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Chemical Speciation Studies of Binary Complexes of Calcium (II) and Magnesium (II) with L-Glutamine and Succinic Acid in Urea-Water Medium

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ABSTRACT

The nature of complexes formed by calcium(II) and magnesium(II) with L-glutamine and succinic acid has been investigated at an ionic strength of 0.16 mol L^{-1} and a temperature of 298 ± 0.1 K in 0-36.83% w/v urea-water mixtures. The formation constants have been determined experimentally by monitoring hydrogen ion concentration. The distribution of the metal ion amongst the complexes formed with the title ligands has also been computed. The formation constants have been refined with the computer program, MINIQUAD75 using the primary alkalimetric data. The species distribution with pH at different compositions of urea in the medium and the plausible equilibria for the formation of the species is discussed. The probable structures of the complexes are also given.

Keywords: Speciation, Stability constants, Metal Ligand Complexes, L-Glutamine, Succinic acid, Urea

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INTRODUCTION

The role of calcium(II) and magnesium(II) in biological systems is well recognized[1-6] and any variation in their concentrations leads to metabolic disorders. Calcium is associated with blood coagulation, neuromuscular and membrane excitability, neurotransmitter secretion, transmission of nerve impulses, maintenance and function of cell membranes, cellular adhesiveness, activation of enzyme reactions and hormone secretion. Magnesium is a necessary co-factor for numerous

enzymatic reactions. L-Glutamine (Gln) and succinic acid (Suc) are involved in citric acid and glyoxalate cycles. Gln is utilized in the brain for the respiration and biosynthesis of substances related to neuronal functions such as γ-aminobutyric acid (GABA).Gln-antioxidant nutrient supplementation can increase body weight, body cell mass and intracellular water when compared with placebo supplementation[7]. Suc can be used[8] in medicaments or nutritional supplements that are effective for treating insulin resistance. Hence, there is every likelihood for the above metal ions and ligands to interact. The authors had investigated the speciation studies of Ca(II) and Mg(II) complexes of Gln and Suc under conditions comparable to those existing in biological systems in urea-water mixtures. The presence of urea considerably increases the dielectric constant and the present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behaviour of protein residues with the metal ion.
The effect of co-solvent (urea or DMF) on the protonation equilibria of Gln and Suc was reported[9] earlier.

MATERIALS AND METHODS

Materials

Aqueous solutions of calcium chloride, magnesium chloride, L-Glutamine and succinic acid (E. Merck, G.R.) were prepared using triple distilled water. The stock solutions were rendered slightly acidic to suppress the hydrolysis of metal ions[10] and increase the solubility of the ligands. The free hydrogen ion concentration in the stock solution was determined by Gran plot method [11]. Urea of 99.5% purity (BDH, A.R.) has been used without further purification and the solutions were never allowed to stand at room temperature continuously for more than 24 hours. All the solutions were standardized by standard methods. The assessment of errors that might have crept during the determination of concentrations has been detailed elsewhere [12].

Alkalimetric Titrations

The titrations have been carried out in the medium containing varying concentrations of urea maintaining an ionic strength of 0.16 mol L^{-1} with sodium chloride at 298.0 ± 0.1 K. A systronics (Model 335) pH meter was used. The glass electrode has been equilibrated in a well stirred urea-water mixture containing inert electrolyte. The effects of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and/ or dissolved carbon dioxide on the response of the glass electrode were taken into account in the form of correction factor[13] which was computed from the simulated acid-base titration data calculated by SCPHD program[14]. A correction was applied to pH meter dial reading to account for the solvent effect on pH. Strong acid was titrated with alkali at regular intervals to check whether complete equilibrium was achieved. The calomel electrode was refilled with urea-water mixture of equivalent composition as that of the titrand. In each titration, the titrand consisted of approximately 1mmol of mineral acid (HCl) in a total volume of 50cm^3. Solutions containing metal ions and ligands (metal to ligand ratios being in the range of 1:2.7 to 1:5.8) were titrated with 0.4 mol L^{-1} sodium hydroxide.

