Ionophoretic Technique for the Determination of Stability Constants of Mixed Complexes (M-Nitrilotriacetate-5-Amino Pentanoate Systems)

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A new method, involving the use of paper electrophoresis is described for the study of the equilibria in mixed ligand complex systems in solution. This technique is based on the movement of a spot of metal ion under an electric field with the complexants added in the background electrolyte at pH = 8.5. Concentration of the primary ligand (NTA) was kept constant while that of the secondary ligand (5-amino pentanoic acid) was varied. The plots of-log [5-amino pentanoic acid] against mobility were used to obtain information on the formation of mixed complex and to calculate its stability constants. The binary equilibria M(II)-(5--amino pentanoic acid) and M(II)-NTA have also been studied since this is a prerequisite for the investigation of mixed complexes. The stability constants of the complexes, metal-nitrilotriacetate-5-amino pentanoate have been found to be 5.85, 5.50, 5.22, 3.96 and 3.90 (log K values) for Cu(II), UO₂(II), Ni(II), Co(II) and Zn(II) complexes, respectively, at $\mu = 0.1$ mol/L and a temperature of 35 °C.

INTRODUCTION

Paper electrophoresis was applied to the study of metal complexes in solution and attempts were made to determine the stability constant of the complex species.[1,2] In the recent work at these laboratories a new method has been developed for the study of stepwise complex formation.[3-6] Although the use of paper electrophoresis for the study of metal complex systems with a single ligand seems to be well established, there is no systematic study for mixed complexes. However , Czakis-Sulikowska[7] made some observations on the formation of mixed halide complexes of Hg(II), but it is only qualitative and clarifies neither the nature of the species nor their stabilities. Publications[8-11] from our laboratories described a new method for the study of mixed complexes. The present work is an extension of the technique and reports our observation on the mixed systems viz. Cu(II)/UO₂(II)/Ni(II)/Co(II)/ /Zn(II)-nitrilotriacetate-5-amino pentanoic acid.

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Apparatus. — Electrophoresis equipment (Systronics model, 604 India) has been used. It has a built-in power supply (A.C.-D.C.) which is directly fed to a paper electrophoretic tank.

In most electrophoresis instruments no attention is paid to the control of temperature. The electric current running through paper strips generates heat which causes evaporation of background electrolyte leading to serious error. In order to eliminate it two hollow metallic plates, coated outwardly with thin paper plastics, have been used for sandwitching paper strips and thermostated water (35 °C) is circulated through them.

pH measurements were made with an Elico model L_{1-10} pH meter using glass electrodes.

Chemicals. — Cu(II), UO_2(II), Ni(II), Co(II) and Zn(II) perchlorates were prepared by precipitating the corresponding carbonates with sodium carbonate from solutions of nitrates washing the precipitates thoroughly with boiling water and treating them with a suitable amount of 1% perchloric acid. The resulting mixture were heated to boiling on a water bath and then filtered. The metal contents of filtrates were determined as usual and final concentrations were kept at 5.0×10^{-3} M.

1-(2-pyridylazo)-2-naphthol (PAN), 0.1% (w/v) in ethanol was used for detecting the Cu(II), UO_2(II), Ni(II), Co(II) and Zn(II) ions. A saturated aqueous solution of silver nitrate (0.9 mL) was diluted with acetone to 20 mL. Glucose was detected by spraying this solution and then 2% ethanolic sodium hydroxide.

Background electrolyte. — Stock solution of 9.0 M perchloric acid, 2.0 M sodium hydroxide and 0.5 M 5-amino pentanoic acid were prepared from AnalaR samples (B.D.H., Poole, Great Britain); 0.01 M nitrilotriacetic acid (NTA) was prepared from a sample obtained from E. Merck (Darmstadt G.F.R.). Each solution was standardized as usual. The background electrolyte in the study of binary complexes consisted of 0.1 M perchloric acid and 1.0×10^{-2} M 5-amino pentanoic acid/ /1.0×10^{-3} M NTA while in the study of ternary complexes it consisted of 1.0 M sodium perchlorate 1.0×10^{-3} M NTA and varying amounts of 1.0×10^{-2} M 5-amino pentanoic acid; it was maintained at pH = 8.5 by addition of sodium hydroxide.

