STUDIES ON THE INTERACTION OF TRANSITION METAL IONS WITH SOME BIOLOGICALLY ACTIVE LIGANDS BY PAPER IONOPHORETIC METHOD

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ABSTRACT. The binary equilibria of Cu(II)-methionine, Co(II)-methionine, Cu(II)-nitrilo-triacetate, Co(II)-nitrilotriacetate and the ternary equilibria Cu(II)-nitrilotriacetate-methionine, Co(II)-nitrilotriacetate-methionine have been studied by using paper ionophoretic method (PIM). The proportion of ionic species of nitrilotriacetate and methionine were varied by changing pH of the background electrolyte. The logarithm stability constants values of the mixed complexes of Cu(II)-nitrilotriacetate-methionine and Co(II)-nitrilotriacetate-methionine were found to be 4.72 ± 0.05 and 3.72 ± 0.03, respectively, at ionic strength 0.1 M $HClO_4$ in aqueous medium and a temperature of 35 °C.

KEY WORDS: Ionophoretic technique, Overall mobility; Mixed complexes, Stability constants; Copper(II) complexes, Cobalt(II) complexes, Nitrilotriacetic acid, Methionine

INTRODUCTION

The role of mixed ligand complexes in biological processes have been well recognized [1]. The knowledge of stabilities of these complexes is known to play an important role in many metabolic and toxicological functions. Several attempts have been made to correlate stabilities of the metal-ligand complexes with their biocidal activities [2, 3]. Nitrilotriacetic acid (NTA) behaves as a unique tridentate or tetradentate ligand [4, 5]. The significance of sulphur-containing amino acids is enhanced by the fact that they display independent therapeutic activity [6]. In recent years they have been utilized in connection with rheumatoid arthritis [7]. The importance of metal ions to vital function in living systems and for the well-being of living organisms is now well-established. Copper and cobalt both are essential metals for living being without which life can not sustain [8]. Copper(II) and cobalt(II) have significant biomedical applications but are toxic at higher concentration [9-18]. Studies on the feasibility of controlling the level of Cu^{2+} and Co^{2+} ions by complexation are seems to be very interesting.

The PIM usually suffers from a number of defects. Temperature during electrophoresis, capillary flow on the paper, electroosmosis, adsorption and molecular sizing affect the mobilities of charged moieties [19, 20]. The method described here is almost free from these vitiating factors. It gives results in fair agreement with the accepted literature values. Communications [21-25] from our laboratory described a new method for study of mixed complexes. The present work is an extension of this method and reports observations on mixed systems, viz; Cu^{++}/Co^{++}-NTA-methionine.

EXPERIMENTAL

The apparatus and procedure used were as described previously [21- 25]. Solutions of copper(II) and cobalt(II) metal perchlorate were prepared by the precipitation of metal carbonates from a

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0.1 M solution of Cu(II) and Co(II) nitrates with the solution of sodium carbonate (chemically pure grade, BDH, Poole, UK). The precipitates were thoroughly washed with water and treated with a calculated amount of analytical-reagent grade perchloric acid. These were boiled in a water bath and then filtered to get a stock solution of metal perchlorate 0.005 M. The 0.005 M glucose (BDH, AnalaR) solution was prepared in water and used as an electro-osmotic indicator for the correction due to electro-osmosis.

A 0.1% (w/v) solution of 1-(2-pyridylazo)-2-naphthol (PAN) (E. Merck, Darmstadt, Germany) in ethanol was used for detecting the Cu(II) and Co(II). A saturated aqueous solution (0.9 mL) of silver nitrate was diluted with acetone to 20 mL. Glucose was detected by spraying with this silver nitrate solution and then with 2% ethanolic sodium hydroxide, when a black spot was formed.

Stock solution of 5.0 M perchloric acid was prepared from 70% perchloric acid (SDS, AnalaR grade). 2.0 M sodium hydroxide (AnalaR grade) and 0.5 M methionine (BDH, Poole, UK) were prepared. A 0.01 M NTA solution was prepared from the compound obtained from E. Merck (Darmstadt, Germany). Each solution was standardized using the appropriate method.