Modeling Strategy The best-fit chemical models consisting of stoichiometric coefficients and logarithm of stability constants (log β) were arrived at by using a computer program

MINIQUAD75[15]. Someheuristics[16] were followed in the refinement of stability constants and validation of models[17].

RESULTS AND DISCUSSION

Complex Equilibria

A preliminary investigation of alkalimetric titration of mixtures of Suc and Gln solutions, in different mole ratios in the presence of mineral acid and inert electrolyte confirmed that these two ligands do not form[9] condensed species. Moreover, dimeric and trimeric species are ruled out since the experimental conditions are not favourable for the formation. Existence of species was determined by performing exhaustive modelling[18]. Models containing various number and combination of species were generated using an expert system package CEES [19] and these models were refined using MINIQUAD75.

Table 1 Best fit chemical models of Ca(II) and Mg(II) binary complexes in urea-water media at 298 K and ionic strength, I = 0.16 mol L^{-1}.

% w/v urea	log β_{mlh} (SD)		NP	U_{corr} x 10^7	Skewness	Kurtosis	χ^2	R-factor
	120	**122**						
Ca(II)-glutamine complexes (pH range: 8.5-11.0)								
00.00	5.01(5)	20.39(4)	28	2.577	-0.17	7.82	35.41	0.025
05.80	3.10(3)	20.50(5)	27	2.874	-1.77	10.04	31.30	0.012
11.52	3.50(2)	20.62(2)	28	3.790	-2.94	17.96	76.00	0.013
20.31	3.46(3)	19.81(3)	28	2.824	-3.14	18.30	60.95	0.011
29.64	3.88(4)	19.74(4)	27	5.192	-2.14	9.04	28.33	0.015
36.83	3.31(7)	19.23(7)	28	2.902	-3.52	21.59	96.38	0.012
Mg(II)-glutamine complexes (pH range: 8.3-10.2)								
	120	**111**						
00.00	3.40(3)	10.82(3)	28	8.185	-0.11	6.47	67.38	0.025
05.80	3.25(2)	10.22(2)	28	1.826	-1.48	14.88	78.86	0.009
11.52	3.90(3)	10.67(2)	27	0.503	-0.61	6.46	30.70	0.004
20.31	4.21(4)	10.40(5)	26	0.369	-0.24	5.36	15.13	0.004
29.64	5.09(3)	10.98(3)	28	1.348	-1.90	9.26	42.10	0.008
36.83	--	10.21(2)	27	1.154	-1.92	8.53	40.78	0.007
Ca(II)-succinic acid complexes (pH range: 3.0-9.0)								
	121	**122**						
00.00	--	--	62	1.626	-0.06	6.13	62.78	0.034
05.80	14.17(5)	9.49(4)	61	3.200	-2.22	13.22	57.66	0.008
11.52	14.11(6)	9.81(7)	62	1.406	-0.57	4.37	45.05	0.006
20.31	13.14(2)	8.74(2)	57	0.948	-0.97	6.87	43.78	0.005
29.64	12.96(6)	8.73(6)	58	1.531	-1.31	5.84	57.45	0.006
36.83	11.83(3)	7.66(2)	59	1.025	-1.17	6.65	68.83	0.005
Mg(II)-succinic acid complexes (pH range: 3.0-9.0)								

	121	**122**						
00.00	9.43(1)	--	62	4.498	-3.79	23.48	109.70	0.016
05.80	7.48(1)	--	61	8.136	-1.46	8.95	118.60	0.014
11.52	8.94(1)	--	62	1.337	-0.82	5.14	41.18	0.005
20.31	7.92(2)	--	57	2.316	-1.50	8.06	59.22	0.008
29.64	8.01(3)	--	59	0.576	-0.75	5.98	57.80	0.004
36.83	9.68(3)	--	60	0.268	-1.75	10.20	56.80	0.002

NP = Number of experimental points; SD = Standard deviation in log β

The final model in urea-water media for Gln-Ca(II) system contained ML_2 and ML_2H_2 and that for Suc-Ca(II) contained ML_2H_2 and ML_2H. Similarly MLH and ML_2 were refined for Gln-Mg(II) system and only ML_2H species for Suc-Mg(II) system. The model parameters along with the statistical parameters as detailed elsewhere [12] is given in Table 1.

