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POTENTIOMETRIC STUDY OF THE BINARY AND TERNARY COMPLEXES OF METAL IONS WITH CHLOROQUINE PHOSPHATE

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ABSTRACT

Potentiometric studies of complexes of Cu (II) and Ni (II) with amino acid (L- isoleucine, L-leucine, L-serine) and chloroquine phosphate (CQ) are conducted in aqueous solution. Change in potential value reflected the formation of complex. Stability constants of the binary and ternary complexes are determined at 35 ± 0.5°C and at ionic strength I = 0.1M KNO_3. Titration graphs were plotted between H^+ ion concentration and moles of base added. Irving-Rosotti method was applied for calculation of stability constants at each point for binary and ternary complexes. In optimum pH condition, stoichiometric relationship of 1 : 1 metal and CQ binary complex and 1 : 1 : 1 metal : amino acid and CQ ternary complexes are formed. Δ log K values were also calculated for the system. It indicates greater stabilities of binary complexes over the ternary ones.

Key words: Amino acids, Complex, pH-metric study, Nickel, Copper, Chloroquine phosphate

INTRODUCTION

"Inorganic Pharmaceuticals"[1] provide an exciting possibility for oral delivery of a wide variety of peptide-based drugs, which currently have to be given intravenously because of digestive degradation and poor absorption. Many anti-inflammatory and anti-tumour drugs are more potent when co-administered or complexed with copper than they are given alone[2] Although the molecular mechanism by which chloroquine exerts its effects on the malarial parasite remains unclear, the drug has previously been found to interact specifically with the glycolytic enzyme lactate dehydrogenase from the parasite. Specific interactions between the drug and amino acids unique to the malarial form of the enzyme suggest that this binding is selective. The structure of this enzyme-inhibitor complex provides a template

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from which the quinoline moiety might be modified to develop more efficient inhibitors of the enzyme[3].

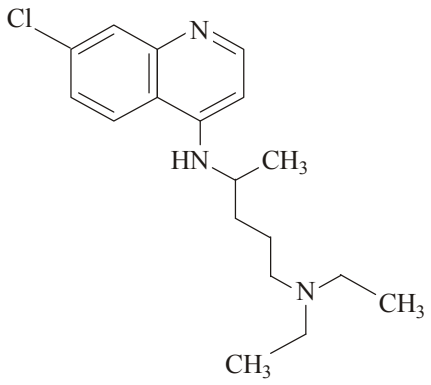

Fig. 1: Chloroquine

Interactions of chloroquine, quinine, and 9-epiquinine with Fe (III)PPIX are shown to remain strong at pH 5.6; the approximate pH of the food vacuole of the malaria parasite, which is believed to be the locus of drug activity[4]. Conductometric method and indirect atomic absorption spectrometric method have been used to measure the excess metal ion present in supernatant solutions after precipitation of the ion associates[5]. Electron donor – acceptor interaction between drugs, which acts as electron donors and some electron deficient compounds have also been studied spectrophotometrically[6].

Study of complexes by varied techniques could provide an invaluable boost to the design of new drugs urgently needed to overcome developing drug resistance. Thus, study of stability of binary and ternary complexes of chloroquine with metal ions and amino acids in solution via potentiometry is aimed.

EXPERIMENTAL

Methodology

The study involved potentiometric titration of ligands in the absence and presence of metal ions under controlled experimental conditions. The concentration changes caused by complex formation are reflected in the potential change of suitable chosen electrodes. As the conjugate acids of most ligands are weak; consequently, complex formation means a competition between protons and metal ions for the ligands. During complex formation, changes in the hydrogen ion concentration of solution take place. These changes can be measured with the help of glass and calomel electrodes. By analyzing pH data, metal ligand

concentration can be determined. On the basis of the interaction between a metal ion M^{n+}, ligand A (primary ligand) and L (secondary ligands), protons (H^+) and hydroxide ions (OH^-), the existence of new equilibria can be shown. The method has been reviewed by Marcus[7], Beck[8] and Fridman[9]. The determination of formation constants is based on the measurement of free concentration of metal ion or the ligand at equilibrium.

