Electrophoretic Technique in Determination of Stability Constants of Mixed Complexes (M-NTA-Cysteine)

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Summary: The stability constants of different complex species of some metal ion viz. Hg(II), Be(II) and Ni(II) with cysteine were determined electrophoretically at an ionic strength of 0.1 M percholoric acid and 35°C. Beside binary complexes, ternary complexes involving NTA and amino acid (Cysteine) have also been studied. For studying mixed ligand complexes the background electrolyte is made 1 x 10^{-2}M with respect to NTA and its pH brought to 8.5. This pH is purposely chosen because amino acid and NTA form very stable complexes much a head of this pH. The stability constant of complexes M-NTA- Cysteine have been found to 5.06, 4.93 and 4.88 (log K values) for Hg(II), Be(II) and Ni(II) complexes respectively.

Introduction

Out of all the technique adopted for the study of metal ligand equilibria, we have opted for paper electrophoresis, not much work however, is on record in the application of paper electrophreresis for examing complexation reactions. A significant development on the determination of stability constants of complexes was made by Jokl [1] in 1964. A theoretical treatment similar to that of Jokl was adopted by Biernat [2] for the study of stepwise complex. The use of paper electrophoretic technique [3] for the study of metal complex system with single ligand seems to be well estabilished but there is no systematic study for mixed complexes.

However Czakis-Sulikowski [4] made some observation on the formation of mixed halide complexes of Hg(II), but it is only qualitative and does not throw light either on the nature of the species

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or on their stabilities. Publication [5-6] from our laboratories described a new method for study of mixed complexes. The present work is an extension of the technique and reports our observation on the mixed system viz Hg(II)/Be(II)Ni(II)-Nitrilotriacetate-cysteine.

Discussion

(1) M-cysteine binary system

The electrophoretic mobility of metal spot against pH gives a curve with a number of plateaus shown in Fig. 1. The every plateau indicates the formation of a certain complex species. A plateau is obviously indication of a pH range, where speed is practically constant. The first one in beginning corresponds to a region where metal ions are uncomplexed. The fig. 2, reveals that second plateaus in each case with zero mobility indicating the formation of 1:1 complexes of neutral nature. Further increase of pH mobility decreases giving rise to third plateau lies in negative region anionic nature of metal complexes indicated. Chemical literature also assigns prominent chelating properties to unprotonated anionic species of cysteine ruling out any such property to Zwitter ion [7]. Further increase of pH has no effect on mobility of metal ions. In view of above observation the complexation of metal ion with cysteine anion $[L^{2-}]$ may be represented as:

$$M^{2+} + L^{2-} \underset{}{\overset{K_1}{\rightleftharpoons}} ML$$

$$ML + L^{2-} \underset{}{\overset{K_2}{\rightleftharpoons}} ML_2^{2-}$$

The metal spot on the paper is thus a conglomeration of uncomplexed metal ions, 1:1 complex and 1:2 complex. The spot moving under the influence of electric field, the overall mobility is given by equation

$$U = \sum_n u_n f_n$$

This equation is transformed to the following form on taking into consideration the different equilibria:

$$U = \frac{u_o + u_1 K_1 [L^{2-}] + u_2 K_1 K_2 [L^{2-}]^2}{1 + K_1 [L^{2-}] + K_1 K_2 [L^{2-}]^2}$$

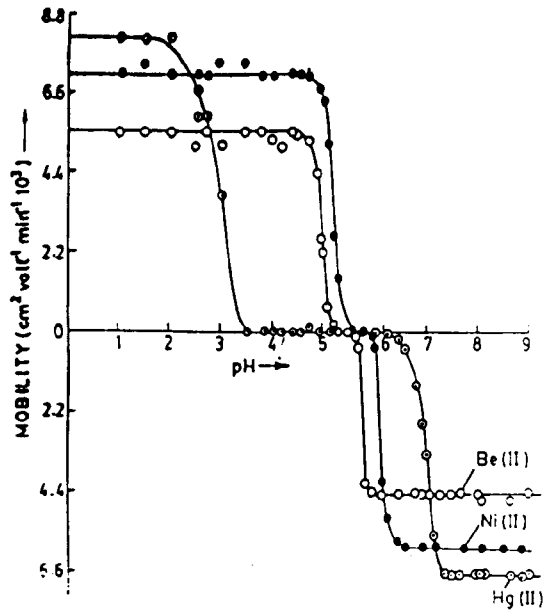

Fig.1: Mobility curve [Hg(II), Be(II) and Ni(II)-cysteine]system.
[Temp. 35°C, ionic strength 0.1]

