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Determination of Stability Constants of Metal-methylcysteine and Metal-methylcysteine-cysteine Complexes by Paper Ionophoretic Technique

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A method, involving the use of an advanced paper ionophoretic technique is described for the study of equilibria in mixed complex systems in solution. The method is based on the migration of a spot of metal ion, with the complexants added in the background electrolyte. The concentration of one of the complexants, methylcysteine, is kept constant while that of the second ligand, cysteine, is varied. A graph of log [cysteine] against mobility is used to obtain information on the mixed-ligand complexes and to calculate their stability constants. Stability constants of metal-methylcysteine-cysteine complexes were found to be 3.05 ± 0.13 and 4.31 ± 0.11 (logarithm of stability constant values) for aluminium(III) and thorium(IV) respectively, at ionic strength of 0.1 mol dm^{-3} and a temperature of 35 °C.

INTRODUCTION

Due to the growing interest in the use of sulphur containing compounds in analytical as well as structural studies of metal complexes, sulphur containing amino acids, methylcysteine and cysteine, have been chosen for the present study of metal complex formation. Ionophoretic technique has been used in determination of stability constants of metal complexes for a long time.[1] Using paper chromatography, Hurnick[2] studied tartrate complexes of Fe^{II}, Co^{II} and Ni^{II} in aqueous media and determined the stability constants, but this study is only qualitative and does not throw light on the nature of species or on their stabilities. Communications[3–9] from our laboratory reported a new method for the study of mixed-ligand complexes, in which two different amino acids act as primary and secondary ligands. Aluminium(III) and thorium(IV) metal ions have significant biomedical applications, but are toxic at higher concentrations.[10–17] It seems to be very interesting to study the feasibility of controlling their level by complexation.

In this respect, attempts were made to establish the optimum conditions for metal-methylcysteine and metal-methylcysteine-cysteine complex formation. Further, this paper describes a paper electrophoretic method for the determination of the stability constants of these complexes.

EXPERIMENTAL

Instruments

Electrophoresis equipment from Systronics (Naroda, India), model 604, was used. The apparatus consists of a polyvinyl chloride (PVC) moulded double tank vessel. In order to avoid errors due to the heat generated during electrophoresis, two hollow rectangular plates were used through which thermostated water circulated. The tanks were covered with a transparent PVC moulded lid to prevent changes in moisture that might upset the equilibrium in the paper strip. Each electrolyte tank contained a separate Pt-wire electrode. Applied voltage was from a stabilized source.

pH measurements were made with an Elico (Hyderabad, India) model L_{1-10} pH meter using a glass and calomel electrodes assembly, working at 220 V/50 Hz established a.c. mains.

Chemicals

Solutions of aluminium(III) and thorium(IV) metal perchlorate were prepared by preliminary precipitation of metal carbonates from 0.1 mol dm^{-3} 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} mol dm^{-3}.

Metal spots were detected on the paper using aluminon (BDH, England) – an ammonium acetate mixture in water for aluminium(III) and a 0.1 % solution of 1-(2-pyridylazo)-2-napthol (PAN) (E. Merck, Darmstadt, Germany) for thorium(IV). 5.0×10^{-3} mol dm^{-3} 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, as the black spot, was detected by spraying with this solution and then with 2 % ethanolic solution of sodium hydroxide.

Background Electrolytes

The background electrolytes used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M methylcysteine. For the study of mixed-ligand systems, the background electrolytes were 0.1 M perchloric acid, 0.01 M methylcysteine and 0.01 M cysteine. The mixed system was maintained at pH = 8.5 by addition of sodium hydroxide.

Stock solution of 5.0 M perchloric acid was prepared by 70 % solution (SDS, AnalaR grade). 2.0 M sodium hydroxide (AnalaR grade), 0.5 M methylcysteine and 0.5 M cysteine (BDH, Poole, UK) solutions were prepared. Each solution was standardized using appropriate methods.

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 containing 0.1 mol dm^{-3} of perchloric acid and 0.01 mol dm^{-3} of methylcysteine. 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 °C) was circulated into the plates to keep the temperature constant. The lid was then placed on the instrument to make it airtight. It was left for 10 minutes to ensure strip wetting. A direct 200 V potential was subsequently applied between the electrodes.

Electrophoresis 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. A plot of mobility *versus* pH is shown in Figure 1.

Mixed-ligand 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, electrophoresis 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 2).