Materials

Cu (II), and Ni (II) metal ions were used for chelation. Amino acids (L-leucine, L-isoleucine, L-serine) and chloroquine phosphate (CQ) were used as primary and secondary ligands, respectively. Potassium nitrate and buffer solutions were used for maintaining the ionic strength and for calibrating the pH meter, respectively. Solutions of above metal ions and ligands were titrated pH metrically with potassium hydroxide solution.

Preparation of stock solutions

All the reagents used in the present investigations are either AR/GR samples. All the solutions used in the pH metric studies were prepared in CO_2 free conductance water. It was obtained freshly by the redistillation of distilled water over alkaline $KMnO_4$. The distillate was boiled off to expel the dissolved CO_2 and O_2 and stored in air tight bottles. In order to avoid adverse effects like evaporation, hydrolysis, oxidation and ageing, all the solutions were prepared freshly just before the titrations.

Metal ions solutions

(i) Copper (II) solution

The copper (II) solution (0.01 M) was prepared by dissolving required quantity of $CuSO_4.5H_2O$ (GR E. Merck) in double distilled water. The strength of this solution was determined by iodometric titration using 0.1 N sodium thiosulphate solution in presence of starch as indicator. The details of procedure are available in literature[10].

(ii) Nickel (II) solution

Nickel (II) solution (0.01 M) was prepared by dissolving required amount of $Ni(NO_3)_2$ (GR E. Merck) in conductance water. The standardization of nickel nitrate solution was done volumetrically with EDTA in the alkaline region of pH (pH ~ 10). Murexide was used as an indicator and titration was carried out as per details discussed by Vogel[10].

Primary ligand solutions: Amino acid solutions

Biochemical grade amino acids, AA (where AA = L-leucine, L-isoleucine and L-serine) were recrystallised and the purity was checked by chromatographic method using

acetone and perchloric acid as solvents. Stock solutions of amino acids were prepared by dissolving the accurately weighed amount of substance in conductance water. The strength of stock solutions of these amino acids was estimated according to the procedure described by Gowda and Mahadevappa[11].

Secondary ligand solutions: Chloroquine phosphate solution

Chloroquine phosphate was prepared by dissolving requisite quantity in minimum amount of hydrochloric acid and making up the solution with conductance water.

Potassium hydroxide solution

The carbonate free 0.1 M solution of potassium hydroxides (GR E. Merck) was prepared as per the method suggested by Vogel[12] and stored in an automatic microburette assembly. It was standardized against 0. 1 M potassium hydrogen phthalate (KHP).

Potassium nitrate solution

1.0 M Potassium nitrate solution was prepared by dissolving the required quantity of potassium nitrate (GR E. Merck) in conductance water. It was used for maintaining the constant ionic strength.

Buffer solution

Analytically pure samples of potassium hydrogen phthalate and borax (GR E. Merck) were used for the preparation of 0.05 M buffer solutions. They were respectively employed to calibrate the pH meter in acidic and alkaline region of the pH.

Nitric acid

Nitric acid GR (E.Merck) was used to prepare 0.1 M stock solution. The acid was standardized against standard KOH solution.

Apparatus

pH Meter: All the measurements were made with an Elico Model digital pH meter (readable accuracy of ± 0.01 pH unit) equipped with a combined glass and calomel electrodes at 35 ± 0.5˚C for the measurement of hydrogen ion concentration.

Thermostat: A Toshniwal thermostat provided with a pump arrangement for circulating the thermostat water through an external titration cell was used. The thermostat was capable of maintaining the temperature within ± 0.1˚C.

Potentiometric titrations

Primary ligands

Potentiometric titrations of primary ligands were performed by mixing 10.0 mL of 0.01 M solution of amino acids AA (where AA = L-leucine and L-isoleucine, L-serine) with 5 mL of 1.0 M potassium nitrate and 1.0 mL of 0.1 M nitric acid against 0.1 M KOH solution after diluting the solutions to 50 mL.

Secondary ligand

The secondary ligands i. e. CQ was titrated by taking 10 mL of 0.01 M fresh solution with 2.0 mL of 0.1 M HNO_3 and 5 mL of 1.0 M KNO_3. It was then diluted to 50 mL with water and titrated against 0.1 M KOH solution.

