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DETERMINATION OF MIXED STABILITY CONSTANTS OF LEAD(II)/URANYL(II)-NTA-CYSTEINE COMPLEXES BY PAPER ELECTROPHORESIS

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ABSTRACT. A method involving the use of paper ionophoresis is described for the study of equilibria in mixed – ligand complex systems in solution. The technique is based on the movement of a spot of metal ion under an electric field with the complexants added to the background electrolyte at pH 8.5. The stability constants of the complexes Pb(II) – nitrilotriacetate – cysteine and UO_2(II) – nitrilotriacetate – cysteine are found to be 5.35 ± 0.02 and 6.27 ± 0.07 (logarithm of stability constant values) at ionic strength 0.1 M and a temperature of 35 °C.

KEY WORDS: Paper ionophoresis, Lead(II) complexes, Uranyl(II) complexes, Nitrilotriacetic acid, Cysteine, Stability constants.

INTRODUCTION

For a mononuclear binary complex, if a central atom (central group) M (the 'metal') and a ligand L have been defined, then in the following expressions K_n is the stepwise formation constant, and β_n is the cumulative formation constant for the complex ML_n. They can both be referred to as stability constants (stepwise and cumulative) [1].

$$K_n = K\,(ML_{n-1} + L \leftrightarrows ML_n)$$
$$\beta_n = K\,(M + nL \leftrightarrows ML_n)$$

Sulfur containing amino acids have several biomedical applications [2, 3]. The main biochemical aspects of sulphur containing amino acids have been reviewed by Jocelyn [4]. Lead(II) and uranyl(II) are toxic metal ions because they are harmful even at very low concentration. Therefore work on removal of these metal ions from living systems with interaction of bioactive nitrilotriacetic acid and cysteine seems to be very interesting. The studies on complexation reactions of divalent lead and uranyl is also of interest because of their nutrient properties and toxicity [5-14]. The recommended normal level of lead and uranyl per day human diet are 0.06-0.5 mg and 0.001-0.002 mg, respectively [15].

The ionophoretic technique had been introduced in 1964 for investigating the formation of complexes and determination of the stability constants of mononuclear complexes [16, 17]. The procedure was to study the mobility of a metal cation spot on a paper strip soaked with background electrolyte buffered at a fixed pH containing progressively increasing concentration of ligand. In our modified method the concentration of ligand in solution is kept constant but the hydrogen ion concentration of the background electrolyte is progressively decreased by the addition of alkali solution. The previous technique did not take into account the effect of changes of relative concentration upon the different ionic species which might be formed.

The ionophoretic technique typically suffers from a number of defects. Adsorption, capillary flow on paper, electro-osmosis and temperature during electrophoresis affect the mobility of the charged moieties [18]. Electro-osmosis refers to the movement of liquid as a result of an applied

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*Corresponding author. E-mail: brijtew@yahoo.com electromotive force either through a porous diaphragm or through a capillary. The motion of the liquid may be regarded as the direct opposite of electrophoresis. Electro-osmosis arises from the influence of the electric field on the counter ions in the double layer adjacent to the capillary wall. The electro-osmotic mobility of a liquid can serve as a basis for characterizing it, for separating it and for estimating zeta potential describing its double layers [19-21].

The technique described in the present work is free from above mentioned vitiating factors. It gives results in fair agreement with the accepted literature values. The experimental set up for paper electrophoresis equipment and dried paper strips showing spots of metal ion and glucose after electrophoresis are shown in Figures 1 and 2, respectively.

