using the computer program SCPHD[15, 16].

Other experimental details are given elsewhere[17]. The approximate protonation constants were calculated using SCPHD. By following some heuristics[18] 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 arrived at using the computer program MINIUAD75[19].

1.1 Results and Discussion

The amino and carboxyl groups of L-glutamine and the two carboxyl groups of succinic acid are protonated. Alkalimetric titration curves in TBAB-water mixture revealed that the active forms of Gln and Suc are in the pH ranges 2.0–10.0 and 2.0-7.0 respectively[20].

Models containing various numbers and combination of cobalt with Gln and Suc are generated using an expert system package CEES[21]. These models were inputted to MINIUAD75, along with the alkalimetric titration data and the best-fit model, were obtained. The final model in TBAB-water mixture for Cobalt with Suc contains ML, ML_2 and MLH and ML and ML_2H for Gln are given in Tables 1 and 2, along with the statistical parameters.

The skewness is between zero to four for succinic acid and closeness to zero for L-glutamine indicates that the residuals follow Gaussian distribution and so least squares technique can be applied. The low standard deviation in the model parameters ($\log \beta$) illustrates the adequacy of the models.

3.2 Solute-Solvent Interactions

The linear variation of Suc species with increasing % TBAB indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions. The decrease in formation constants with % TBAB for Gln indicate that the equilibrium process is destabilising due to interaction of metal ion with TBAB (Fig 1).



Table 1: Best fit table for the binary complexes of Cobalt(II) in 0.0 to 3.0%w/v TBAB-water mixtures of Succinic acid

%w/v TBAB	log β ML (SD)	log β ML ₂ (SD)	log β MLH (SD)	U corr X 10 ⁶	χ^2	Skewness	Kurtosis	R-factor
0.0	2.4852 (0.4927)	Rej	6.6739 (0.8011)	8.66	41.57	3.88	15.97	0.1856
0.5	2.2651 (0.8516)	4.9970 (0.6172)	7.4747 (0.4902)	3.43	34.97	4.57	22.47	0.1061
1.0	2.5398 (0.8935)	5.4683 (0.5113)	7.6420 (0.5367)	3.34	42.9	4.32	20.16	0.1219
1.5	Rej	Rej	8.0124 (0.2134)	4.16	29.89	0.35	2.23	0.1228
2.0	Rej	Rej	8.5177 (0.2511)	2.37	56.43	1.06	5.10	0.0973
2.5	Rej	Rej	8.4385 (0.35266)	1.66	41.5	1.08	7.26	0.1036
3.0	rej	rej	8.6691 (0.2508)	9.79	21.06	3.93	20.75	0.0823

Table 2: Best fit table for the binary complexes of Cobalt(II) in 0.0 to 3.0%w/v TBAB-water mixtures of L-Glutamine

%w/v TBAB	log β ML (SD)	log β ML ₂ H (SD)	U corr X 10 ⁶	χ^2	Skewness	Kurtosis	R-factor
0.0	12.6864 (0.2646)	25.2511 (0.2231)	1.56	46.76	0.76	2.62	0.09722
0.5	12.6812 (0.2110)	24.9962 (0.2639)	1.43	45.32	0.84	3.09	0.08759
1.0	11.4023 (0.6112)	25.1865 (0.7525)	1.36	24.01	0.70	3.52	0.08814
1.5	10.1011 (0.2951)	23.8032 (0.2604)	1.93	14.77	0.07	2.50	0.03312
2.0	9.05752 (0.3111)	22.70	3.68	34.10	0.20	2.37	0.04714
2.5	9.9312 (0.2478)	23.933	1.15	26.17	-0.04	2.36	0.03387
3.0	9.6534 (0.3171)	22.56	8.74	49.05	0.10	1.75	0.1064