1 : 1 Binary system of metal ion and primary ligand

The solutions for the titration of 1 : 1 metal-ligand binary system (MA) were prepared by mixing solutions of 10 mL of 0.01M metal ion M (where M = Cu (II), Ni (II), with 10 mL of 0.01 M primary ligand (L-leucine and L-isoleucine, L-serine), 1 mL of 0.1 M HNO_3 and 5 mL of 1 M KNO_3. In each case, total volume was made up to 50 mL with conductance water then titrated against 0.1 M KOH.

1 : 1 Binary systems of metal ion and secondary ligand

Binary system for secondary ligand was titrated by using the same procedure as for primary ligand except that CQ was used in place of amino acids.

1 : 1 : 1 Ternary system

10 mL of 0.01 M metal ion solution, 10 mL of 0.01 M primary ligand solution, 10 mL of 0.01 M secondary ligand, 5 mL of 1 M KNO_3 and 3 mL of 0.1 M HNO_3 were mixed to prepare ternary solution in 1 : 1 : 1 molar ratio (MAL). This solution was diluted to 50 mL by adding conductance water and titrated against 0.1 M KOH solution.

Determination of formation constants

Binary complexes

The ionization constants of the ligands and metal-ligand formation constants have been determined pH-metrically using method due to Irving and Rossotti[13]. The procedure involves the titration of the following sets of solutions

(i) Mineral acid (Acid titration)

(ii) Mineral acid + Ligand (Ligand titration)

(iii) Mineral acid+ Ligand + Metal ion (Metal titration)

The reaction mixture was taken into a double walled glass titration vessel provided with an inlet and outlet tube for the circulation of thermostat water. The electrodes were dipped into the reaction mixture .The solution is stirred constantly with the aid of a magnetic stirrer. Sufficient time was allowed for the equilibrium to be established as shown by the constancy of the pH meter reading. The titrations were carried out by adding the standard CO_2 free potassium hydroxide solution from a micro-burette and noting the pH after each addition. In all these sets, the total initial volume ($V° = 50$ mL), total ionic strength [I] and temperature were kept constant. The concentrations of ligand (T°_{L}) and that of metal ion (T°_{M}) were taken in the ratio 1 : 1.

The acid, ligand and metal titration curves were obtained by plotting the obtained readings against the volume of alkali added.

The successive proton ligand formation constants of the ligands and those of metal-ligand formation constants of binary complexes have been evaluated by computational techniques discussed by Irving - Rossotti as well as by Rossotti and Rossotti[13].

Terary complexes

Formation of ternary complexes may take place either by step-wise coordination or by simultaneous coordination of the ligands (Eq.1-3).

$$M + A \rightleftharpoons MA \quad ...(1)$$

$$MA + L \rightleftharpoons MAL \quad ...(2)$$

$$M + A + L \rightleftharpoons MAL \quad ...(3)$$

The formation constants of mixed ligand complexes have been determined using the procedures due to Ramamurthy and Santhappa[14]. It involves the titration of following mixtures against CO_2 free alkali.

(i) Mineral acid (Acid titration)

(ii) Mineral acid + Ligand (A) (Ligand [A] titration)

(iii) Mineral acid + Ligand (A) + Metal ion (1 : 1 Binary complex / MA/ titration)

(iv) Mineral acid + Ligand (L) (Ligand [L] titration)

(v) Mineral acid + Ligand (L) + Metal ion (1 : 1 Binary complex [ML] titration)

(vi) Mineral acid + Ligand (A) + Ligand (L) + Metal ion (1 : 1 : 1 Ternary complex titration)

A molar ratio of 1 : 1 : 1 with respect to M : A : L was maintained.