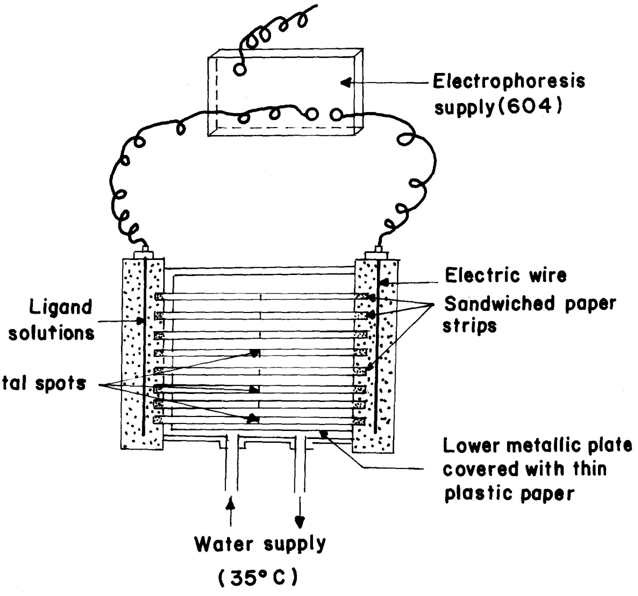

Figure 1. Experimental set up for paper electrophoresis equipment.

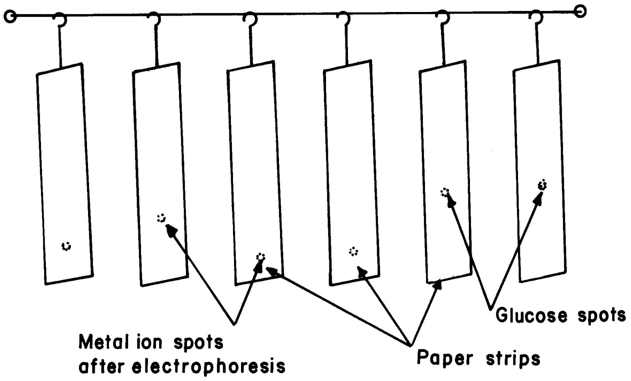

Figure 2. Paper strips showing spots of metal ions after electrophoresis.

Paper electrophoresis technique (PET) is being increasingly used for studies of metal complexes. Hurnick [22] studied tartrate complexes of Fe(II), Ce(II) and Ni(II) in aqueous media and determined stability constants. Soni and Bertusek [23] investigated Mo(II) complexes of *o*-diphenol. In the majority of investigation reported, the studies are largely qualitative and did not throw light either on the nature of species or on their stabilities. Publications [24-26] from our laboratories described a new method for the study of mixed complexes. The present work is an extension of this techniques and reports observations on mixed systems, viz: Pb(II)/UO_2(II) – nitrilotriacetic acid (NTA) – cysteine.

EXPERIMENTAL

Instrument

Electrophoresis equipment from Systronics (Naroda, India), model 604, was used. The equipment has a built in power supply (a.c.– d.c.) that is fed directly to the paper electrophoresis tank. The potential in each experiment was kept 200 V and electrophoresis was carried out for 60 minutes. An Elico (Hyderabad, India) model $L_{1\text{-}10}$, pH meter with glass and calomel electrodes assembly and working on 220 V/50 Hz established a.c. mains, was used for pH measurement. The pH meter was calibrated with buffer solution of pH 7.0.

Chemicals

Solutions of lead(II) and uranyl(II) metal perchlorate were prepared by preliminary precipitation of metal carbonates from 0.1 M solution of sodium carbonate (AnalaR grade; BDH, Poole, UK). The precipitate was thoroughly washed with boiling water and treated with calculated amounts of 1% perchloric acid. The resulting mixture was heated to boiling on a water bath and then filtered. Metal contents of the filtrates were determined and the final concentration was kept at 5.0×10^{-3} M.

Lead(II) and uranyl(II) metal spots after electrophoresis were detected on the paper using 0.1% (w/v) solution of 1-(2-pyridylazo)-2-napthol (PAN) (E. Merck, Darmstadt, Germany) in ethanol. 0.005 M glucose (BDH, AnalaR) solution was prepared in water and used as an electro-osmotic indicator for correction due to electro-osmosis. 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.

Background electrolyte

The background electrolytes (BGEs) used in the study of metal(II)-cysteine and metal(II)-NTA binary complexes were 0.1 M perchloric acid, 0.01 M cysteine and 0.1 M perchloric acid 0.001 M NTA, respectively. For the study of mixed systems the BGEs used consisted of 0.1 M percholoric acid, 0.001 M NTA and various amounts of 0.01 M cysteine. The mixed system was maintained at pH 8.5 by the addition of sodium hydroxide. Stock solutions of 5.0 M perchloric acid, 2.0 M sodium hydroxide and 0.5 M cysteine were prepared from AnalaR reagents (BDH, Poole, UK). A 0.01 M nitrilotriacetic acid solution was prepared from a sample obtained from E. Merck (Darmstadt, Germany).

