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A pH metric investigation of chemical speciation of binary complexes of manganese(II) with L-glutamine and succinic acid in TBAB-water mixtures

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ABSTRACT
Chemical speciation of binary complexes of Mn(II) with L-glutamine and succinic acid in varying concentrations (0.0-3.0%, w/v) of Tetrabutylammonium bromide (TBAB)-water mixtures has been studied pH metrically at an ionic strength of 0.16 mol dm^{-3} and 303 K temperature. The active forms of Mn(II) are ML, ML_2 and MLH for succinic acid and ML, ML_2 and ML_2H for L-glutamine are refined and confined using the computer programs SCPHD and MINIQUAD75. The variation of stability constants with %TBAB is explained on the basis of electrostatic and non-electrostatic forces. The species distribution with pH at different solvent composition, plausible equilibria and structures of the species are also presented.

KEY WORDS*: Chemical speciation, manganese, L-glutamine, succinic acid, TBAB, MINIQUAD75*

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I. INTRODUCTION

Manganese, among the first-row transition metals biologically important and performs a remarkable range of catalytic functions[1], especially in the Mn clusters. For example, the unique redox properties of Mn are essential to the generation of dioxygen from water in the oxygen-evolving complex[2,3]. It is also useful in the production of the deoxyribonucleotide building blocks needed for DNA synthesis[4]. Mn(II) ions can be used as Lewis acid catalysts by numerous enzymes of metabolic importance[5], including glutamine synthetase.

The human body cannot produce manganese, but it can store it in the liver, pancreas, bones, kidneys, and brain[6]. It helps to support the bones by activating crucial enzymes involved in the formation of bone, cartilage and collagen. A person usually obtains manganese from their diet. The human body contains about 12 mg of manganese, mostly in the bones. The soft tissue remainder is concentrated in the liver and kidneys[6]. Along with vitamin K, manganese aids the formation of blood clots. Blood clotting, which keeps the blood in a damaged blood vessel, is the first stage of wound healing[7].

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. It is believed that Mn(II) plays biologically important roles and appear to be five enzymes possessing a non-heme, mononuclear Mn(II) center, which changes oxidation state during the catalytic cycle, that have been mechanistically and structurally characterized: Mn-dependent superoxide dismutase(MnSOD)[8], oxalateoxidase(OxOx)[9], oxalate-decarboxylase (OxDC)[10], Mn-dependent homoprotocatechuate 2,3-dioxygenase (MndD)[11] and lipoxygenase[12] (MnLOX). Mn-induced neurotoxicity leads to a degenerative brain disorder, referred to as manganism[13]. Only a few instances of Mn deficiencies have been reported in humans, with symptoms including dermatitis, slowed growth of hair and nails, decreased serum cholesterol levels and decreased levels of clotting proteins[14].

Amino acids plays important role in biological functions, L-Glutamine (Gln) and succinic acid (Suc) are biologically important ligands[15]. Gln is normally considered to be a "conditionally essential" during inflammatory conditions such as infection and injury under appropriate conditions. Gln can enhance the stimulation of immune cells[16] and it can act as a respiratory fuel. 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]. Gln is highly conserved outer sphere residue in the active site of Escherichia coli (E. Coli) manganese superoxide dismutase[20].

Suc is involved in citric acid cycle[21] and Glyoxalate cycle. In neurotransmission, Gamma aminobutyric acid (GABA) is inactivated by transamination to succinic semi-aldehyde, which is then oxidized to succinate. Suc can be used in the manufacture of medicaments or nutritional supplements effective for treating insulin resistance[22] in mammals. The concentration of Suc in human blood plasma is 0.1-0.6 mg/dl. Succinate stimulates insulin secretion and pro-insulin biosynthesis[23]. Tetrabutylammonium 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 commonly used as a phase transfer catalyst and used to prepare many other tetrabutylammonium salts via salt metathesis reactions[24].

Protonation and complexation equilibria of Gln and Suc in urea-water[25], dimethylformamide-water[25], ethyleneglycol-water[26], acetonitrile-water[15] and TBAB-water[27] media were studied to thoroughly understand the speciation of their complexes. The protonation constants of Gln and Suc were correlated[25] with the dielectric constant of the medium using various solvents. Effect 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. Because of the importance Mn(II), Gln, Suc and TBAB, the author has studied the speciation of Mn(II) complexes with Gln and Suc in TBAB-water mixtures, no such studies are reported in the literature.