(i) *Binary Complexes*

The level of hollow base plate in the instrument was made horizontal with a spirit level. 150 mL of 0.1 M perchloric acid was taken in each tank of the electrophoretic apparatus. The levels of the two tank solutions were made equal by siphoning them through a bent tube. These precautions were taken to check any gravitational or hydrodynamic flow. Paper strips (Whatman No. 1) of 30 cm × 1 cm in size were soaked in the background electrolyte and then the excess of electrolyte solution was blotted by pressing them gently within the folds of dry filter paper sheets. The strips in duplicate were then spotted with metal ion solution in the centre with a micropipette and were subsequently placed on the base plate and sandwitched under the upper hollow metallic plate with the end of strips allowed for 15 minutes to remain as such. Then a 200 volt potential difference was applied between the tank-solutions to initiate electrophoresis.

The electrolysis was carried on for 60 minutes. All the time the hollow plates were well circulated with thermostated water (35 °C). Afterwards, strips were taken out by means of glass and dried on a horizontal platform and the spots were detected. The observation was repeated at different pH's of the background electrolyte (variation in pH was made by addition of caustic soda solution). The distance recorded in duplicates differed within ± 5% and the average distances in duplicates were noted for calculation. The distance travelled towards the anode was assumed to be negative and those

towards the cathode positive. The actual distance of the sample spot was measured after taking into account the distance travelled by the reference glucose spot. The potential gradient through the strip was found to be 7.5 V/cm by dividing the movement by potential gradient and time yield mobility which are plotted in Figures 1. and 2.
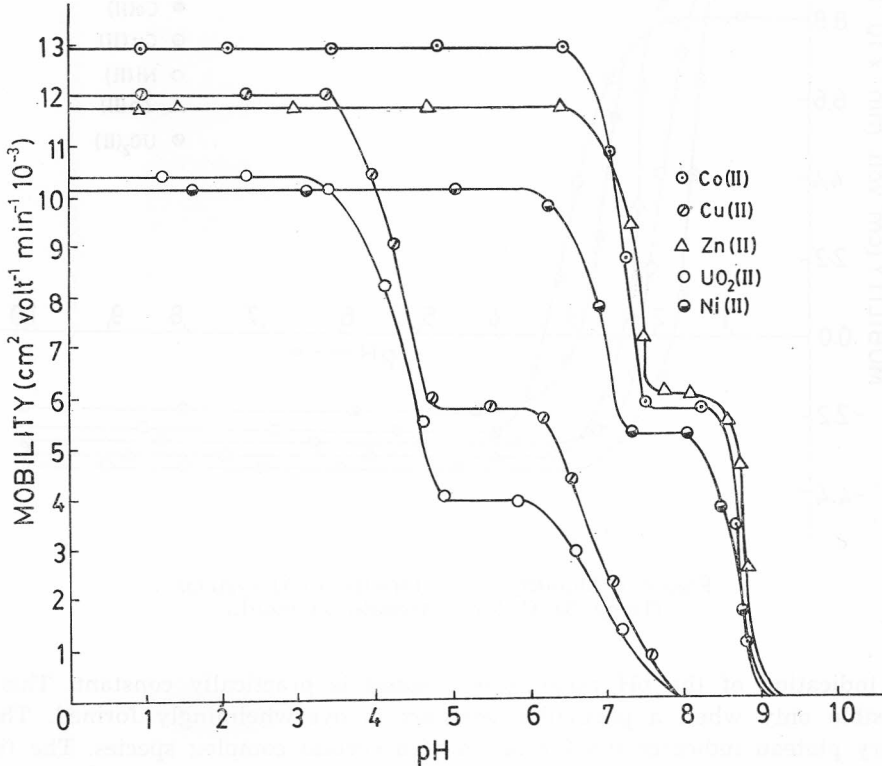

Figure 1. Mobility curves of [M(II)-4-amino pentanoic acid (Temp. 35 °C, Ionic strength 0.1 mol/L)

(ii) *Ternary Complexes*

Strips were marked with metal ions in duplicate along with an additional one marked with glucose. After drenching the strips with background electrolyte the electrophoresis was carried on for one hour at the same potential difference as in case of binary complexes. For subsequent observation, the 5-amino pentanoic acid solution, maintained at pH = 8.5, was added progressively and ionophoretic mobility was recorded. A plot of mobility against-log [5-amino pentanoic acid] was made which is shown in Figure 3.