RESULTS AND DISCUSSION

Titration of binary system of chloroquine phosphate is shown graphically (CQ in Figs. 2 - 4). The titration curves show two inflections. These curves exhibit no large pH jump, but abrupt change in pH resulted, when one and two moles of potassium hydroxide were added per mole of chloroquine phosphate. So, in case of chloroquine phosphate, dissociation should take place as follows:

$$H_2CQ^{2+} \overset{K_{1a}}{\rightleftharpoons} HCQ^{+} + H^{+} \quad ...(4)$$

$$HCQ^{+} \overset{K_{2a}}{\rightleftharpoons} CQ + H^{+} \quad ...(5)$$

The two nitrogen in the side chain are supposed to be protonated due to phosphate group present in the molecule (Fig. 1)

The potentiometric titration curves (Ni-CQ in Figs. 2 - 4) of solutions containing equimolar quantities of Cu (II)/Ni (II) and chloroquine phosphate in 1 : 1 molar ratio, show a single inflection at two moles of base. The reaction between the metal (II) ion and CQ may be speculated as -

$$M^{2+} + H_2CQ^{2+} \rightleftharpoons [M(HCQ^{+})]^{+} + H^{+} \quad ...(6)$$

$$[M(HCQ^{+})]^{+} + OH^{-} \rightleftharpoons [M(CQ)] + H_2O \quad ...(7)$$

The formation constant for these complexes can be given as -

$$K^{M}_{M\text{-}CQ} = \frac{[M(CQ)]}{[M\ [H_2CQ^{2+}]} \quad ...(8)$$

The values of log $K^{M}_{M\text{-}CQ}$ have been calculated by Irving and Rossotti method[13] at each titration point and are given in Table 1.

A comparative account of the first dissociation constants of the secondary ligands along with the formation constants of ML has been presented in Table I.