Procedure

Binary complexes. Whatman No. 1 filter paper for chromatography was used for electrophoresis. To record observation of a particular metal ion, two paper strips were spotted with the metal ion solution along with additional two spotted with glucose using a 1.0 μL pipette and then mounted on an insulated plate. Each of the two electrolyte vessels was filled with 150 mL of background electrolyte. The paper became moistened with the background electrolyte solutions due to diffusion. Another insulated plate was placed on paper strips and then thermostated water (35 ºC) was circulated into the plates to keep the temperature constant. The lid was then placed on the instrument to make it air tight. It was left for 10 minutes to ensure strip wetting. A direct 200 V potential was subsequently applied between the Pt-wire electrodes.

Ionophoresis was carried out for 60 minutes and then the strips were removed from the tank and dried. The metal ion and glucose spots were detected by specific reagents. Observations were repeated for different pH values of the background electrolyte. The differences in the distances recorded in duplicates were within ±5% and the average distances in the duplicates were noted for calculation. The actual distance that the sample spot moved was corrected for the distance travelled by the reference glucose spot. The potential gradient through the strips was found to be 7.5 V cm^{-1}. Mobility was calculated by dividing the movement by the potential gradient and time. Plots of mobility versus pH are shown in Figures 3 and 4.

Mixed complexes. Paper strips were marked with metal ions in duplicate along with additional two strips marked with glucose. After drenching the paper strips with background electrolyte, ionophoresis was carried on for 60 minutes at the same potential difference as in the case of binary complexes. For subsequent observations, the cysteine solution was maintained at pH = 8.5. Mobility of metal ions was recorded against log [cysteine] (Figure 5).

RESULTS AND DISCUSSION

Metal(II) – cysteine binary system

The overall electrophoretic mobility of metal ions as a function of pH is given in Figure 3. There are three plateaus each in the curves of lead(II) and uranyl(II) cations, respectively. The first plateau in each case with positive mobility at lower pH range is due to non-complexed cations, in this pH range cysteine present as a non-complexing species $[H_2C(SH)CH(NH_3^+)COOH]$. The second plateau in each case with zero mobility indicates 1:1 $[M\{H_2C(S)CH(NH_2)COO\}]$, complex of neutral nature. The ligand present is the anionic species of cysteine $[H_2C(S^-)CH(NH_2)COO^-]$. At higher pH range formation of third plateau with negative mobility was observed in each case, which indicates formation of 1:2 $[M\{H_2C(S)CH(NH_2)COO\}_2]^{2-}$, anionic complex. Further increase of pH has no effect on the mobility of metal ions. The $[M\{H_2C(S)CH(NH_2)COO\}_2]^{2-}$ is the highest complex in the experimental region of various pH values of the Pb(II) and UO_2(II). The complexation of metal ions with cysteine anion may be represented as

$$M^{2+} + L^{2-} \overset{K_1}{\leftrightarrows} ML \tag{1}$$

$$ML + L^{2-} \overset{K_2}{\leftrightarrows} ML_2^{\;2-} \tag{2}$$

where M^{2+} is the Pb^{2+} and UO_2^{2+} metal cations; $[L^{2-}]$ is the cysteine anion; K_1, K_2 are the first and second stability constants, respectively.