(1) *Metal-5-amino Pentanoic Acid Binary Systems*

The plot of overall electrophoretic mobility of metal spot against pH gives a curve with a number of plateaus shown in Figure 1. A plateau is obviously
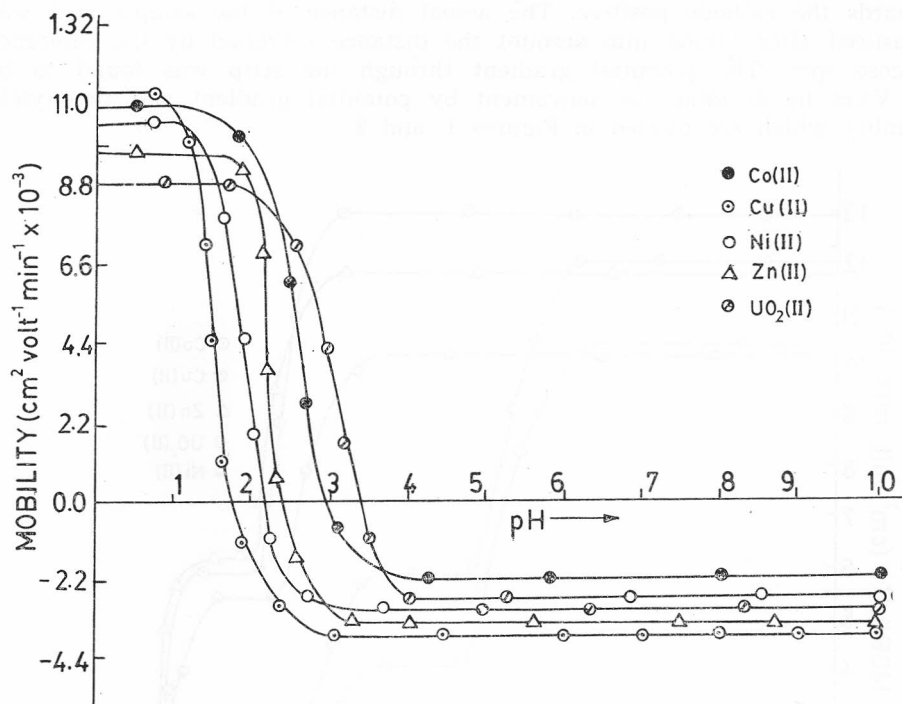
Figure 2. Mobility curve [M-(II) NTA] systems
(Temp. 35 °C, Ionic strength 0.1 mol/L)

an indication of the pH range where speed is practically constant. This is possible only when a particular complex is overwhelmingly formed. Thus, every plateau indicates the formation of a certain complex species. The first one at the beginning corresponds to a region in which metal ions are uncomplexed. It lies in a low pH region where concentration of protonated species of 5-amino pentanoic acid is obviously maximum. Hence, it is concluded that this protonated species of 5-amino pentanoic acid is non-complexing. Beyond this range the metal ion spot has a progressively decreasing velocity and hence complexation of metal ion should be taking place with other ionic species of 5-aminopentanoic acid whose concentration increases progressively with the increase of pH. The figure reveals a second plateau in each case with positive mobility indicating the formation of 1 : 1 complex of cationic nature. Further increase in pH gives rise to a third plateau with zero mobility in each case which indicates formation of an electrically neutral metal complex. This is possible only when two anionic species of 5-amino pentanoic acid combine with one bivalent metal ion. Chemical ltierature also assigns a prominent ligating property to unprotonated anionic species of 5-amino pentanoic acid, ruling out any such property to the Zwitter ion.[12-14] Further increase of pH has no effect on the mobility of metal ions.
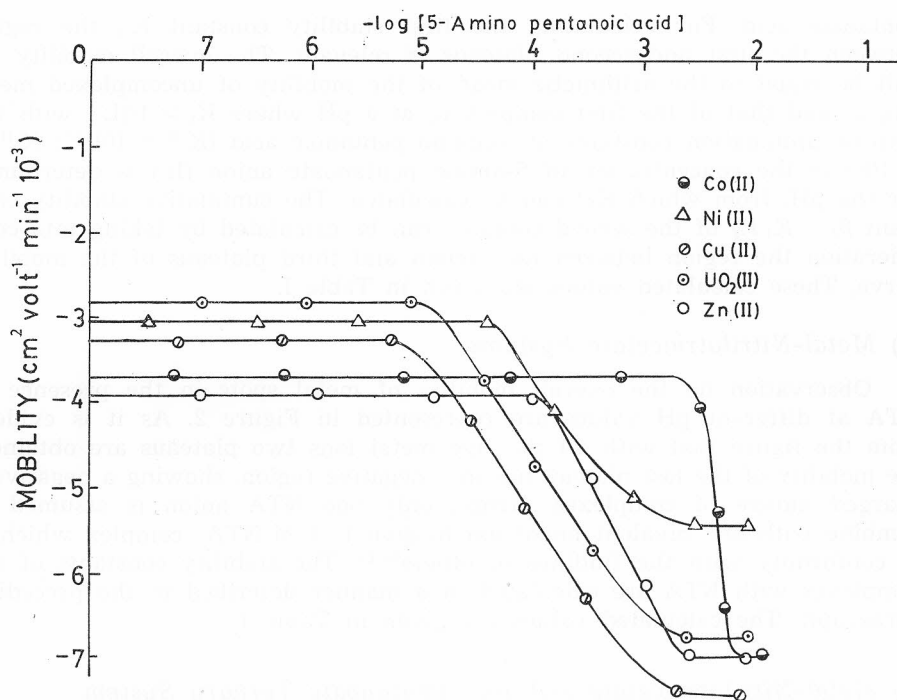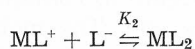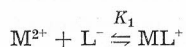
Figure 3. Mobility curves [M-NTA-5-Amino Pentanoic acid]
(Temp. 35 °C, Ionic strength 0.1 mol/L)
— log [5-Amino pentanoic acid]

