

Paper Ionophoretic Technique in the Study of Mixed Complexes

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Stability constants of complexes of aluminium(III) and thorium(IV) with methionine and cysteine have been determined by modified paper electrophoretic technique at $\mu = 0.1$ M. The proportion of ionic species of methionine and cysteine were varied by changing pH of background electrolyte. The stability constants of the complexes metal-methionine-cysteine have been found to be 4.31 ± 0.12 and 5.40 ± 0.19 (log K values) for Al^{3+} and Th^{4+} complexes, at temperature 35°C , respectively.

Keywords : Paper electrophoresis, Overall mobility, Mixed complexes, Stability constants.

Introduction

Mixed metal complexes play an important role in various biological systems¹ and in different fields of chemistry.^{2,3} Hence, the formation stability and reactivity of these complexes have been an active field of research.⁴ Due to growing interest in the use of sulphur containing compounds in analytical as well as structural studies of metal complexes, sulphur containing amino acids methionine and cysteine are taken in present study. The significance of these amino acids enhanced by the fact that it displays independent therapeutic activity.⁵ Its most valuable use is for the treatment of Wilsons disease, cause by accumulation of copper. The aluminium (III) and thorium(IV) metal ions are important for biological systems but are toxic at higher concentration.⁶⁻¹⁰

Ionophoretic technique has been applied for the study of metal complexes in solution.^{11,12} Recently the technique has been modified in our laboratory to facilitate the study of mixed metal complexes.¹³⁻¹⁶ The technique employed in these laboratories is almost free from the common defects of electrophoretic techniques like temperature variation during electrophoresis, capillary flow on the paper, electro-osmosis, adsorption and molecular sieving affecting the mobility of charged moieties.

In the present work the technique has been employed to determine the nature and stability constants of $\text{Al}^{3+}/\text{Th}^{4+}$ -methionine-cysteine mixed complexes.

Experimental Section

Apparatus. A Systronic (Naroda, India) Model 604 electrophoresis system was used. It has a built-in power supply (a.c.-d.c.) that is fed directly to a paper electrophoresis tank. 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 these

plates. pH measurements were made with an Elico (Hyderabad, India) Model L₁₋₁₀ pH meter using a glass electrode.

Chemicals. Solutions of aluminium(III) and thorium(IV) metal perchlorate were prepared by preliminary precipitation of metal carbonates from 0.1 M solution of sodium carbonate. Metal perchlorate solutions were kept in plastic coated bottles and avoid to contact with skin, eyes and clothing. Metal spots were detected on the paper using aluminon (BDH, England) ammonium acetate mixture in water for Al(III) and 1-(2-Pyridylazo)-2-naphthol (PAN) (E. Merck, Germany) for Th(IV). A saturated aqueous solution (0.9 mL) of silver nitrate was diluted with acetone to 20 mL. Glucose, as the black spot, was detected by spraying with this solution and then with 2% ethanolic sodium hydroxide.

Background Electrolytes. The background electrolytes used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M methionine. For the study of mixed system the background electrolyte used were 0.1 M perchloric acid, 0.01 M methionine and 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 (SDS, AnalaR), 2.0 M sodium hydroxide (AnalaR grade), 0.5 M methionine and 0.5 M cysteine (BDH, Poole, U.K.) were prepared.

Procedure. The hollow base plate in the instrument was verified to be horizontal with spirit level. 150-mL volume of background electrolyte was placed in each tank of the electrophoretic apparatus. The levels of the two tank solutions were equalized by a siphon. These precautions were taken to stop any gravitational hydrodynamic flow. Paper strips (Whatman No. 1) of 30×1 cm² size were soaked in background electrolyte and then excess of electrolyte solution was blotted by pressing them gently within the folds of dry filter paper sheets. Paper strips in duplicate were spotted with metal ions and glucose in the center 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 sides. Then a potential difference of 200 V was applied between the tank solutions and electro-

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phoresis was carried out for 60 min. Afterward the strips were taken out with the help of glass rod and dried. The spots were developed. The distance recorded in duplicate strips differed $\pm 5\%$ and the average distance was calculated. The distance traveled towards anode were given a negative sign and those towards cathode to be positive. The actual distance of the sample spot moved was corrected for the distance traveled by the reference glucose spot. The mobilities were calculated by dividing the movement by the potential gradient and expressed in $\text{cm}^2 \text{V}^{-1} \text{min}^{-1}$.

