

Short Communication

Ionophoretic technique in the study of mixed-ligand complexes of biochemical importance in the Co(II)/Cu(II)–adenosine diphosphate nitrilotriacetate system

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ABSTRACT

A method involving the use of an ionophoretic technique (paper electrophoresis) is described for the study of equilibria in mixed-ligand complex systems in solution. The method is based on the migration of a spot of a metal ion (m), with the complexants added to the background electrolyte (0.1 M perchloric acid). For the study of ternary complexes, the concentration of one of the complexants, adenosine diphosphate (ADP), is kept constant, while that of the second ligand, nitrilotriacetate (NTA), is varied. A graph of mobility against $-\log[\text{MTA}]$ is used to obtain information on the formation of the mixed-ligand complex and to calculate the stability constants. The overall stability constants of M-ADP and M-ADP-NTA complexes were found to be $10^{5.9}$ and $10^{11.9}$ for Cu(II) and $10^{4.3}$ and $10^{9.7}$ for Co(II) complexes at $\mu = 0.1$ and 30°C .

INTRODUCTION

Paper electrophoresis has previously been applied to the study of metal complexes in solution and attempts have been made to determine the stability constants of the complex species [1,2]. In previous work a method was developed for the study of step-wise complex formation [3–5]. Although the use of paper electrophoresis for the study of metal complex systems with a single ligand seems to be well established, there has been no systematic study on mixed-ligand complexes. However, Czakis Sylikowska [6] made some observations on the formation of mixed

halide complexes of Hg(II), but the studies were only qualitative and did not throw light either on the nature of the species or on their stabilities. Previous papers [7–10] described a method for the study of mixed-ligand complexes. The present work represents an extension of that technique and this paper reports our observation on the mixed-ligand system Cu(II)/Co(II)–adenosine diphosphate (ADP)–nitrilotriacetate (NTA).

EXPERIMENTAL

Horizontal-vertical-type electrophoresis equipment (Systronics Model 604) was used together with various accessories. In each instance electrophoresis was carried out for 60 min at 200 V at 30°C . Whatman No. 1 paper strips (25 × 1 cm) were used.

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pH measurements were made with an Elico Model L₁₋₁₀ pH meter using a glass electrode.

Metal perchlorates were prepared by an appropriate method and the final concentrations were kept at $5.0 \cdot 10^{-3}$ M. 1-(2-Pyridylazo)-2-naphthol (PAN) [0.1% (w/v) in ethanol] was used for detecting Cu(II) and Co(II) ions. A saturated solution of silver nitrate in acetone was sprayed on the paper and subsequently fumed with ammonia to detect glucose spots.

The background electrolyte for the study of binary complexes was 0.1 M perchloric acid–0.002 M ADP– $3.3 \cdot 10^{-3}$ M NTA with sodium hydroxide added to produce the desired pH, and for the study of ternary complexes it was 0.1 M perchloric acid–0.002 M ADP with various amounts of NTA, maintained at pH 8.5 by addition of sodium hydroxide solution.

Procedure for binary complexes

Whatman No. 1 paper strips (25 × 1 cm) in duplicate are spotted in the middle with metal ion solutions. An extra strip is marked with glucose. The strips are sandwiched between two insulated hollow metal plates and the temperature of the system is maintained by water supply within the plates at a fixed temperature. The plates are then mounted on the electrophoresis equipment with the end of the paper strips dipping in the two tanks of the instrument. Electrophoresis is carried out for 60 min. The strips are then removed from tank and dried and the migrated spots are detected with specific reagents. The movement of a metal spot towards the negative electrode is taken as positive mobility and in the reverse direction as negative mobility. Duplicate strips always recorded less than a 5% variation in the distance travelled and the mean of the two was taken for calculation of mobility. The movement of glucose is used as a correction factor for electroosmosis. The electrophoretic migration of metal spots on the paper was observed at different pH values of the background electrolyte. Movements divided by potential gradient yield mobilities, which are plotted in Figs. 1 and 2.