The background electrolytes (BGEs) used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M methionine. For the study of mixed systems the BGEs used consisted of 0.1 M perchloric acid, 0.001 M NTA and various amounts of 0.01 M methionine. The mixed system was maintained at pH 8.5 by addition of sodium hydroxide.

RESULTS AND DISCUSSION

Metal(II)-methionine binary system

Literature reveals that an ionic species of amino acids are sole co-ordinating species in complex formation with metal ions [26, 27]. Hence a metal ion spot on paper strip show a variation in composition of different ionic species of the amino acids in BGEs. So the mobility of metal ion spot would depend upon the pH of BGEs. As is evident from Figure 1, the plot of overall electrophoretic mobility of metal ion spot against pH give a curve with two plateaus in each case viz., Cu(II)-methionine and Co(II)-methionine. The first plateau corresponds to a region in which metal ions are uncomplexed. In this low pH region protonated species of methionine is non-complexing. Beyond this region, metal ion spots have progressively decreasing velocity and hence complexation of metal ions should be taking place with some anionic species of ligand methionine whose concentration increases progressively with increase of pH. Figure 1 shows that second plateau in each case with positive mobility indicating the formation of 1:1 complex of cationic nature. The mobility register a downward trend ultimately resulting in a third plateau obviously corresponds to overwhelmingly formation of 1:2 complex of neutral nature. It is significant that these studies give clear evidence of the complexation of anionic species of methionine with Cu^{2+} and Co^{2+} metal ions forming two varieties of binary complexes of 1:1 and 1:2 in composition. In general complexation of metal ions with methionine anion may be represented as

$$M^{2+} \quad + \quad L^- \quad \overset{K_1}{\underset{}{\rightleftarrows}} \quad ML^+ \tag{1}$$

$$ML^+ \quad + \quad L^- \quad \overset{K_2}{\underset{}{\rightleftarrows}} \quad ML_2 \tag{2}$$

where M^{2+} is Cu^{2+} and Co^{2+} metal ions; L^- is methionine anion; K_1 and K_2 are first and second stability constants, respectively.

For calculating first stability constant, K_1, the region between first and second plateau is pertinent. The overall mobility will be equal to the arithmetic mean of the mobility of the uncomplexed metal ion, u_0, and that of first complex, u_1, at a pH where $K_1 = 1/[L']$. The concentration, of methionine anion [L'] is determined for a particular pH by the use of protonation constants of methionine (ionophoretically obtained value, $pk_1 = 2.25$; $pk_2 = 9.00$) from which K_1 can be calculated. The concentration of liganding methionine anion is calculated with the help of equation

$$[L'] \quad = \quad \frac{[L_T]}{1 + [H]/k_2 + [H]^2/k_1 \, k_2} \tag{3}$$

where $[L_T]$ is total concentration of methionine; k_1 and k_2 are first and second dissociation constants of pure methionine, respectively.