A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} indicate that the models represent the experimental data. The Kurtosis values between 4.37 and 23.48 indicate that the residuals form leptokurtic pattern. The values of skewness between -3.79 and -0.06 shows that the residuals form a part of normal distribution and 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 which indicate the need for inclusion of additional species in the model. χ^2 is a special case of γ distribution which measures the probability of residuals forming a part of standard normal distribution.

Protonation constants of the ligands were retrieved from the metal-ligand titrations fixing the stability constants of the binary complexes and compared (Table 2) with those obtained from proton-ligand titration data. The proximity of the former with the latter confirms the accuracy of the titration data.

Table 2. Comparison of protonation constants determined from proton-ligand (P-L) and metal-ligand (M-L) titration data.

System (%w/v urea)	**P-L**		**M-L**	
	Log			
	β_1	**β_2**	**β_1**	**β_2**
Mg(II)-Suc (5.80%)	5.31	9.25	5.28	9.22
Ca(II)-Suc (11.52%)	4.90	8.47	4.87	8.51

Effect of Systematic errors

The computer programs refine the stability constants by minimizing the random errors in the data. But in the presence of considerable systematic errors, not only the stability constants are in error, even some species may be rejected. MINIQUAD75 has no provision to vary the dangerous or influential parameters. Hence, some representative systems were studied in order to have a cognizance of the effect of errors in the concentrations of ingredients on the stability constants of binary metal complexes. Typical results given in Table 3 show that the order of affecting the magnitudes of the stability constant is alkali > ligand > acid > total volume > metal

ion. The increased standard deviation in stability constants and even rejection of some species on the introduction of errors confirms the correctness of the proposed models and this investigation has significance because the data acquisition was done under varied experimental conditions with different accuracies.

Table 3. Effect of errors in influential parameters on the stability constants of Ca(II)- succinic acid complexes in 20.31% w/v urea-water mixtures

Ingredient	% error	log β_{mlh} (SD)	
		122	121
Acid	0	8.74(4)	13.13(2)
	+1	8.70(3)	13.03(3)
	-1	8.79(2)	13.22(2)
Ligand	+1	8.72(2)	12.99(2)
	-1	8.77(2)	13.24(2)
Metal	+1	8.74(2)	13.13(2)
	-1	8.74(2)	13.13(2)
Alkali	+1	8.09(5)	13.87(3)
	-1	8.37(7)	Rejected
Total volume	+1	8.76(2)	13.14(3)
	-1	8.73(2)	13.12(2)
All parameters	+1	8.05(4)	13.75(3)
	-1	8.41(6)	11.79(2)

Effect of Urea

Urea acts as denaturant [20] of DNA, proteins, insulin and other macromolecules but this action is reversible. Urea influences the equilibria due to change in the dielectric constant of the medium that varies the relative contribution of electrostatic and non-electrostatic interactions which in turn change the magnitudes of the stability constants.

A plot of log β versus 1/D (D is dielectric constant) should be linear if Born's classical treatment holds good indicating that electrostatic forces alone operate. The linear variation (Figure 1) of the log β values of Ca(II) and Mg(II) complexes of Gln and Suc with 1/D reveals that the electrostatic forces are dominating the equilibrium process under the present experimental conditions. In urea-water mixtures, the dielectric constant increases from 79.4 to 92.48 as the percentage of urea increases from 5.83-36.83 %(w/v). Hence, the stability of the species decreases with increased urea content. Since complex formation can be viewed as a competition between pure and solvated forms of ligand and metal ion, the solute-solvent interactions, relative thermodynamic stabilities and kinetic labilities are also expected to play an important role. The slight deviations from the linearity at certain composition indicate the blend of electrostatic and non-electrostatic interactions.