3.3 Effects of Surfactant:

Dielectric constant is one of the most and prominent solvent properties that could be altered by surfactants in the given titration mixtures. The anisotropic water distribution within micellar structure causes non-uniform micropolarity, microviscosity and degree of hydration within the micellar media. The degree of stability of complexes could be measured in terms of the magnitude of the overall stability constant 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. In the present study results of the stability constants were found to be linearly decreasing as the percentage of surfactants increases progressively for Gln-Metal complexes. The destabilization of the metal ligand complexes could be attributed mainly to the low dielectric constant of the surfactant mediated solvent compared to aqueous medium. Moreover, the destabilization effect of the low dielectric constant is synergized by the cationic surfactant, TBAB, which causes the $\log \beta$ values to decrease linearly as shown in Fig. 1[B]. On the other hand, the proton accepting ability of the ligand increases in acidic environment (in TBAB). Hence, the metal ion, the protons and TBAB, as a cationic surfactant, compete for the ligand decreasing the full availability of the electron pairs of the ligand making difficult to easily donate to vacant shell of the metal ion. As a result of these competing processes, the stability of the complex and values of the stability constant seems to decrease in TBAB-water mixture. This concept is in good agreement with the linearity of plots of $\log \beta$ values vs. % TBAB (low dielectric constant effect of surfactant modified medium). On the other hand stability constants for Suc were increased as the percentage of TBAB Fig. 1[A]. Here the destabilisation nature of TBAB decreases for metal-Suc interactions. Hence, Suc forms more stable complexes at basic conditions ($\text{pH} > 5.5$) and $\log \beta$ values for Suc were increasing linearly. This concept is in good agreement with the linearity of plots of $\log \beta$ values vs. % TBAB (low dielectric constant effect of surfactant modified medium).

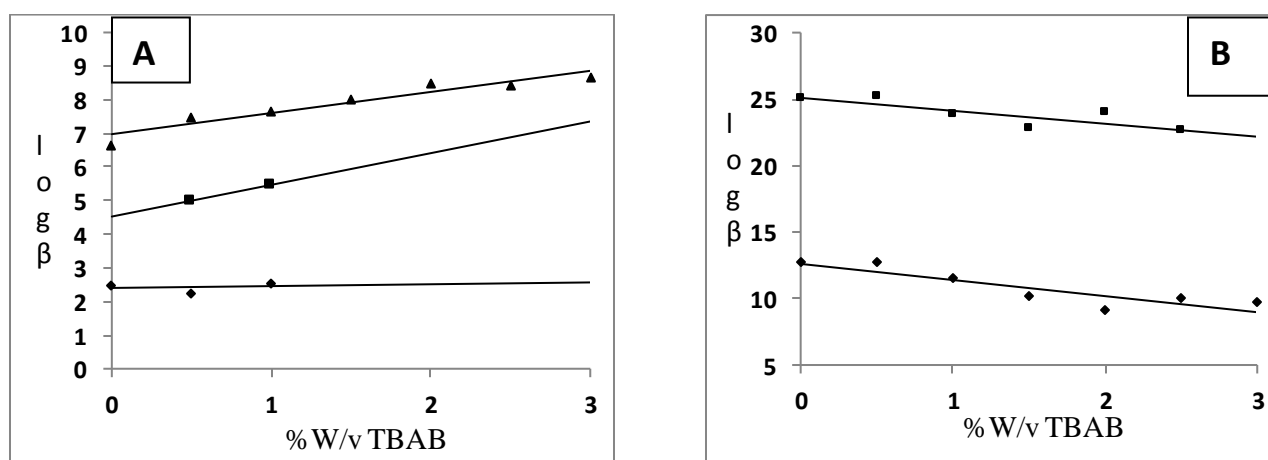


Fig 1: Variation of $\log \beta$ with %w/v TBAB-water mixtures of (A) Succinic acid (◆) ML (■) ML_2 and (▲) MLH and (B) L-glutamine (◆) ML and (■) ML_2

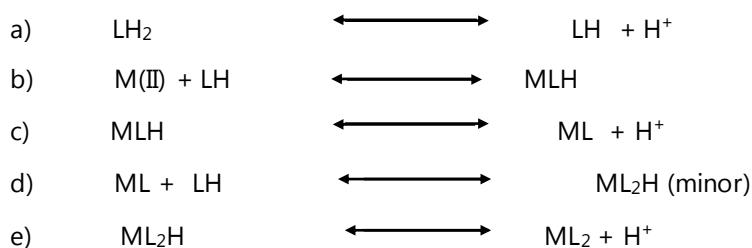
3.4 Distribution Diagrams

L-glutamine has three functional groups (amino, carboxyl and amido) but only amino and carboxyl groups can associate with protons. Succinic acid has two carboxyl groups and both are protonated. The various forms of ligands exist in the pH range of study (2.0-10.0) 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 an extent of 90% in the pH range 2.5-8.5, which are confirmed by MINIQUAD75. Perusal of the models indicates that the species (ML_2H and ML) concentration is highly stable and constant between pH 2 to 10 for Gln and for Suc the species (ML and ML_2) concentration is almost