The second stability constant 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.
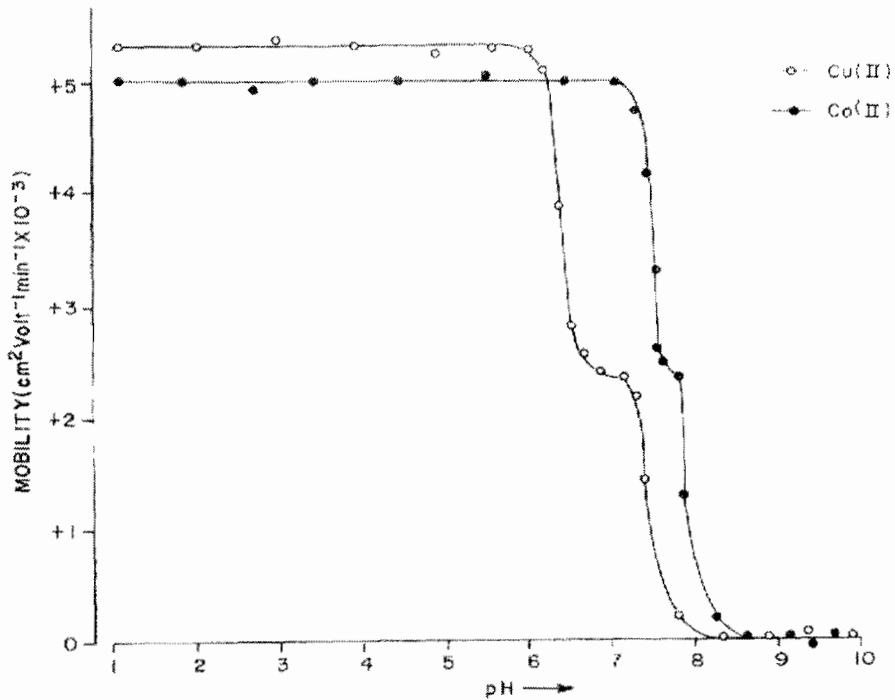

Figure 1. Mobility curve for the metal(II)-methionine system. o = Cu(II)-methionine; ● = Co(II)-methionine. Concentration of methionine = 0.01 M. Concentration of Cu(II) and Co(II) = 0.005 M. Ionic strength = 0.1M; temperature = 35 ^{0}C.

Table 1. Stability constants of binary and ternary complexes of copper(II) and cobalt(II).

Values	Metal ions	Stability constants[*]			
		a	b	c	d
Calculated (present	Cu^{2+}	6.40 ± 0.01	4.50 ± 0.03	12.37 ± 0.04	4.72 ± 0.05
work)	Co^{2+}	4.40 ± 0.03	4.20 ± 0.05	10.39 ± 0.03	3.72 ± 0.03
Literature	Cu^{2+}	-	-	12.94 [28]	
				13.30 [29]	
				13.60 [29]	
				12.75 [29]	
	Co^{2+}	-	-	10.38 [28]	-
				10.38 [29]	

[*] $a = \log K_1 \overset{M}{_{ML}} = [ML]/[[M][L]]; \quad b = \log K_2 \overset{M}{_{ML2}} = [ML_2]/[ML][L];$

$c = \log K_3 \overset{M}{_{M\text{-}NTA}} = [M\text{-}NTA]/[M][NTA]; \quad d = \log K_4 \overset{M\text{-}NTA}{_{M\text{-}NTA\text{-}L}} = [M\text{-}NTA\text{-}L]/[M\text{-}NTA][L];$

M = metal cation; L = ligand (methionine); NTA = nitrilotriacetic acid, NTA anion = $[N(CH_2COO)_3]^{3-}$; methionine anion = $[(CH_3)_2C(SCH_3)CH(NH_2)COO^-]$; ionic strength = 0.1 M; temperature = 35 °C.

Metal(II)-nitrilotriacetic acid binary system

Figure 2 shows the mobility curve of metal spots with NTA. The mobility of last plateau in each case is in negative region showing the anionic nature of metal(II)-NTA complexes. Hence, only one NTA anion is assumed to combine with an divalent metal ion to give 1:1 metal(II)-NTA complexes. The complexation of metal ion with NTA may be represented as

$$M^{2+} + [N(CH_2COO)_3]^{3-} \overset{K_3}{\leftrightarrows} [M\{N(CH_2COO)_3\}]^- \tag{4}$$

The stability constants K_3 of binary complexes with NTA are calculated in the same manner as for amino acid (the ionophotically obtained protonation constants value for pure NTA are $pK_1 = 1.66$, $pK_2 = 2.67$, $pK_3 = 9.49$). The calculated values of K_3 are given in Table 1.

Metal(II)-nitrilotriacetic acid-methionine mixed system

Beside binary complexes, mixed complexes involving NTA as primary ligand and methionine as secondary ligand have also been studied. The study of these mixed complexes is carried out in presence of 0.001 M NTA with progressive addition of various concentrations of secondary ligand methionine to the BGE fixed at pH 8.5.