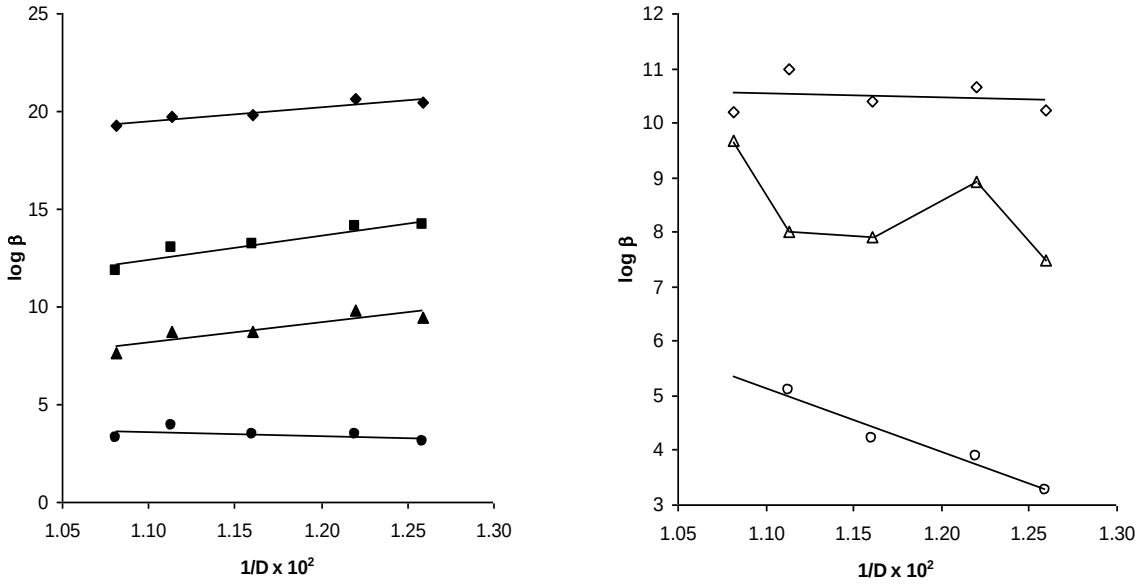


Figure 1. Variation of log β with reciprocal of dielectric constant in urea-water mixtures

Ca(II)-Succinic acid: (▲) log β_{122} (■) log β_{121} Mg(II)-Succinic acid: (△) log β_{121}

Ca(II)-L-Glutamine: (●) log β_{120} (◆) log β_{122} Mg(II)-L-Glutamine: (○) log β_{120} (◇) log β_{111}

Distribution diagrams

Gln has three functional groups (amino, carboxyl and amido) but only amino and carboxyl groups participate in protonation equilibria. The forms of Gln are LH_2^+, LH and L^- in the pH regions 1.5-4.5, 1.5-9.5 and 8-11.0 respectively[9]. Since the present study is confined to the pH ranges 8.5-11.0 and 8.3-10.2 for Ca(II) and Mg(II) complexes of Gln, the plausible metal-ligand species are ML_2, MLH^+ and $ML_2H_2^{2+}$. The species ML_2 and $ML_2H_2^{2+}$ for Ca(II) and ML_2 and MLH^+ for Mg(II) were confirmed by MINIQUAD75. The equilibria for the formation of these species in the pH ranges of the study can be represented as follows:

i) $M(II) + LH_2^+ \rightleftharpoons MLH^{2+} + H^+$

ii) $MLH^{2+} + LH_2^+ \rightleftharpoons ML_2H_2^{2+} + H^+$

iii) $ML_2H_2^{2+} \rightleftharpoons ML_2H^+ + H^+$ (minor process)

iv) $ML_2H^+ \rightleftharpoons ML_2 + H^+$

Since ML_2H^+ species is not refined in the present study, process (iii) must be minor or ML_2 is readily formed from ML_2H^+.