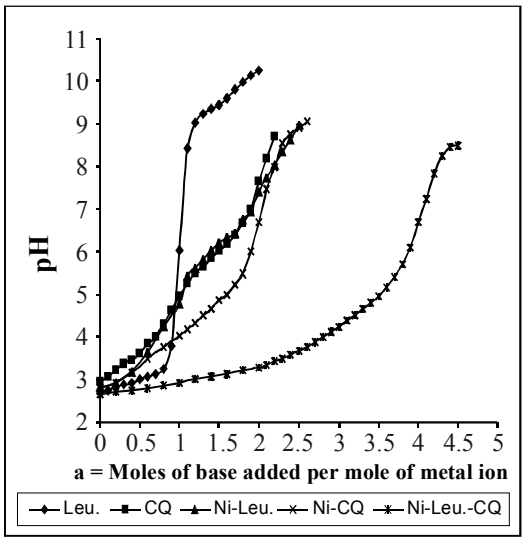

Fig. 2: Potentiometric titration of Ni (II)-L-Leu.-CQ system

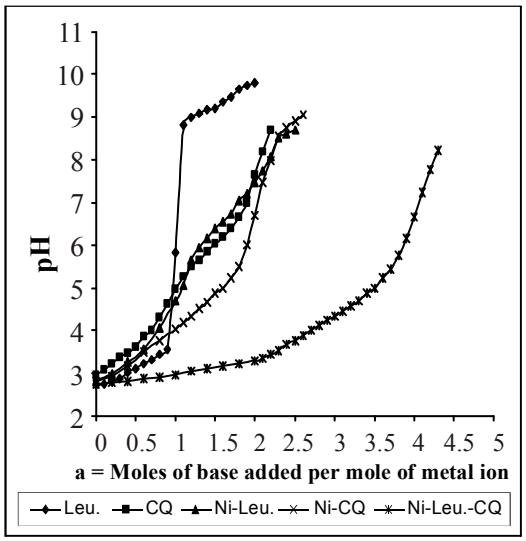

Fig. 3: Potentiometric titration of Ni (II)-L-IIeu.-CQ system

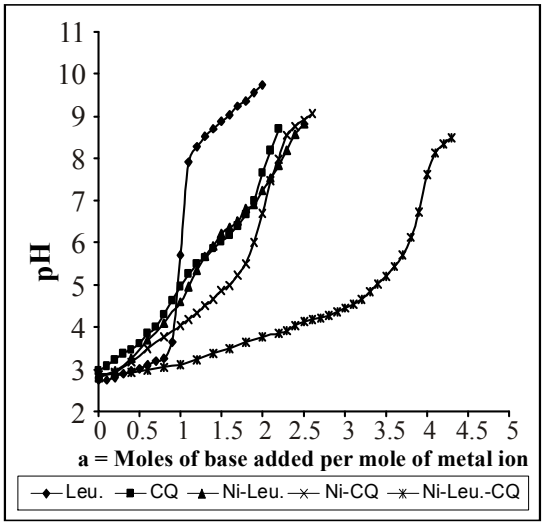

Fig. 4: Potentiometric titration of Ni (II)-L-Ser.-CQ system

The potentiometric titration curves (Figs. 2 - 4 to curves Ni-AA-CQ) of Cu (II)/Ni (II) in presence of equimolar solutions of amino acids and chloroquine phosphate, show an

inflection at 3 moles of base added per mole of metal ion. The curve for ternary systems lie below the curves for MA or ML binary systems. These observations suggest the formation of a mixed complex MAL.

Table 1: Acid dissociation constants of secondary ligands and stability constants of their metal complexes [μ = 0.1 M (KNO_3), T = 35 ± 0.5 °C]

Metal ion	Ligand	pK_{1a}	pK_{2a}	log K^{M}_{M-CQ}
Cu (II)	CQ	3.206	5.542	3.626
Ni (II)	CQ	3.206	5.542	2.661

Thus, the pH metric studies indicate the formation of mixed species in aqueous solutions according to the following equations:

$$M^{2+} + H_3N^+CHRCOO^- \rightleftharpoons [M(NH_2CHRCOO)]^+ + H^+ \quad ...(9)$$

$$[M(NH_2CHRCOO)]^+ + H_2CQ^{2+} \rightleftharpoons [M(NH_2CHRCOO)CQ] + 2H^+ \quad ...(10)$$

Due to chelate effect, the formation of 1 : 1 M- AA complex is preferred in presence CQ. However, the formation of $[M(CQ)]^+$ can not be ruled out as amino acids are more basic than CQ.

The values of stability constants can be calculated at each titration point by employing mass balance equation as follows:

$$K^{MA}_{MACQ} = \frac{[M(AA)((CQ)]\,[H]^2}{[M(AA)[H_2CQ]} \quad ...(11)$$

The overall stability constant (log β)[15] can be evaluated as –

$$\log \beta = \log K^{MA}_{MAL} + \log K^{M}_{MA} \quad ...(12)$$

The values of stepwise stability constant (log K) and overall stability (β) are reported in Table 2. A constant value of log K for each titration point between a = 3 to 4, has been calculated employing an Rossotti method[13].

Table 2: The stepwise and overall stability constants of 1 : 1 : 1 M-AA-CQ complexes and corresponding Δ log K values in aqueous medium [μ = 0.1 M (KNO_3), t = 35 ± 0.5 °C]

Metal ion	Ligand	Log K_{MAL}^{MA}	log β_{MAL}^{MA}	Δ log K
Cu (II)	L-Leucine	3.37	11.505	-0.235
Cu (II)	L-Isoleucine	3.32	9.184	-0.28
Cu (II)	L-Serine	3.21	8.827	-0.368
Ni (II)	L-Leucine	2.57	10.706	-0.069
Ni (II)	L-Isoleucine	2.38	8.188	-0.193
Ni (II)	L-Serine	2.20	7.895	-0.293

CONCLUSION

Potentiometry is a reliable tool to measure the stability of complexes. During the course of present investigation, the metal ion in +2 oxidation state forms complex with amino acid in preference to chloroquine as amino acid are strongly basic in character as compared to chloroquine. However, the formation of metal and chloroquine binary complexes is supported by the log K values. Mixed ligand ternary complex can be formed step-wise or simultaneously. Complexes of copper were found to be more stable than nickel. Ternary complexes have been found to be less stable as compared to binary ones. Their relative stabilities have been compared in terms of Δ log K[16]. The negative values of Δ log K in the present investigations, may allow us to reach to the firm conclusion that the ternary complexes are significantly less stable than the binary complexes and the equilibria lie toward left hand side of following reaction:

$$MA + H_2L \rightleftharpoons MAL + 2H \qquad ...(13)$$

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