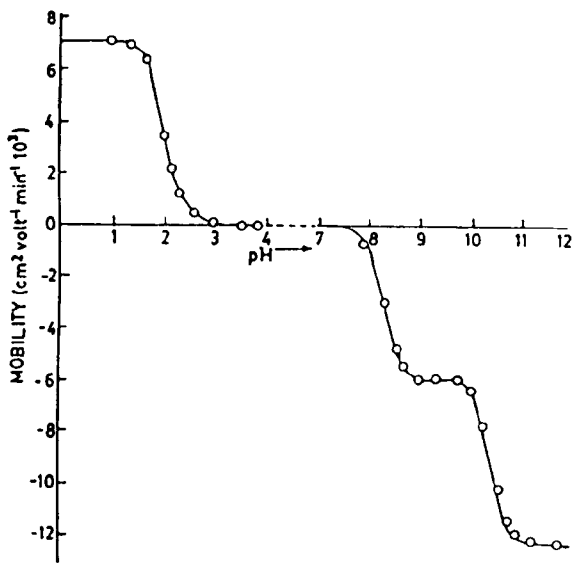

Fig.2: Mobility curve H^+ - cysteine (temp 35°C ionic strength 0.1)

where, u_o, u_1 and u_2 are mobility of uncomplexed metal ion, 1:1 metal complex and 1:2 metal complex.

The equation has been used for calculating stability constants of the complexes of metal ions with the cysteine. For calculating first stability con-

stant K_1 the region between first and second plateau is partinent. The overall mobility 'U' will be equal to the arithmetic mean of mobility of uncomplexed metal ion, u_o and that of first complex u_1 at a pH where $K_1 = 1/[L^{2-}]$. With the help of dissociation constants of cysteine ($k_1 = 10^{2.00}$, $k_2 = 10^{8.25}$, $k_3 = 10^{10.35}$), which is shown in fig. 2, the concentration of cystein anion $[L^{2-}]$ is determined for the pH, from which K_1 can be calculated. The concentration of chelating amino acid (cysteine) species $[L^{2-}]$ is calculated with the help of equation.

$$[L^{2-}] = \frac{[L_T]}{1 + \frac{[H]}{K_3} + \frac{[H]^2}{K_2 K_3} + \frac{[H]^3}{K_1 K_2 K_3}}$$

Where, $[L_T]$ = total concentration.

The stability constants K_2 of second complex can be calculated by taking into consideration, the region between second and third plateau of mobility curve, their calculated values are given in Table 1.

Ternary system

The overall mobility of metal ion spot has been plotted against pH of background electrolyte containing NTA shown in Fig. 3. With increases of pH the mobility of metal spot decreases and finally become constant (second plateau). The dedcrease in mobility is due to progressive complexation of metal ion with triple charged anionic species of NTA till the last plateau. The complex formed with bivalent metal ions should bel:1 in composition with a net negative charge. The stability constant of these complexes have been calculated in a manner as complexes with other amino acid. These values are recorded in Table 1.

(3) M-NTA Cysteine ternary system

On the plot of mobility against log of concentration of added amino acid a curve is obtained, which is shown in Fig. 4. The first constant values of mobility obviously corresponds to mobility of M-NTA complex, whereas second constant mobility would corresponds to the mobility of second complex. The new complex can not be 1:1 or 1:2 metal amino acid complex for mobilities of latter complex species are different from the mobility of the new complex.

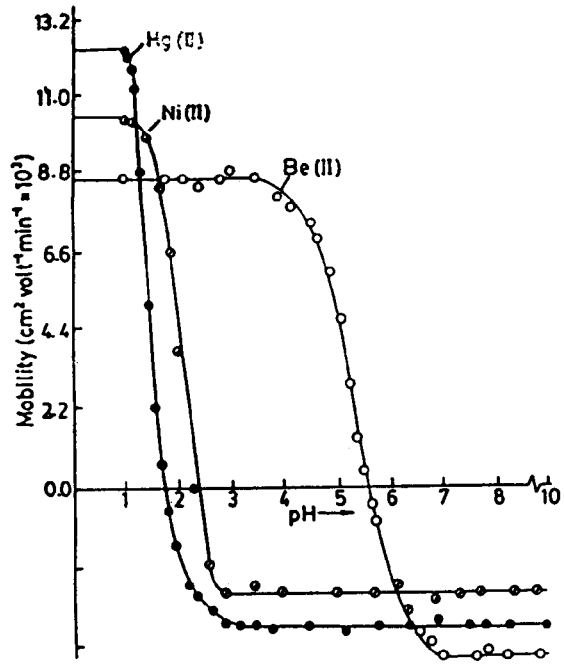

Fig.3: Mobility curve [Hg(II), Be(II) and Ni(II)-NTA] System
[Temp. 35°C ionic strength 0.1]

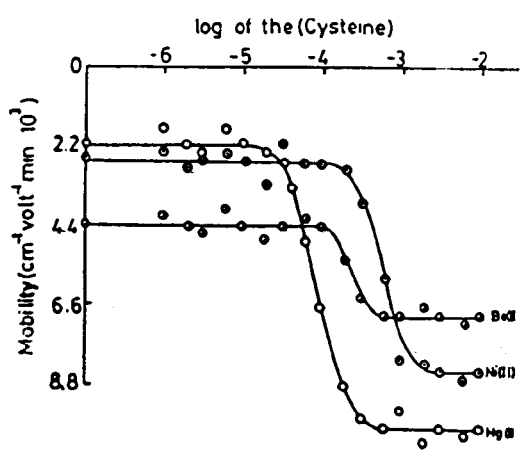

Fig.4: Mobility curve [Hg(II), Be(II) and Ni(II)-NTA-Cysteine
[Tempe 35°C ionic strength 0.1]