II. RESULTS AND DISCUSSION

The active forms of Gln and Suc are revealed from alkalimetric titration curves in the pH ranges 2.0–10.0 and 2.0-7.0, respectively[27]. Amino and carboxyl groups of Gln and carboxyl groups of Suc are protonated in these pH ranges. Models containing various numbers and combinations of complexes of manganese with Gln and Suc were generated using an expert system package CEES[32]. These models were inputted to MINIQUAD75[33], along with the alkalimetric titration data, to obtain the best-fit models. The final models of manganese for Suc are ML, ML_2 and MLH and for Gln are ML, ML_2 and ML_2H as given in Tables 1 and 2 along with the statistical parameters.

Table 1: Best fit models for binary complexes of Mn(II) with Succinic acid in TBAB-water mixtures (pH 2.0 – 7.0). No of titrations in each percentage is 6.

%w/v TBAB	$Log\beta_{mlh}$ (SD)			NP	Skewness	Kurtosis	χ^2	Ucorr x10^6	R-factor
	110	120	111						
0.0	2.83(1)	4.36(2)	7.56(2)	110	0.92	2.86	62.0	1.58	0.0269
0.5	3.11(1)	4.28(1)	7.84(1)	148	-0.07	1.84	37.7	2.34	0.0225
1.0	rej	rej	7.63(2)	128	0.02	2.70	29.4	2.99	0.0776
1.5	rej	rej	7.53(3)	104	-0.05	2.58	37.3	2.84	0.0950
2.0	2.66(1)	3.72(1)	7.45(1)	130	-2.17	1.03	29.5	2.99	0.0268
2.5	2.42(1)	rej	7.40(1)	123	-1.76	2.84	17.7	2.69	0.0256
3.0	2.28(9)	rej	7.38(2)	70	-2.06	3.42	15.3	1.90	0.0276

Table 2: Best fit models for the binary complexes of Mn(II) with L-Glutamine in TBAB-water mixtures (pH 2.0 to 8.0). Number of titrations in each percentage is 6.

%w/v TBAB	$log\beta_{mlh}$ (SD)			NP	Skewness	Kurtosis	χ^2	Ucorr x10^6	R- factor
	110	120	121						
0.0	9.08(1)	16.64(1)	20.25(1)	115	-0.59	1.21	98.80	5.84	0.0122
0.5	9.05(1)	16.47(1)	20.21(1)	110	-0.25	1.56	37.85	7.59	0.0150
1.0	8.94(3)	16.14(3)	20.12(3)	93	-1.08	3.38	19.47	9.37	0.0543
1.5	8.73(2)	rej	20.07(1)	94	-0.66	3.50	56.27	9.72	0.0171
2.0	8.53(1)	15.81(1)	19.92(1)	92	-0.75	3.83	7.65	6.0-7	0.0153
2.5	rej	15.69(2)	19.84(1)	89	0.85	3.92	23.01	8.66	0.0184
3.0	8.13(1)	15.58(2)	19.61(2)	49	-0.78	0.11	1.73	3.13	0.0149

The skewness between -2.17 to 0.92 for Suc and -1.08 to 0.85 for Gln indicates that the residuals follow Gaussian distribution and so least squares technique can be applied. The low standard deviation in the model parameters (log β) illustrates the adequacy of the models.

Effect of TBAB on the solute-solvent interactions

TBAB is a quaternary ammonium salt and acts as surfactant. Surfactants can alter the dielectric constant[34], which is one of the most and prominent solvent properties 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.[35] 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, the stability constants were found to linearly decrease as the percentage of surfactant increased progressively for both Mn-Suc and Mn-Gln complexes (Fig 1). Linear variation of the species with increasing %w/vTBAB indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions. 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[36] by the cationic surfactant, TBAB, which causes the log β values to decrease linearly.

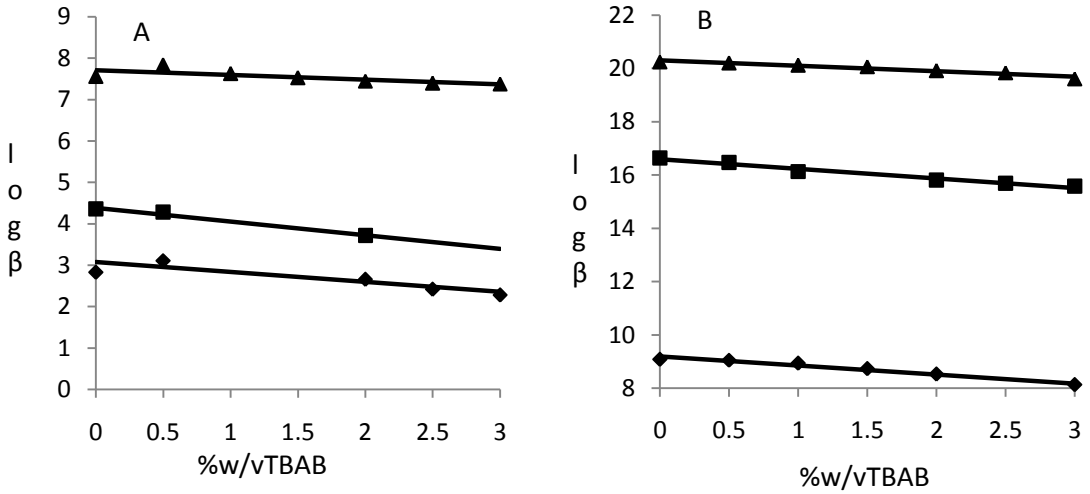

Fig 1: Variation of log β with TBAB-water mixtures of (A) Mn-Suc (♦) ML, (■)ML_2 and (▲) MLH and (B)Mn-Gln (♦) ML, (■)ML_2 and (▲)ML_2H