The plot of overall mobility against logarithm of concentration of methionine for Cu(II) and Co(II) are shown in Figure 3. As is evident from the Figure 3, two plateaus are obtained in each case. The mobilities of first plateau corresponds to the mobility of 1:1 metal(II)-NTA complex. The mobilities of second plateau in each case are seen to be more negative than that of first plateau and this shows the formation of more negatively charged complex species. It is inferred, therefore, that methionine ion has co-ordinated with the 1:1 metal(II)-NTA complex resulting in the formation of 1:1:1 metal(II)-NTA-methionine mixed ligand complex. The complexation of metal(II)-NTA with methionine anion may be represented as

$$[M\{N(CH_2COO)_3\}]^- + L^- \overset{K_4}{\leftrightarrows} [M\{N(CH_2COO)_3\}L]^{2-} \tag{5}$$

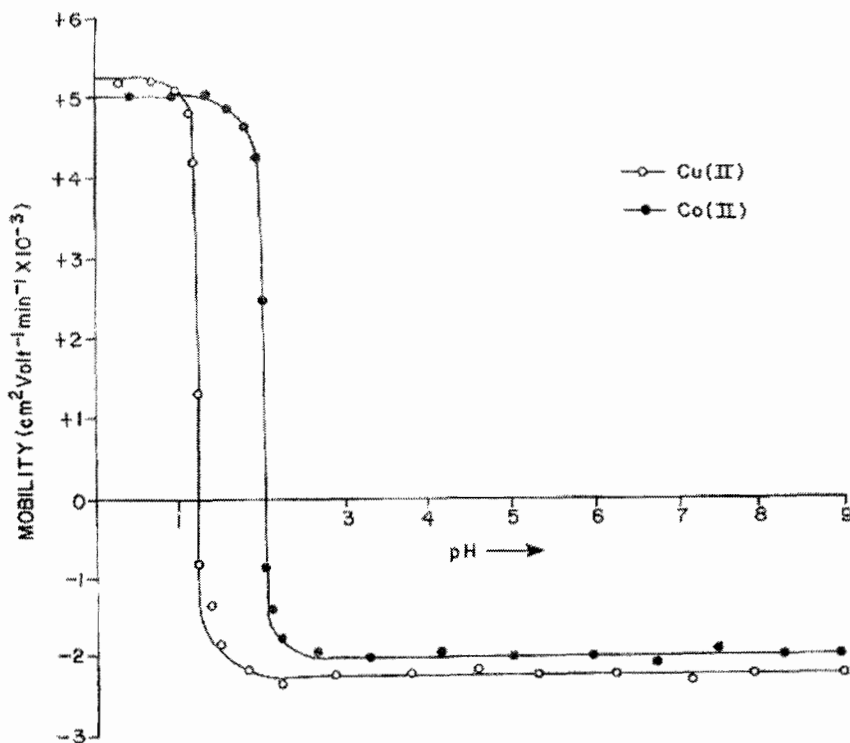where L^- is the methionine anion and K_4 is the stability constants of mixed complexes.

Figure 2. Mobility curves for metal(II)-nitrilotriacetate system. 0 = Cu(II)-nitrilotriacetate; • = Co(II)-nitrilotriacetate. Concentration of nitrilotriacetate = 1.0×10^{-3} M. Concentration of Cu(II) and Co(II) = 5.0×10^{-3} M. Ionic strength = 0.1 M; temperature = 35 ^{0}C.

There is no third plateau and no further interaction with methionine anion possible. In present electrophoretic study the transformation of a simple complex into a mixed complex takes place, hence overall mobility U of this complex is given by

$$U = \frac{u_0 + u_1 K_4[(CH_3)_2C(SCH_3)CH(NH_2)COO^-]}{1 + K_4[(CH_3)_2C(SCH_3)CH(NH_2)COO^-]} \qquad (6)$$

where u_0 and u_1 are mobilities of the [M-NTA]$^-$ and [M-NTA-L]$^{2-}$ complexes, respectively.