Results and Discussion

(i) Metal-Methionine Binary Systems. Mobilities of metal ion complexes against pH give a curve containing three and two plateaus in case of Al(III) and Th(IV) metal ions, respectively (Figure 1). The first plateau in each case at low pH region present uncomplexed metal ion while remaining indicate metal complexes. It is obvious that the protonated ionic species of the ligand methionine viz; $[(\text{CH}_3)_2\text{C}(\text{SCH}_3)\text{CH}(\text{NH}_3^+)\text{COOH}]$, while arounds in low pH range are non-complexing. The second plateau in each case with positive mobility indicates the formation of 1 : 1 complex of cationic nature, the ligand being the anionic species of methionine $[(\text{CH}_3)_2\text{C}(\text{SCH}_3)\text{CH}(\text{NH}_2)\text{COO}^-]$. In case of Al(III) metal ion with further increase of pH decreases the mobility and gives rise to a third plateau in positive region indicates formation of 1 : 2 metal complex of cationic nature. Further increase of pH in case of Th(IV) metal ion has no effect on the mobility of metal ions which indicates formation of only

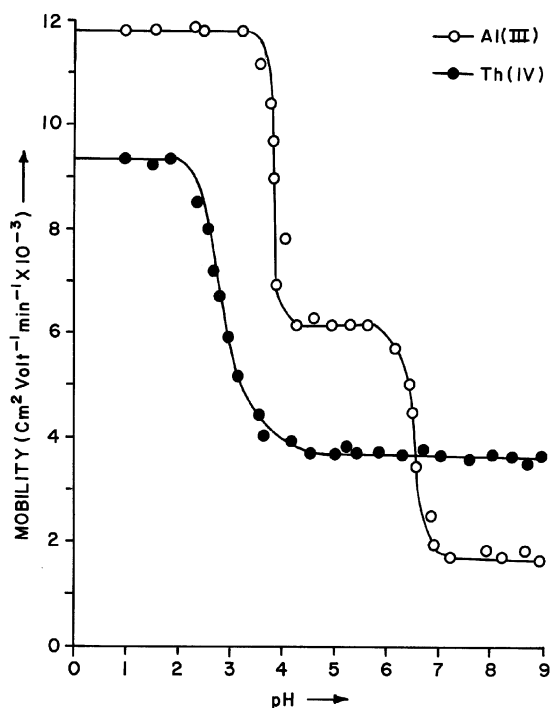


Figure 1. Mobility curves for M-methionine systems. \circ = Al(III)-methionine; \bullet = Th(IV)-methionine. Concentration of Al(III) & Th(IV) = 5.0×10^{-3} M. The paper strips were spotted with $0.1 \mu\text{L}$ of sample solution and glucose (for making osmotic correction).

1 : 1 metal complex.

Using protonation constant of methionine (Electrophoretically obtained value, $\text{p}K_1 = 2.25$; $\text{p}K_2 = 9.00$), the concentration of methionine anion $[\text{L}^-]$ is determined at the pH between first and second step, from which K_1 can be calculated, $K_1 = 1/[(\text{CH}_3)_2\text{C}(\text{SCH}_3)\text{CH}(\text{NH}_2)\text{COO}^-]$. The stability constants K_2 of second complex can be calculated by taking into consideration the region between second and third plateau of the mobility curve. These calculated values are given in Table 1.