RESULTS AND DISCUSSION

Metal-methylcysteine Binary System

Mobility of metal ion complexes against pH gives a curve containing three and two plateaus in the case of Al^{III} and Th^{IV} cations, respectively (Figure 1). The first plateau in each case, in the low pH region, represents non-complexed cations, while the remaining ones indicate metal complexes. In this low pH range, methylcysteine is present as a non-complexing species. The second plateau in each case, still of positive mobility, indicates a 1:1 complex of cationic nature. The ligand here is the anionic species of methylcysteine. With further increase in pH, the mobility in the case of aluminium(III) ion decreases, giving rise to a third plateau in the positive region, indicating a 1:2 metal complex of positive nature, whereas for thorium(IV) ion, the mobility remains constant with increasing pH, which indicates that only a 1:1 complex is

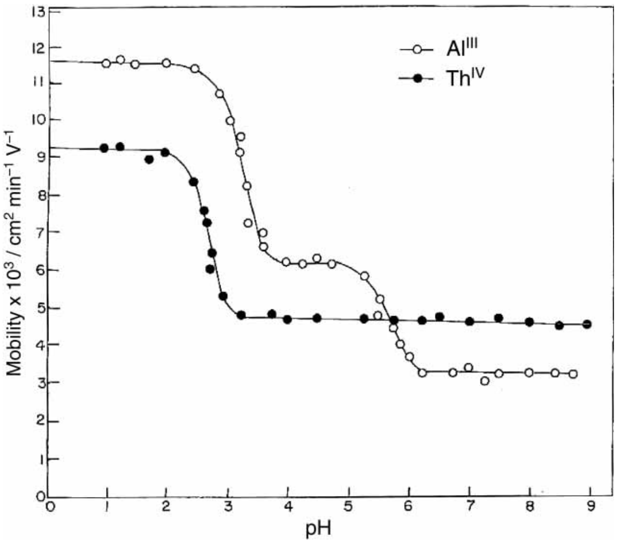

Figure 1. Mobility curves for metal-methylcysteine systems: Al^{III}-methylcysteine (○); Th^{IV}-methylcysteine (●). Concentration of Al^{III} and Th^{IV} is 5.0 × 10^{-3} mol dm^{-3}. Paper strips were spotted with 0.1 µl of sample solution and glucose (for osmotic correction).

formed in the case of thorium(IV)-methylcysteine system. In general, the complexation of metal ions with methylcysteine anion can be represented as:

$$M^{3+} + L^- \overset{K_1}{\rightleftharpoons} ML^{2+} \qquad (1)$$

$$ML^{2+} + L^- \overset{K_2}{\rightleftharpoons} ML_2^{+} \qquad (2)$$

$$M^{4+} + L^- \overset{K_1}{\rightleftharpoons} ML^{3+} \qquad (3)$$

where M^{3+} and M^{4+} are the Al^{3+} and Th^{4+} ions; L^- is the methylcysteine anion; K_1 and K_2 are the first and second stability constants, respectively.

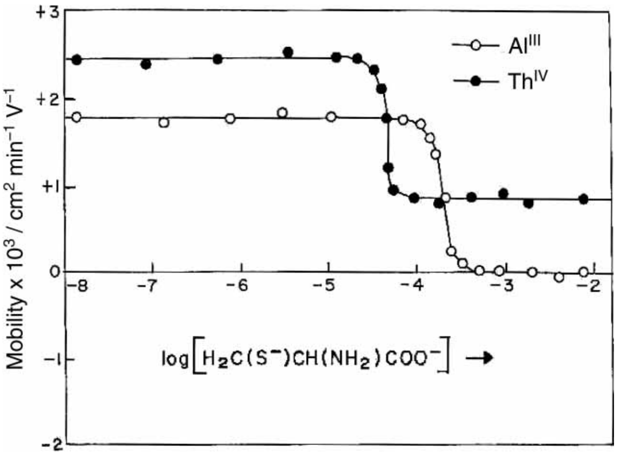

Figure 2. Mobility curves for metal-methylcysteine-cysteine systems: Al^{III}-methylcysteine-cysteine (○); Th^{IV}-methylcysteine-cysteine (●). Concentration of Al^{III} and Th^{IV} = 5.0 × 10^{-3} mol dm^{-3}. Paper strips were spotted with 0.1 µl of sample solution and glucose (for osmotic correction).

The metal spot on the paper is thus a combination of uncomplexed metal ions, a 1:1 complex and a 1:2 complex (in the case of Al^{3+}). The overall mobility U is given by:

$$U = \frac{u_0 + u_1K_1[L^-] + u_2K_1K_2[L^-]^2}{1 + K_1[L^-] + K_1K_2[L^-]^2} \qquad (4)$$

where u_0, u_1 and u_2 are mobilities of uncomplexed metal ions, 1:1 and 1:2 metal complexes, respectively.

The region between the first and second plateau is pertinent for the calculation of the first stability constant K_1. The overall mobility U will be equal to the arithmetic mean of mobility of uncomplexed metal ions, u_0, and that of the first complex, u_1, at a pH value where $K_1 = 1 / [H_2C(SCH_3)CH(NH_2)COO^-]$.

Using protonation constants of pure methylcysteine [$pk_1 = 2.25$; $pk_2 = 8.55$ (values obtained by paper electrophoresis)], the concentration of methylcysteine anion [L^-] is determined for the pH value(s) of interest, from which K_1 can be calculated. The concentration of complexing methylcysteine species [L^-] is calculated with the help of the following equation:

$$[L^-] = \frac{[L_t]}{1 + [H] / k_2 + [H]^2 / k_1k_2} \qquad (5)$$

where [L_t] is the total concentration of the ligand (methylcysteine) and k_1, k_2 are the first and second dissociation constants of pure methylcysteine, respectively.