From Figure 3 concentration of methionine at which overall mobility is the mean of the mobilities of two pleaus is determined. The concentration of anionic species of methionine at pH 8.5 for this methionine concentration is calculated. Mixed stability constant K_4 is obviously equal to $1/[(CH_3)_2C(SCH_3)CH(NH_2)COO^-]$. The values of K_4 are given in Table 1.

The logarithm of formation constants (log K_{ML-NTA}^{ML}) for the interaction

$$[ML]^+ + [NTA]^{3-} \leftrightarrows [ML-NTA]^{2-} \qquad (7)$$

can be computed by the relation

$$\text{Log } K_{ML\text{-}NTA}^{ML} = \text{log } a_{M\text{-}NTA\text{-}L} - \text{log } K_{1\,ML}^{M} \tag{8}$$

$$\text{Log } a_{M\text{-}NTA\text{-}L} = \text{log } K_{3\,M\text{-}NTA}^{M} + \text{log } K_{4\,M\text{-}NTA\text{-}L}^{M\text{-}NTA} \tag{9}$$

The values of ($\text{log } K_{ML\text{-}NTA}^{ML}$) are found to be 10.69 ± 0.10 and 9.71 ± 0.03 for copper(II) and cobalt(II) metal ions, respectively. This shows that NTA does have strong affinity to these metal ions in comparison to methionine.
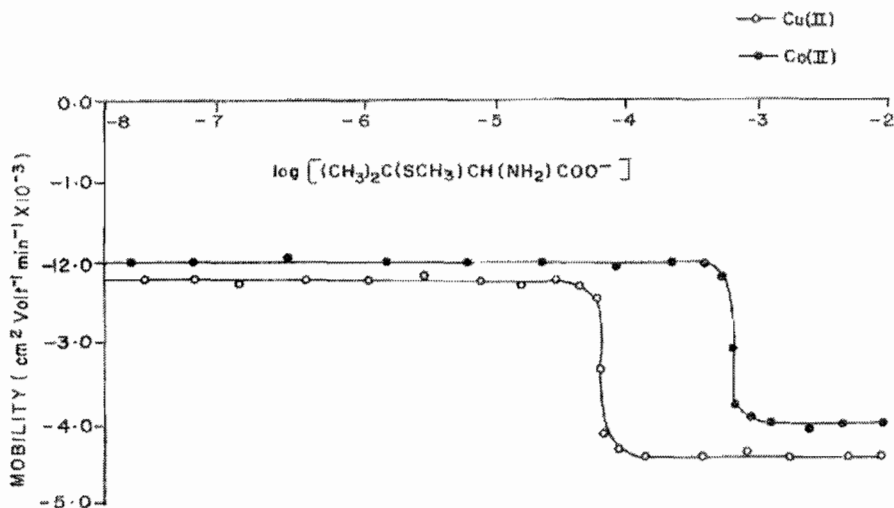

Figure 3. Mobility curves for the metal(II)-nitrilotriacetate-methionine system. $0 = $ Cu(II)-nitrilotriacetate-methionine; $\bullet = $ Co(II)-nitrilotriacetate-methionine. Concentration of nitrilotriacetate $= 1.0 \times 10^{-3}$ M. Concentration of Cu(II) and Co(II) $= 5.0 \times 10^{-3}$ M. Ionic strength $= 0.1$M; temperature $= 35\,^0$C.

The present PIM is limited to charged species and the precision of the method is not as high as other physicochemical methods. However, uncertainty in the result is $\pm$ 5%. As for as of uncertainty is concerned the results reported here are fairly reliable.

CONCLUSION

It may be concluded from these studies that copper(II) and cobalt(II) metal ions are essential for biological systems but as such they are toxic. The methionine and NTA may be used to reduce the level of these metal ions in living cells. The stability constants of metal complexes can be very easily calculated by this method, so the present PIM have significant advantages over other methods reported in chemical literature for the determination of stability constants of metal complexes.

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