Procedure for ternary complexes

Strips are marked with metal ion solutions in duplicate along with an additional strip marked with glucose. After drenching the strips with the back-

ground electrolyte, electrophoresis is carried for 1 h at the same potential difference as for binary complexes. For subsequent observations, the NTA solution (pH 8.5) is added progressively and the ionophoretic mobility is recorded. Mobility is plotted against $-\log$ [NTA] (Fig. 3).

RESULTS AND DISCUSSION

M-ADP system

The plot of the overall electrophoretic mobility of a metal spot against pH gives a curve with two plateaux as shown in Fig. 1. A plateau is obviously an indication of the pH range where the speed is virtually 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 plateau corresponds to the region in which metal ions are uncomplexed. It lies in a low pH range, where the concentration of protonated species of ADP is obviously maximum. Hence it is concluded that this protonated species of ADP is non-complexing. Subsequently the metal ion spot has a progressively decreasing velocity and hence complexation of the metal ion should be taking place with other ionic species of ADP whose concentration increases progressively with increase in pH. The second plateau in each instance with a negative mobility indicates the formation of a 1:1 negatively charged metal complex. Further increases in the pH of the background electrolyte has no effect on the mobility of metal ions. Hence it is concluded that the complexing species of the ligand is ADP^{3-} . This is in conformity with the findings of other workers [11–13].

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

$$U = \sum_n U_n f_n \quad (1)$$

where U_n and f_n are the mobility and mole fraction of a particular complex species, respectively. This equation is transformed into the following form on taking into consideration different equilibria:

$$U = \frac{U_0 + U_1 K_1 [L]}{1 + K_1 [L]} \quad (2)$$

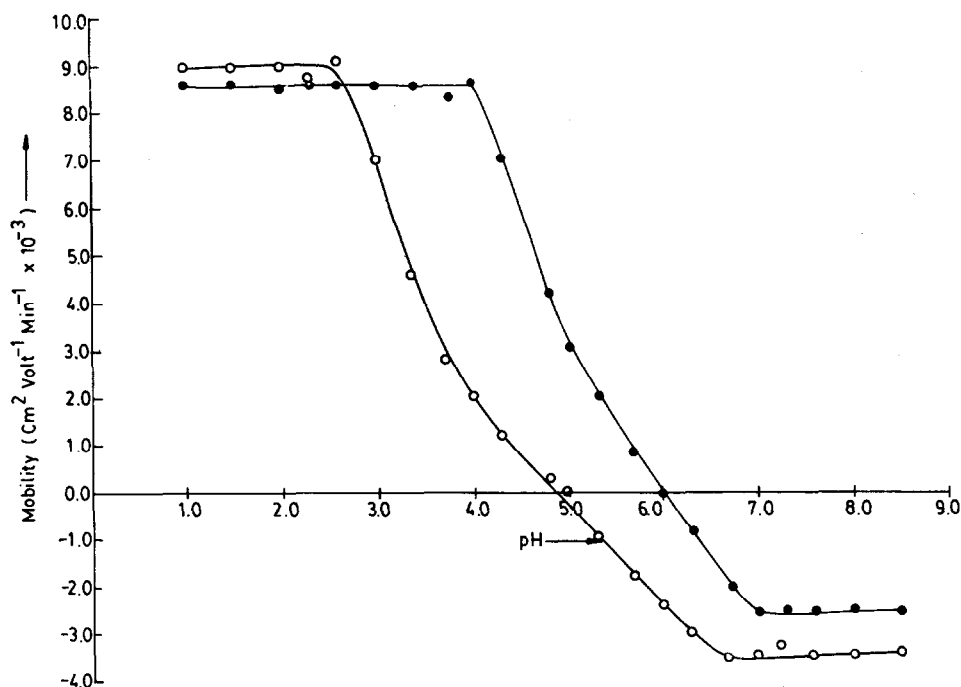


Fig. 1. Plots of mobility versus pH for the metal-ADP system. \circ = Cu(II); \bullet = Co(II).

where U_0 and U_1 are the mobilities of the uncomplexed metal ion and 1:1 metal complex, respectively, and L is the total ligand concentration. This equation was used for calculating the stability constants of the complexes of metal ions with ADP.