The stability constant K_2 of the second complex can be calculated by taking into account the region between the second and third plateaus of the mobility curve. The calculated values of K_1 and K_2 are given in Table I.

Metal-methylcysteine-cysteine Ternary System

It is seen from the mobility curve of the metal-methylcysteine system that binary complexes are formed at pH = 8.5. It was therefore considered necessary to study the transformation of metal-methylcysteine binary complexes into metal-methylcysteine-cysteine mixed complexes at pH = 8.5 to avoid all side interactions.

The plot of mobility *versus* logarithm of concentration of added cysteine gives a curve (Figure 2) containing two plateaus; the mobility in the range of the first plateau is in agreement with the mobility of metal-methylcysteine complexes. The mobility of the last plateau is more negative than that of the first plateau, indicating the formation of a more negatively charged complex. Further, since the mobility in the last plateau does not tally with the mobility of 1:1 and 1:2 metal-methylcysteine complexes, it is inferred that the mobility in the last plateau is due to co-ordination of the cysteine anion to 1:1 metal-methylcysteine moiety, resulting in the formation of 1:1:1 metal-methylcysteine-cysteine mixed complexes:

$$ML^{2+} + (L')^{2-} \xrightleftharpoons{K_3} MLL' \qquad (M = Al^{3+}) \qquad (6)$$

$$ML^{3+} + (L')^{2-} \xrightleftharpoons{K_3} (MLL')^{+} \qquad (M = Th^{4+}) \qquad (7)$$

where $(L')^{2-}$ is the cysteine anion and K_3 is the stability constant of the mixed-ligand complexes.

Mobilities of the last plateau in the case of aluminium(III) and thorium(IV) are in zero and positive regions, indicating the neutral and cationic nature of the complexes formed, respectively. In the present ionophoretic study, transformation of a binary complex into a ternary complex takes place, hence the overall mobility U of the complex is given by:

$$U = \frac{u_0 + u_1 K_3[H_2C(S^-)CH(NH_2)COO^-]}{1 + K_3[H_2C(S^-)CH(NH_2)COO^-]} \qquad (8)$$

where u_0 and u_1 are the mobilities of metal-methylcysteine and metal-methylcysteine-cysteine complexes, respectively.

The cysteine concentration at which the overall mobility is the mean of the mobilities of two plateaus is determined from Figure 2, and the corresponding cysteine anion concentration at pH = 8.5 is calculated. K_3, is obviously equal to $1 / [H_2C(S^-)CH(NH_2)COO^-]$. The calculated values of K_3 are given in Table I.

TABLE I. Stability constants of binary and mixed complexes of aluminium(III) and thorium(IV)[(a),(b),(c)]

Metal ions	Complexes	Stability constants	Log K values
Aluminium(III)	ML^{2+}	K_1	7.68 ± 0.05
	ML_2^{+}	K_2	12.73 ± 0.10
	MLL'	K_3	3.05 ± 0.13
Thorium(IV)	ML^{3+}	K_1	8.37 ± 0.08
	ML_2^{2+}		–
	$(MLL')^{+}$	K_3	4.31 ± 0.11

[(a)] Ionic strength = 0.1 mol dm^{-3}, temperature = 35 °C.

[(b)] Methylcysteine anion = $[H_2C(SCH_3)CH(NH_2)COO^-]$, cysteine anion = $[H_2C(S^-)CH(NH_2)COO^-]$.

[(c)] M = metal cations, L = primary ligand (methylcysteine), L' = secondary ligand (cysteine).

Perusal of Table I reveals that the order of stability constants, *viz.* thorium(IV) > aluminium(III), is the same for metal-methylcysteine binary and metal-methylcysteine-cysteine mixed complexes, but the corresponding stability constant values are lower in mixed complexes. It is therefore inferred that the co-ordinating tendency of a ligand decreases with a higher state of aggregation.[18,19]

CONCLUSION

A simple electrophoretic technique has thus been helpful in finding out whether a complex system is formed or not and if it is formed, its stability constants can be determined. The above studies point to the conclusion that methylcysteine and cysteine can be used to reduce the level of aluminium(III) and thorium(IV) in biological systems.

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SAŽETAK

Određivanje konstanata stabilnosti kompleksa metal-metilcistein i metal-metilcistein-cistein papirnom ionoforezom

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Opisana je tehnika papirne ionoforeze kao metode za izučavanje ravnoteže u sustavima mješovito-ligandnih kompleksa u otopini. Metoda je temeljena na gibanju mrlje metalnoga iona, pri čemu su sredstva za kompleksiranje dodana u pozadinski elektrolit. Koncentracija jednoga od liganada, metilcisteina, držana je konstantom, dok je koncentracija drugoga, cisteina, mijenjana. Dijagram ovisnosti log[cistein] *vs.* pokretljivost iona poslužio je za izračunavanje konstanata stabilnosti nastalih kompleksa. Pri ionskoj jakosti od 0.1 mol dm^{-3} i temperaturi od 35 °C konstante stabilnosti (log K) kompleksa metal-metilcistein-cistein iznose: 3.05 ± 0.13 za aluminij(III) i 4.31 ± 0.11 za torij(IV).