(ii) Metal-Methionine-Cysteine Mixed Systems. It is observed from the mobility curves of metal-methionine system that binary complexes are formed at $\text{pH} < 8.5$. It was therefore considered necessary to study the transformation of metal-methionine binary complexes into metal-methionine-cysteine mixed complexes at $\text{pH} 8.5$ to avoid any side interaction.

The plot of mobility versus $\log [\text{cysteine}]$ gives the curve with two plateaus, the mobility in the range of first plateau corresponds to that of 1 : 1 cation methionine complex. The mobility of the second plateau do not agree with either of metal-methionine complexes. The more negative value of the mobility of the second plateau indicates the formation of more negatively charged complex. It is inferred that the complex moiety formed in the region of second plateau is due to coordination of cysteine anion to 1 : 1 cation-methionine moiety resulting in 1 : 1 : 1 mixed complex. The curves show the transformation of a metal-methionine simple complex to metal-methionine-cysteine mixed complexes.

The concentration of cysteine at which overall mobility is the mean of the mobilities of two plateaus is determined from Figure 2, the corresponding cysteine anion concentration at $\text{pH} 8.5$ is calculated. The stability constant of mixed complexes is given by $K_3 = 1/[\text{H}_2\text{C}(\text{S}^-)\text{CH}(\text{NH}_2)\text{COO}^-]$. These values are given in Table 1.

The order of stability constants values viz; thorium(IV) > aluminium(III), are found in support from the work of Irving and Williams.¹⁷ Comparison of the stability constants shows that values are lower in mixed complexes. It is therefore inferred that the coordination tendency of a ligand decreases

Table 1. Stability Constants of Binary and Ternary Complexes of Aluminum(III) and Thorium(IV)

Ionic strength = 0.1 M; Methionine anion		Temperature = 35 °C Cysteine anion	
		$[(\text{CH}_3)_2\text{C}(\text{SCH}_3)\text{CH}(\text{NH}_2)\text{COO}^-]$; $[(\text{H}_2)\text{C}(\text{S}^-)\text{CH}(\text{NH}_2)\text{COO}^-]$	
Metal ions	Complexes	Log K Values	
		Present study	Literature
Al(III)	ML	7.00 ± 0.05	—
	ML_2	11.50 ± 0.12	—
	MLL'	4.31 ± 0.12	—
Th(IV)	ML	8.08 ± 0.08	—
	ML_2	—	—
	MLL'	5.40 ± 0.19	—

M = Metal cations; L = Primary ligand (methionine); L' = Secondary ligand (cysteine).

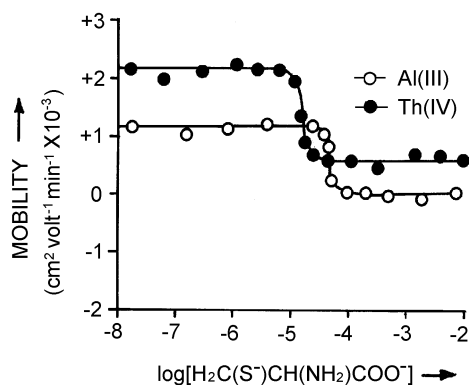


Figure 2. Mobility curves for M-Methionine-cysteine systems. ○ = Al(III)-methionine-cysteine; ● = Th(IV) methionine-cysteine. Concentration of Al(III) & Th(IV) = 5.0×10^{-3} M. The paper strips were spotted with 0.1 μ L of sample solution and glucose (for making osmotic correction).

with higher state of aggregation. In other words, the metal progressively lessen its tendency of linkage with a ligand on progressive filling of vacant orbitals. This conclusion is of universal validity as evident in chemical literature.^{18,19}

Concluding Remarks

The simple electrophoretic technique has thus been helpful in deciding as to whether a mixed complex system is formed or not and if it is formed its stability constants can also be determined. The stability constants of metal complexes can be vary easily calculated by this technique, so the present paper ionophoretic technique have significant advantages over other methods reported in chemical literature for the

determination of stability constants of metal complexes.

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