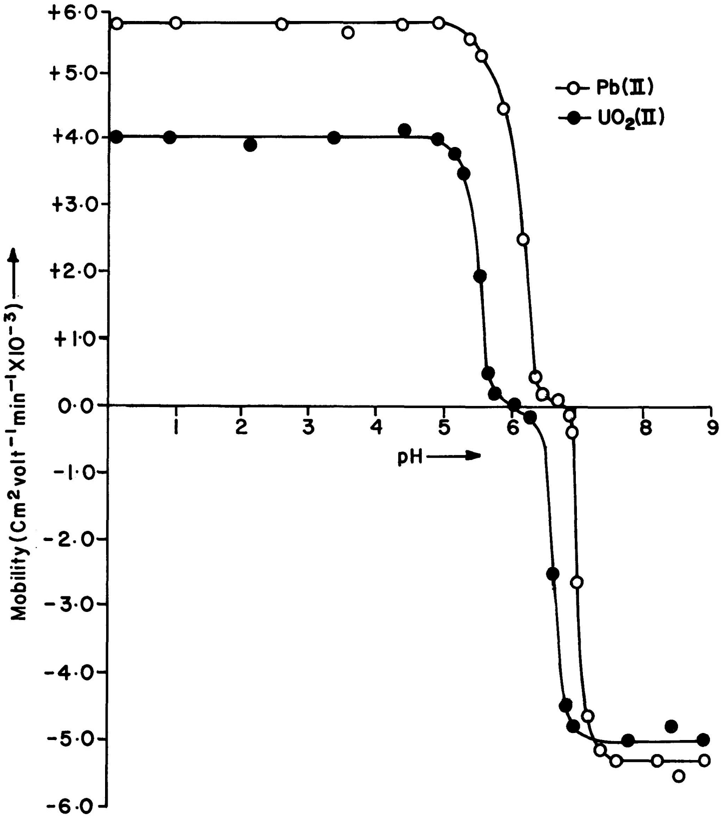

Figure 3. Mobility curve for metal(II) – cysteine binary system. ○ = Pb(II) – cysteine; ● = UO_2(II) – cysteine. BGEs = 0.1 M perchloric acid and 0.01 M cysteine. Concentration of Pb(II) and UO_2(II) = 0.005 M.

Using protonation of pure cysteine [pK_1 = 2.00, pK_2 = 8.25, pK_3 = 10.35 (paper electrophoretically obtained values)] the concentration of cysteine anion [L^{2-}] is determined for the pH values of interest, from which stability constants can be calculated. The mode of protonation of pure cysteine may be represented as

$$\begin{array}{ccc}
H_2C(SH)CH(NH_3^+)COOH & \underset{-H^+}{\overset{k_1}{\leftrightarrows}} & H_2C(SH)CH(NH_3^+)COO^- \\
(a) & & (b) \\
 & & -H^+ \downarrow\uparrow k_2 \\
H_2C(S^-)CH(NH_2)COO^- & \underset{k_3}{\overset{-H^+}{\leftrightarrows}} & H_2C(SH)CH(NH_2)COO^- \\
(d) & & (c)
\end{array}$$

The first stability constant, K_1 and second stability constant, K_2 for the complexes are calculated as previously [24-26]. The results are given in Table 1.

Table 1. Stability constants of binary and mixed complexes of lead(II) and uranyl(II).

Metal ions	Complexes		Stability constants	Log stability constant values
Pb^{++}	$[Pb\{H_2C(S)CH(NH_2)COO\}]$	(binary)	K_1	8.20 ± 0.01
	$[Pb\{H_2C(S)CH(NH_2)COO\}_2]^{2-}$	(binary)	K_2	6.62 ± 0.05
	$[Pb\{N(CH_2COO)_3\}]^-$	(binary)	K_3	8.05 ± 0.03
	$[Pb\{N(CH_2COO)_3\}\{H_2C(S)CH(NH_2)COO\}]^{3-}$	(mixed)	K_4	5.35 ± 0.02
UO_2^{++}	$[UO_2\{H_2C(S)CH(NH_2)COO\}]$	(binary)	K_1	9.45 ± 0.07 (9.04 [29])^a
	$[UO_2\{H_2C(S)CH(NH_2)COO\}_2]^{2-}$	(binary)	K_2	7.21 ± 0.04
	$[UO_2\{N(CH_2COO)_3\}]^-$	(binary)	K_3	8.66 ± 0.08 (7.88 [29])^a
	$[UO_2\{N(CH_2COO)_3\}\{H_2C(S)CH(NH_2)COO\}]^{3-}$	(mixed)	K_4	6.27 ± 0.07

Ionic strength = 0.1 M; temperature = 35 ^{0}C; $[H_2C(S^-)CH(NH_2)COO^-]$ = cysteine anion; $[N(CH_2COO_3]^{3-}$ = NTA anion. ^{a} = literature values.