Since the mobility of the new complex is more negative than the mobility of pure M-NTA complex, it is inferared that anionic species of amino acids has added to M-NTA complex to form mixed complexes. The interaction may be represented as

$$ML' + L \underset{}{\overset{K^1}{\rightleftharpoons}} ML'L$$

Table 1: Stability constants of binary and ternary complexes of Hg (II), Be(II) and Hi(II)
(Ionic strength = 0.1) : (Temperature = 35°C)
NTA Anion = $N(CH_2COO)_3$: Cysteine Anion =

Metal Ions	Calculated $\log K_{1\,ML}^{M}$	Valute of Stability Constants $\log K_{2\,ML_2}^{M}$	$\log K^{M}_{M\text{-}NTA}$	$\log K^{M\text{-}NTA}_{M\text{-}NTA\text{-}L}$	Literature value of stability constants $\log K_{1ML}^{M}$	$\log K_{2ML_2}^{M}$	$\log K_{M\text{-}NTA}^{M}$	$\log K^{M\text{-}NTA}_{M\text{-}NTA\text{-}L}$
Hg(II)	14.64	21.26	13.30	5.06	14.21(10) 14.40(8)	20.50(10)	14.6 -	-
							7.10(13)	-
Be(II)	10.60	19.60	7.10	4.93	-	-	7.11 (12)	-
Ni(II)	10.20	18.80	11.49	4.88	9.64(10)	19.40(10)	10.80(11)	-
					9.00 (10)	20.16(10)	11.90(11)	-
					9.82(8)	20.37(8)	-	-
					9.82(8)	-	-	-

Where $K_{1\,ML}^{M} = \frac{[ML]}{[M]\,[L]}$, $K_{2\,ML_2}^{M} = \frac{[ML_2]}{[ML]\,[L]}$; $K_{M\text{-}NTA}^{M} = \frac{[M\text{-}NTA]}{[M][NTA]}$; $K_{M\text{-}NTA\text{-}L}^{M\text{-}NTA} \frac{[M\text{-}NTA]}{[M\text{-}NTA]\,[L]}$

Under these conditions overall mobility can be given by expression.

$$U = \frac{U_o + u_1 k'[L]}{1 + K'[L]}$$

where, u_o and u_1 are mobility of M-NTA and mixed ML'L complexes respectively.

These mobilities pertain to the two plateau region of the curve. Using again the principle of average mobility K' can be determined to be equal to 1/[L]. All these values of K' are given in Table 1.

On comparing these values with the literature values. It was found that both are not in quite agreement. The difference in result can be attributed to the different experimental conditions i.e. ionic strength and temperature though with certain modification in experimental conditions we have tried to remove the error, even then the precision of the method is limited to that of paper electrophoresis and the range of uncertainity in the results in $\pm$ 5%

Experimental

Instruments

A systronics model 604 electrophoresis equipment system was used, which has a built-in power supply (a.c.-d.c.) that is fed directly to paper electrophoretic tanks. In order to maintain the temperature constant, two hollow metallic plates coated with thin plastic paper on the outer surface were used for sandwiching paper strips and thermostated water (35°C) was circulated through them. pH measurements were made with an Elico Model $L_{1\text{-}10}$ pH meter using-glass electrode.

Chemicals

Hg(II), Be(II) and Ni(II) perchlorate solutions were prepared in the laboratory from the nitrates via. carbonates. The solution was standardized and diluted to 5.0 x 10^{-3}M. Metal spots were detected on the paper using hydrogen sulphide (for Hg^{2+}). Aluminon (for Be^{2+}) and DMG (for Ni^{2+}). Silver nitrate in acetone made alkaline is used for glucose.

Background Electrolyte

The background electrolute in the study of binary complexes consists of 0.1M perchloric acid and 1.0 x 10^{-2}M Cysteine. It was maintained at pH 8.5 by addition of sodium hydroxide. Each solution was standardized, as usual. Stock solution of 9.0 M perchloric acid, 2.0 M sodium hydroxide and 0.5 cysteine were prepared from AnalaR samples (B.D.H., Poole, Great Britain), 0.01 M (NTA) was prepared from a sample obtained from E. Merck (Dramstadt F.R.G).

Procedure

The hollow base plate in the instrument was made horizontal with a spirit level. A 150 - ml volume of background electrolyte was placed in each tank of electrophoretic apparatus. Paper

strips (Whatman No. 1, 30 x 1 cm.) in triplicate were spotted with metal ions and glucose in the centre with a Micropipette and were subsequently placed on the base plate and sandwiched under the upper hollow metallic plate with the ends of the strips immersed in the tank solutions on both side. Then a potential difference of 200 V. was appllied between the tank solutions and electrophoresis was carried out for 60 min. Subsequently the strips were removed and the spots were detected. The averages for triplicate strips were noted for calculations and movement of the glucose spot was used as a correction factor. The mobilities were calculated by dividing the movement by the potential gradient and expressed in $cm^2 V^{-1} min^{-1}$.

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