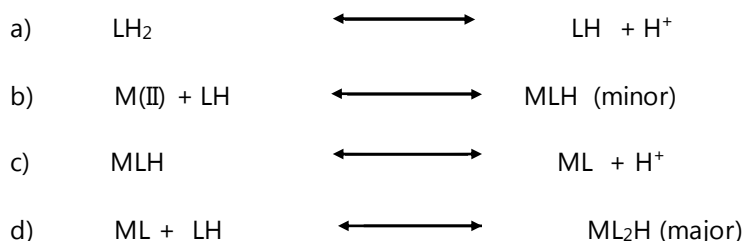


constant at above pH 6 which readily converted from MLH (Fig.2). The formation equilibria, based on the above observations, are represented below.

The plausible equilibria for cobalt with succinic acid



The plausible equilibria for cobalt with L-glutamine



The charges of species are omitted for clarity. The formation of LH and LH₂ for both Gln and Suc are insignificant. The variation of species concentration with pH is shown in Fig. 2 for typical systems. In succinate speciation, concentration of LH₂ decreased with TBAB content and readily converted to LH. In glutamine, the concentration of LH₂ decreased with pH and readily converted to LH. Another notable observation is that the LH concentration of Gln is high about 80% between pH 4 and 8.

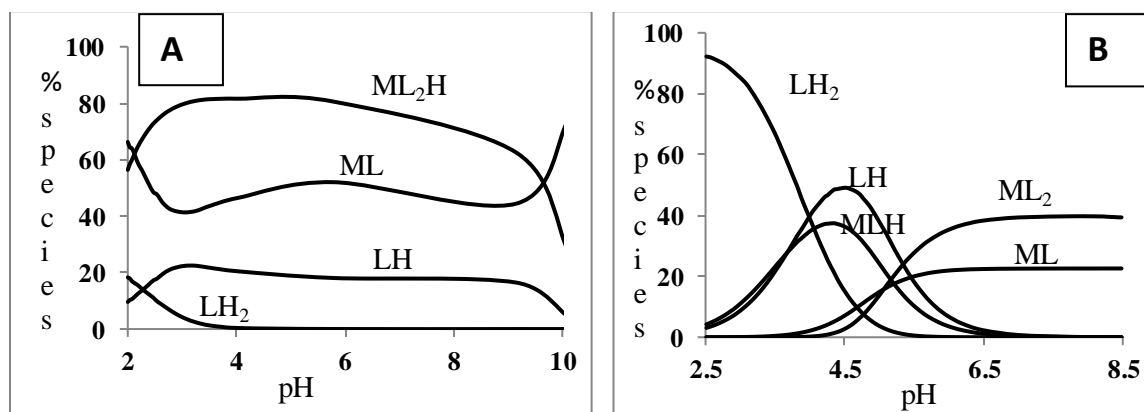


Fig 2: Distribution diagrams Cobalt(II) complexes of (A) L-glutamine and (B) Succinic acid in 0.5%w/v TBAB-water mixtures.

2. Conclusions

The presence of TBAB in aqueous solution 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 behaviour of the protein residues with the metal ion in further studies. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of glutamine and succinate residues.

3. The biomimetic studies of L-glutamine and succinic acid indicate that the both are protonated (LH₂) under acidic pH (2.0-6.0) conditions and readily converted to deprotonated species (LH).
4. Tetra butyl ammonium bromide (TBAB) is a cationic surfactant and has a positively charged head group which plays important role in modifying the behaviour of aqueous media. As a cumulative effect the stabilities of the species have increased with increased TBAB content for Suc and for Gln is almost constant.

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