The mobility of the ions depends upon (1) the strength of the applied electric field, (2) the value of the charge on the ion, and (3) the nature of the medium, that is, the resistance offered by the medium to the movement of the ions (viscosity). A plateau is obviously an indication of a pH range where speed is practically constant. This is possible only when a particular complex is formed in overwhelming proportions. Thus every plateau indicates the formation of certain complex species. Sharp drop or sudden decrease of mobility in the curve is due to the formation of a new complex species.

It is observed from Table 1 that stability constant values of UO_2(II) – cysteine complex is higher than Pb(II) – cysteine complex. Therefore it is to be evident that bond formation within UO_2(II) – cysteine complex is stronger in comparison to Pb(II) – cysteine complex.

Metal(II) – nitrilotriacetic acid binary system

Observations on the overall mobility of metal spots in the presence of NTA at different pH values are presented in Figure 4. It is clear from Figure 4 that in both metal ions two plateaus are obtained. The mobility of last plateau in each case in negative region showing anionic nature of metal(II) – NTA complex. Hence, only one NTA anion is assumed to combine with metal ion to give a 1:1 $[M\{N(CH_2COO)_3\}]^-$ complex. The complexation may be represented as:

$$M^{2+} + NTA^{3-} \overset{K_3}{\leftrightarrows} [M\text{-}NTA]^- \qquad (3)$$

The stability constant (K_3) of the complexes with NTA were calculated as described in the preceding paragraph for complexes with cysteine. The calculated values of K_3 are given in Table 1.

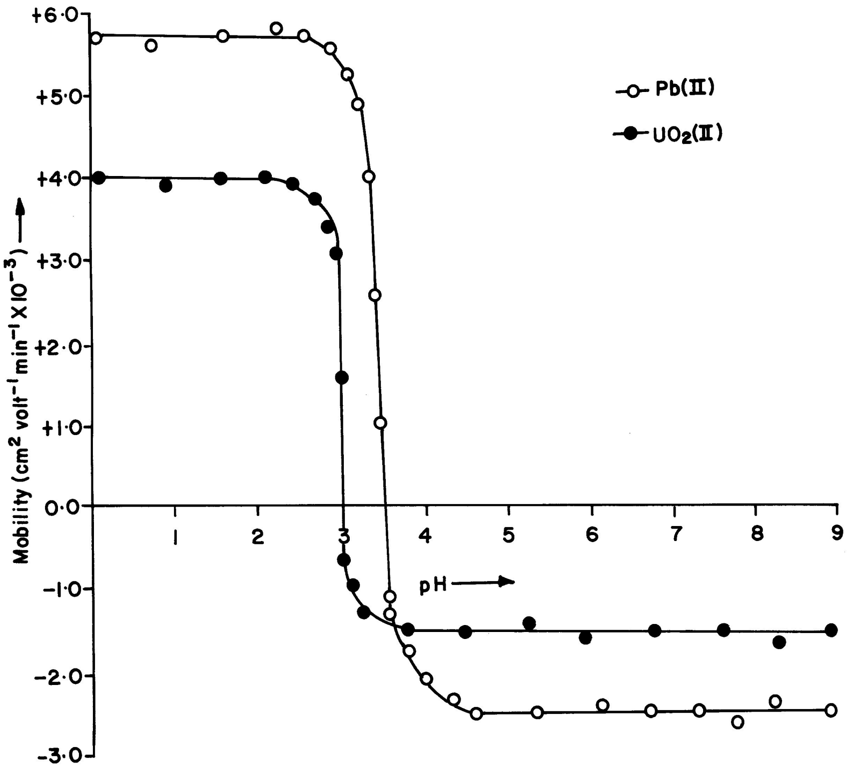

Figure 4. Mobility curve for metal(II) – nitrilotricetic acid binary system. ○ = Pb(II) – NTA; ● = UO_2(II) – NTA. BGEs = 0.01 M perchloric acid and 0.001 M NTA. Concentration of Pb(II) and UO_2(II) = 0.005 M.