In view of the above observation the complexation of metal ion with 5-amino pentanoate ion (L^-) may be represented as:

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

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

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

$$U = \underset{n}{\Sigma}\, u_n f_n$$

where u_n and f_n are mobility and molefraction of a particular complex species.
This equation is transformed into the following form on taking into consideration different equilibria:

$$U = \frac{u_0 + u_1 K_1\,[L^-] + u_2 K_1 K_2\,[L^-]^2}{1 + K_1\,[L^-] + K_1 K_2\,[L^-]^2}$$

where u_0, u_1 and u_2 are mobilities of uncomplexed metal ion 1 : 1 metal complex and 1 : 2 metal complex, respectively. This equation has been used for calculating stability constants of the complexes of metal ions with 5-amino

pentanoic acid. For calculating the first stability constant K_1, the region between the first and second plateaus is relevant. The overall mobility 'U' will be equal to the arithmetic mean of the mobility of uncomplexed metal ion, u_0 and that of the first complex u_1 at a pH where $K_1 = 1/[L^-]$ with the help of protonation constants of 5-amino pentanoic acid ($K_1^H = 10^{10,51}$; $K_2^H = 10^{3.45}$)[15] the concentration of 5-amino pentanoate anion (L^-) is determined for the pH, from which K_1^H can be calculated. The cumulative stability constant $\beta_2 = K_1 K_2$ of the second complex can be calculated by taking into consideration the region between the second and third plateaus of the mobility curve. These calculated values are given in Table I.

(2) Metal-Nitrilotriacetate Systems

Observation on the overall mobility of metal spots in the presence of NTA at different pH values are represented in Figure 2. As it is evident from the figure that with all the five metal ions two plateaus are obtained, the mobility of the last plateau lies in a negative region, showing a negatively charged nature of complexes. Hence, only one NTA anion is assumed to combine with one bivalent metal ion to give $1:1$ M-NTA$^-$ complex which is in conformity with the findings of others[16-18]. The stability constants of the complexes with NTA are calculated in a manner described in the preceding paragraph. The calculated values are given in Table I.

(3) Metal-Nitrilotriacetate 5-Amino Pentanoate Ternary System

The study of this system has been purposely carried out at pH $= 8.5$. It is observed from the mobility curves of M-5-amino pentanoic acid and M-NTA binary systems that binary complexes, M-5-amino pentanoic acid and M-nitrilotriacetate are formed at a pH lower than 8.5. Thus, it would be proper to study the transformation of M-NTA complex into M-NTA-5-amino pentanoate complex at pH $= 8.5$ in order to avoid any side interaction.