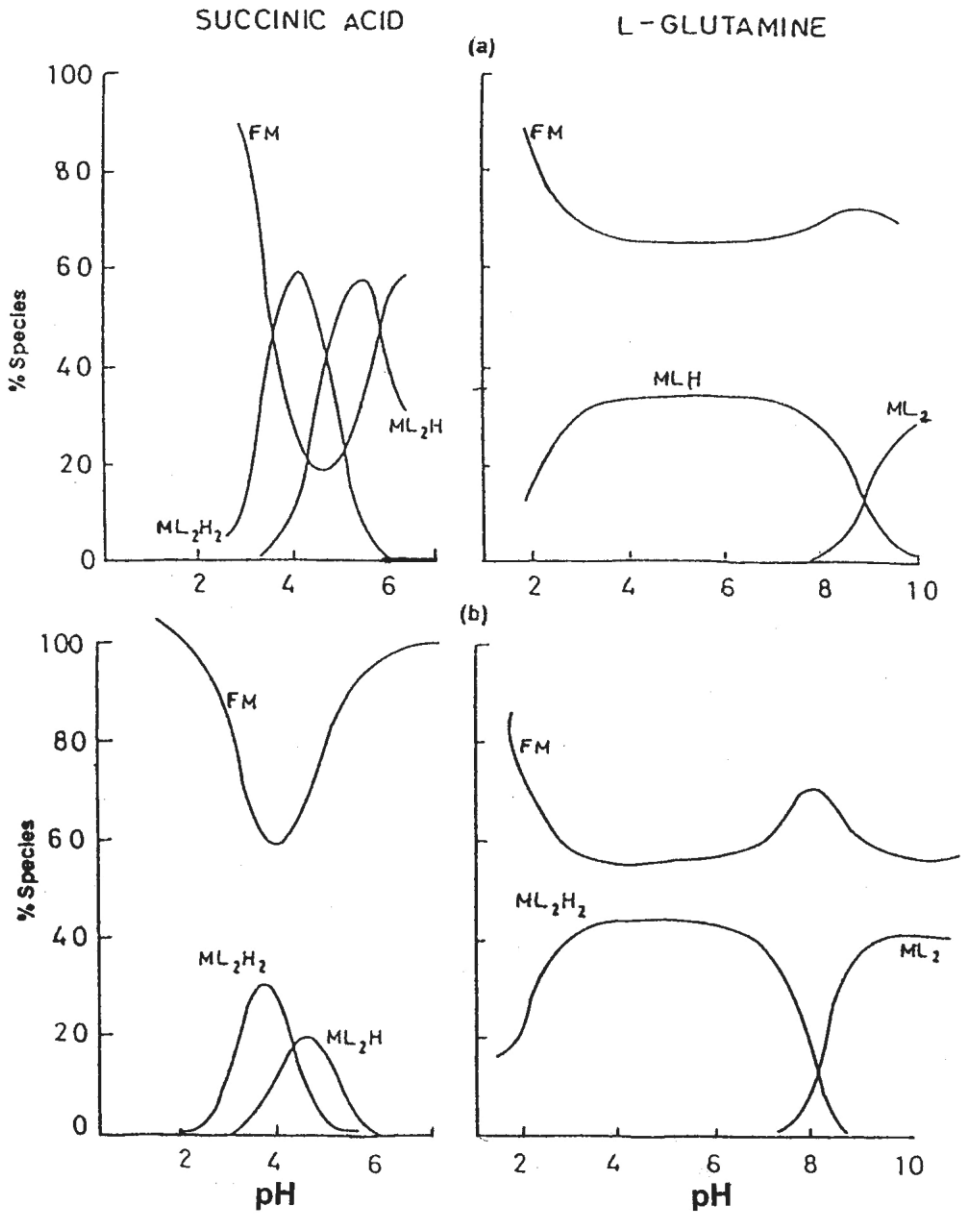


Fig. 2. Distribution diagrams of binary complexes of Ca(II) with succinic acid and L-glutamine in (a) 5.80% and (b) 36.83% urea-water mixtures.