Metal(II) – nitrilotriacetic acid – cysteine ternary system

This system was studied at pH 8.5 for the same reason as given previously [24-26]. The plot of mobility versus added log [cyseine] gives a curve (Figure 5) containing two plateaus – one at the beginning and the other at the end. The mobility of the range of the first plateau corresponds to the mobilities of 1:1 metal – NTA system. The mobility of last plateau is more negative than that of the first plateau, which indicates the formation of a more negatively charged complex. Furthermore, since the mobility in the last plateau does not tally with the mobility of 1:1 and 1:2 metal – cysteine complex (observed in our study of binary metal(II) – cysteine system), it is inferred that the species in the last plateau is formed by coordination of cysteine anion $[H_2C(S^-)CH(NH_2)COO^-]$ to 1:1 $[M\{N(CH_2COO)_3\}]^-$ complex, resulting in the formation of 1:1:1 $[M\{N(CH_2COO)_3\}\{H_2C(S)CH(NH_2)COO\}]^{3-}$ mixed complex as

$$[\text{M-NTA}]^- + [\text{L}]^{2-} \overset{K_4}{\leftrightarrows} [\text{M-NTA-L}]^{3-} \qquad (4)$$

where $[L]^{2-}$ is the cysteine anion; K_4 is the stability constant of mixed complexes.

The stability constant K_4 of mixed complexes are calculated as described earlier [17-19]. All these calculated values of K_4 are given in Table 1.

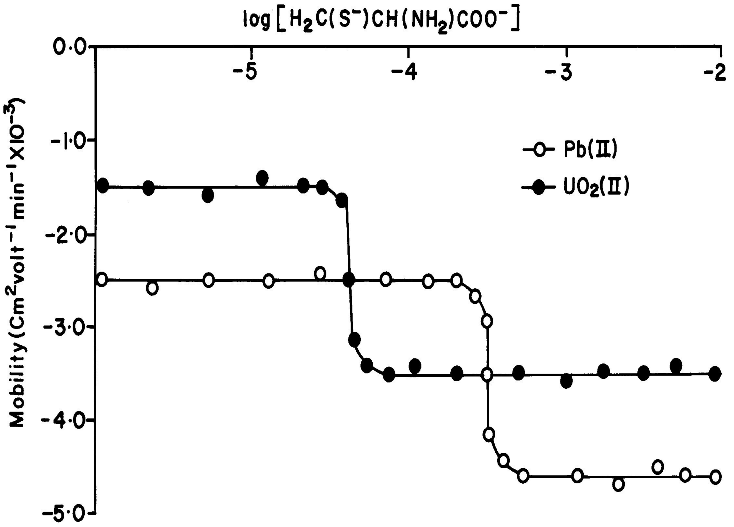

Figure 5. Mobility curve for metal(II) – nitrilotricetic acid – cysteine mixed system. ○ = Pb(II) – NTA – cysteine; ● = UO_2(II) – NTA – cysteine. BGEs = 0.01 M perchloric acid and 0.001 M NTA and various amounts of 0.01 M cysteine. Concentration of Pb(II) and UO_2(II) = 0.005 M. pH 8.5 (maintained by additionof sodium hydroxide).

It is observed from the Table 1 that the order of stability constants viz: UO_2(II) > Pb(II) is same for metal(II) – cysteine, metal(II) – NTA binary and metal(II) – NTA – cysteine ternary complexes. The corresponding stability constant values are lower in mixed complexes. It is therefore inferred that coordinating tendency of a ligand decreases with a higher state aggregation [27, 28]. Our experimental values resemble with the available literature values, the slight difference may be due to the change in temperature, ionic strength and experimental conditions [29].

CONCLUSION

It is concluded from the present studies that present paper electrophoretic technique may be used for the determination of stability constant of metal complexes. The present modified technique is free from destroying factors (e.g. adsorption, capillary flow on paper, electro-osmosis and temperature during electrophoresis) and results reported are fairly reliable. It may also be concluded from present studies that lead(II) and uranyl(II) metal ion are toxic, the nitrilotriacetate and cysteine may be used to reduce the level of these metal ions in biological systems.

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