For calculating the stability constant K_{M-ADP}^M , the region between the first and second plateaux is pertinent. The overall mobility U will be equal to the

arithmetic mean mobility of the uncomplexed metal ion U_0 and that of the 1:1 metal complex U_1 at a pH where $K_{M-ADP}^M = 1/[L]$. With the help of the dissociation constants of ADP ($k_{ADP^{2-}}^{ADP^-} = 10^{4.1}$ and $k_{ADP^{3-}}^{ADP^{2-}} = 10^{6.4}$) [14], the concentration of the ADP anion $[L^{3-}]$ is determined at a particular pH, from which K_{M-ADP}^M can be calculated. These calculated values are given in Table I.

TABLE I

STABILITY CONSTANTS OF SOME BINARY AND TERNARY COMPLEXES OF Cu(II) AND Co(II) WITH ADP AND NTA
Ionic strength = 0.1 M; temperature = 30°C.

Stability constant	Cu(II)		Co(II)		Ref.
	Calculated value	Literature value	Calculated value	Literature value	
Log K_{M-ADP}^M	5.9	5.90	4.3	4.20	19
Log K_{M-NTA}^M	12.2	12.94	10.1	10.38	20
Log $K_{M-ADP-NTA}^M$	6.0	—	5.4	—	—
Log $K_{M-ADP-NTA}^M$	11.9	—	9.7	—	—

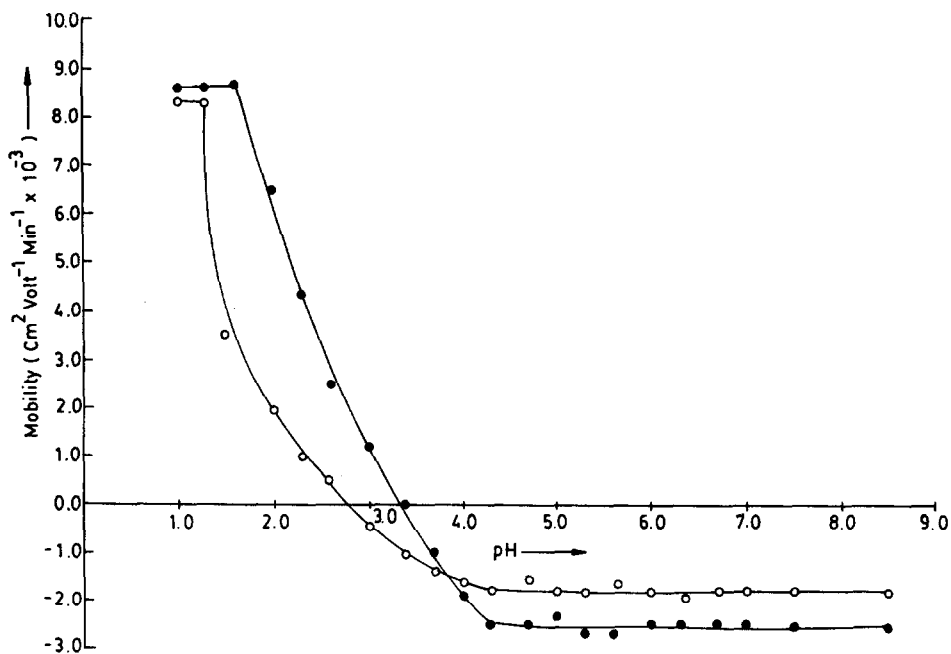


Fig. 2. Plots of mobility versus pH for the metal-NTA system. \circ = Cu(II); \bullet = Co(II).