Succinic acid has two carboxylate groups and both are protonated. The various forms of Suc are LH_2, LH and L^{2-} in the pH ranges 3.0-6.5, 3.0-7.0 and 4.5-7.0 respectively. The species confirmed in the pH range of the present study (3.0-9.0) wherein the species confirmed are $ML_2H_2^{2+}$and ML_2H^+. The formation equilibria for these two species can be represented as:

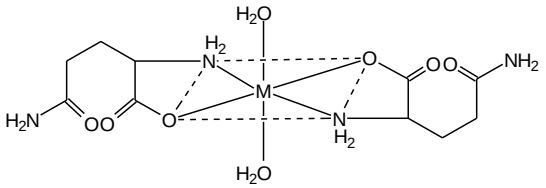
v) $M(II) + LH_2 \rightleftharpoons MLH^+ + H^+$ (minor process)

vi) $MLH^+ + LH_2 \rightleftharpoons ML_2H_2 + H^+$

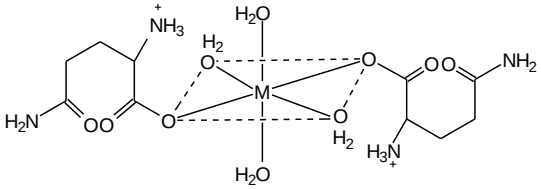
vii) $MLH^+ + LH^- \rightleftharpoons ML_2H^- + H^+$

viii) $ML_2H_2 \rightleftharpoons ML_2H^- + H^+$

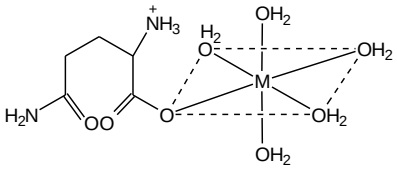
The species MLH^+ could not be detected as step (v) can be a minor process or the equilibria (v)-(vii) might be simultaneous so that the species MLH^+ formed instantaneously converted to ML_2H_2 and ML_2H^-. The distribution of species with pH is shown in Figure 2 for some typical systems. The species concentration decreased with increase in urea content. Based on the above equilibria, the possible structures of the complexes can be represented as given in Figure 3.



ML_2

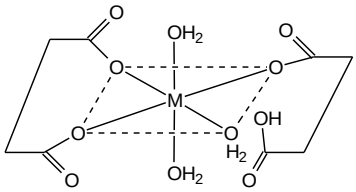


ML_2H_2

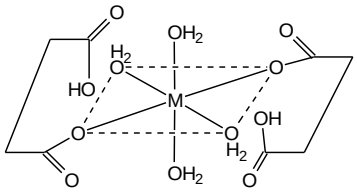


MLH

L-glutamine complexes with Ca(II) or Mg(II)



ML_2H



ML_2H_2

Succinic acid complexes with Ca(II) or Mg(II)

Fig 3. Structures of Ca(II) and Mg(II) complexes

APPLICATIONS

The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex formed by the metal ion and (iii) the bonding behaviour of protein residues with the metal ion.

CONCLUSION

The following conclusions result from the modeling studies:

1. The effect of errors in the concentration of ingredients on stability constants of metal complexes is in the order:
   alkali > ligand > acid > total volume > metal ion.

2. The protonation constants determined from proton ligand titration data are close to those retrieved from metal ligand titration data confirming the existence of the reported species only.
3. The final model in urea-water media for glutamine complexes of Ca(II) contained ML_2 and ML_2H_2 and that for succinic acid complexes of Ca(II) contained ML_2H_2 and ML_2H. Similarly MLH and ML_2 were refined for Gln-Mg(II) system and ML_2H species for Suc-Mg(II) system.
4. The linear variation of log β values of Ca(II) and Mg(II) complexes of glutamine and succinic acid with inverse of dielectric constant of the medium reveals that electrostatic forces are dominating the equilibrium process. The slight deviation from linearity at certain compositions of the co-solvent indicates the blend of both electrostatic and non-electrostatic interactions in some systems.

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