The formation equilibria are represented below based on the above observations. The plausible equilibria for manganese with Suc are (a)-(c) and those for Gln are (d) - (f). Here equations (d) and (e) are simultaneous reactions.

$M(II) + LH_2$	$MLH + H^+$	….(a)
$M(II) + LH$	$ML + H^+$	….(b)
$ML + LH$	$ML_2 + H^+$	….(c)
$M(II) + LH$	$ML + H^+$	….(d)
$M(II) + 2LH_2$	$ML_2H + 3H^+$	….(e)
ML_2H	$ML_2 + H^+$	….(f)

The charges of species are omitted for clarity. Proton accepting ability of the ligand increases in 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 TBAB-water mixture. This is in good agreement with the linearity of plots of log β values with % w/vTBAB.

DISTRIBUTION DIAGRAMS

Succinic acid has two carboxyl groups and both are protonated. 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. 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 an extent of 90% in the pH range 2.5-8.5. The species formed ML, ML_2 and MLH for Suc and ML, ML_2 and ML_2H for Gln are confirmed by MINIQUAD75. Perusal of the models indicates that the species MLH is highly stable at lower pH and concentrations of ML, ML_2 are constant at higher pH for Suc. For Gln, ML concentration is about 80% and the concentration of ML_2 is almost constant at above pH 7 (Fig 2).

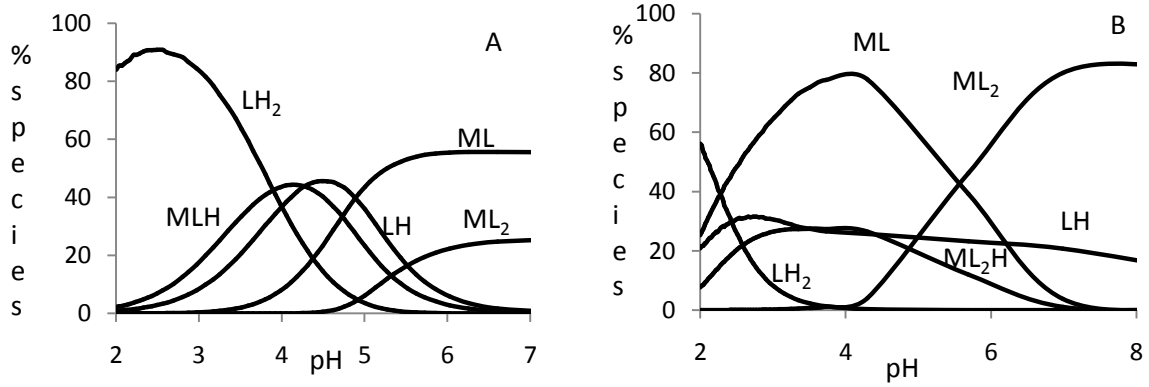

Fig 2: Distribution diagrams Mn(II) complexes of (A) Succinic acid and (B) L-glutamine in 0.5%w/v TBAB-water mixtures.

The plausible structures for succinic acid and L-glutamine complexes are proposed based on the best fit models and equilibria, as given in Figs 3 and 4.

Fig 3: Plausible structures of MLH, ML and ML_2 species of succinic acid complexes with Mn(II).

Fig 4: Plausible Structures of ML, ML_2 and ML_2H species L-glutamine complexes with Mn(II).

Biological significance of present study

The present study is useful to understand the role played by the active site cavities in biological molecules and 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. TBAB presence in aqueous solutions considerably decreases the dielectric constant and these solutions are expected to mimic the physiological conditions.

EXPERIMENTAL

Manganese 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 the determination of the concentrations of above solutions, the data were subjected to ANOVA[37]. The strength of alkali (NaOH) was determined using the Gran plot method[38]. The glass electrode was equilibrated in inert electrolyte.

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 correction factor, log F to correct the pH meter dial reading, was determined using the computer program SCPHD[39,40]. Other experimental details are given elsewhere[37]. The approximate step-wise stability constants were calculated using SCPHD. By following some heuristics[41] 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 MINIQUAD75.

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