M-NTA system

The overall mobilities of the metal spots in the presence of NTA at different pH values are represented in Fig. 2. It is evident that with all the metal ions two plateaux are obtained, the mobility of the second plateau lying in the negative region, showing the negatively charged nature of the complex. Hence only one NTA anion is assumed to combine with one bivalent metal ion to give a 1:1 M-NTA complex, which is in conformity with the findings of other workers [15-18]. The stability constants of complexes with NTA (K_{M-NTA}^M) were calculated as described for the M-ADP system and are given in Table I.

M-ADP-NTA system

This system was studied at pH 8.5 with a specific purpose. It was observed from the mobility curves for M-ADP and M-NTA binary systems that binary complexes $(M-ADP)^-$ and $(M-NTA)^-$ are formed up to pH 8.5. Hence it would be desirable to study the transformation of the $(M-ADP)^-$ complex into the $(M-ADP-NTA)^{4-}$ complex at pH 8.5 in order to avoid any side interactions.

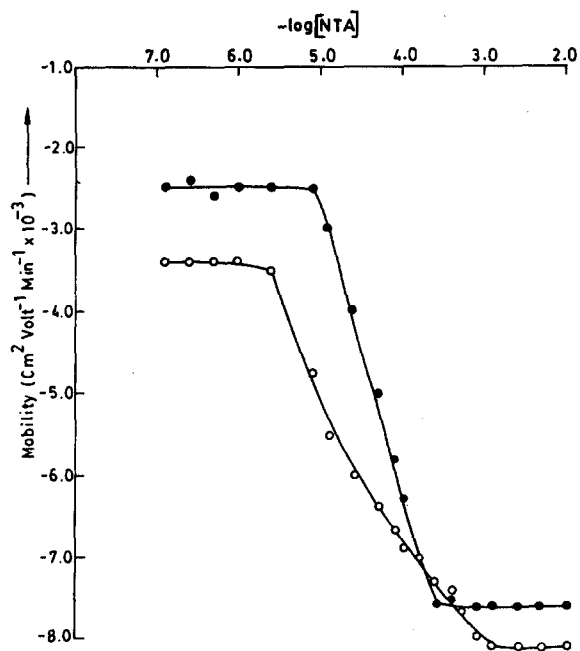
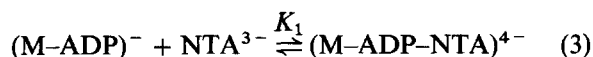


Fig. 3. Plots of mobility versus $-\log[NTA]$ for the metal-ADP-NTA system. \circ = Cu(II); \bullet = Co(II).

The plots of mobility against logarithm of concentration of added NTA are shown in Fig. 3 and exhibit two plateaux. The negative mobility in the region of the first plateau is obviously due to 1:1 M-ADP⁻ complexes (see Fig. 1 at pH 8.5). The mobility corresponding to the second plateau is more negative than that of the first plateau, indicating the formation of a more negatively charged complex. However, as the mobility for the last plateau does not agree with the mobility of a 1:1 M-NTA complex (Fig. 2), it is inferred that the species corresponding to the second plateau is due to the coordination of the NTA³⁻ anion to a 1:1 M-ADP⁻ moiety, resulting in the formation of a 1:1:1 (M-ADP-NTA)⁴⁻ mixed complex according to



In the present electrophoretic study, the transformation of a simple complex into a mixed complex takes place, the overall mobility being given by

$$U = U_0 f_{M-ADP} + U_1 f_{M-ADP-NTA} \quad (4)$$

where U_0 and U_1 are the mobilities and f_{M-ADP} and $f_{M-ADP-NTA}$ the mole fractions of M-ADP and M-ADP-NTA complexes respectively.

From Fig. 3, the total concentration of NTA at which the overall mobility is mean of the mobilities of the two plateaux was determined. For this the concentration of the NTA³⁻ anion at pH 8.5 was calculated. $K_{M-ADP-NTA}^M$ is obviously equal to $1/[L]$. For the calculation of the overall stability constants ($K_{M-ADP-NTA}^M$), the stability constant of the M-ADP complex was multiplied by $\log K_{M-ADP-NTA}^M$. All these values of stability constants are given in Table I.

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