The plot of mobility against logarithm of concentration of added 5-amino pentanoic acid gives a curve (Figure 3) 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 $1:1$ M-NTA complexes. The mobility in this range is also in agreement with mobility of $1:1$ M-NTA complex as evidenced in the study of binary M-NTA system. The mobility of the last plateau is more negative than that of the first plateau, so it 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-5--amino pentanoate complex (observed in our study of binary M-5-amino pentanoate system), it is inferred that the species in the last plateau is formed by co-ordination of 5-amino pentanoate anion to $1:1$ M-NTA complex, resulting in the formation of $1:1:1$ mixed complex (M-NTA-5-amino pentanoate) as

$$\text{M-NTA}^- + \text{L}^- \overset{K_{mix}}{\rightleftharpoons} \text{M-NTA-L}^{2-}$$
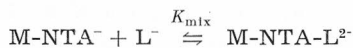
In the present electrophoretic study the transformation of a simple complex into a mixed complex takes place. Hence, the overall mobility is given by:

TABLE I

Stability Constants of Some Binary and Ternary omplexes Cu(II), UO₂(II), Ni(II), Co(II), and Zn(II)
(Ionic Strength = 0.1 mol/L); Temperature = 35 °C
NTA anion = N(CH₂COO)⁻³; 5-Amino pentanoate anion = NH₂—CH₂—CH₂—CH₂—CH₂—COO⁻

Metal ion	Calculated value of stability constants				Literature values			
	$\log K^M_{1_{ML}}$	$\log \beta_{ML_2}$	$\log K^M_{M-NTA}$	$\log K^M_{M-NTA-L}$	$\log K^M_{1_{ML}}$	$\log \beta_{ML_2}$	$\log K^M_{M-NTA-L}$	$\log K^M_{M-NTA}$
Cu(II)	7.23	11.94	12.24	5.85	—	—	—	12.94(15) 13.60(20) 12.75(20)
UO₂(II)	7.15	12.66	9.77	5.50	—	—	—	9.50(15) 7.88(20) 9.50(20)
Ni(II)	5.18	8.99	11.42	5.22	—	—	—	11.54(19) 10.50(15) 11.53(15) 11.26(20)
Co(II)	5.31	9.12	10.32	3.96	—	—	—	10.38(15) 10.38(20)
Zn(II)	5.11	8.82	10.59	4.04	—	—	—	10.45(20) 10.69(20)

where $K^M_{1_{ML}} = \dfrac{[ML]}{[M][L]}$; $\beta_{ML_2} = \dfrac{[ML_2]}{[M][L]^2}$; $K^M_{M-NTA} = \dfrac{[M-NTA]}{[M][NTA]}$ and $K^M_{M-NTA-L} = \dfrac{[M-NTA-L]}{[M-NTA][L]}$

where u_0, u_1 and f_{M-NTA}; $f_{M-NTA-L}$ are the mobilities and the mole fractions of M-NTA-L^{2-} complexes, respectively. Substituting the values of mole fractions, the overall mobility is given by

$$U = \frac{u_0 + u_1 K_{mix} [L^-]}{1 + K_{mix} [L^-]}$$

u_0 and u_1 are obviously the mobilities in the regions of the two plateaus of the curve. From the figure the concentration of 5-amino pentanoic acid, whose overall mobility is the mean of the mobilities of the two plateaus, is determined. The concentration of 5-amino pentanoate anion at pH = 8.5 for this 5-aminopentanoic acid concentration is calculated. K_{mix} is obviously equation $1/[L^-]$. All these values of K'_{mix} are given in Table I. Since the stability constants of these complexes are determined for the first time, no comparison can be made.

The precision of the method is limited to that of paper electrophoresis. However, uncertainty in the result is 5⁰/₀. No doubt it cannot replace the most reliable methods even though it is a new approach worth developing.

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SAŽETAK

Ionoforetska tehnika određivanja konstanti stabilnosti mješovitih kompleksa

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Opisana je elektroforetska metoda za određivanje konstanti stabilnosti mješovitih kompleksa s različitim ligandima. Metoda se zasniva na motrenju pomicanja mrlje metalnog iona pri stalnom pH i koncentraciji jednoga od liganda, dok se koncentracija drugog liganda sustavno mijenja. Na taj su način određene konstante stabilnosti za sustave $M + NTA + 5$-amino pentanoat ($M = Co^{2+}$, Ni^{2+}, Cu^{2+}, Zn^{2+}, UO_2^{2+}).