

Short Communication

Electrophoretic determination of stability constants of Zn(II)- and Cd(II)-nitrilotriacetate-penicillamine mixed complexes

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ABSTRACT

A paper electrophoretic method is described for the study of equilibria in mixed ligand [nitrilotriacetate (NTA)-penicillamine] complex systems in solution. The stability constants of Zn(II)-NTA-penicillamine and Cd(II)-NTA-penicillamine complexes were found to be 5.36 and 5.18 (log *K* values), respectively, at $\mu = 0.1$ and 35°C.

INTRODUCTION

The properties and more important chemical reactions of naturally occurring penicillamine have already been subjected to wide investigations. The main biochemical aspects have been reviewed by Jocelyn [1]. The biological importance is attributed to the capabilities of the mercapto group to undergo various complex formation processes, primarily in **non-haem** iron proteins [2] and the blue copper proteins [3]. Sorensen [4] has demonstrated the anti-inflammatory activity of the copper-penicillamine complex. The complexes of Zn(II) and Cd(II) with penicillamine and other sulphur-containing amino acids are of great importance because of the interesting biological role of Zn(II) and the

toxic nature of Cd(II). The significance of investigations relating to these complexes is further enhanced by the recognition that penicillamine can be advantageously employed for the treatment of metal ion poisoning (Pb, Hg, Cd, Zn, etc.). A search of the literature [4-7] indicated that there is only one report on Zn(II) and Cd(II) binary complexes with penicillamine and no information on ternary (mixed complex) systems.

In view of this, attempts were made to establish the optimum conditions for metal (M)-penicillamine, M-nitrilotriacetate (NTA) and M-NTA-penicillamine complex formation. In addition, this paper describes an electrophoretic method for the determination of the stability constants of these complexes.

EXPERIMENTAL

Apparatus

A Systronic (Naroda, India) Model 604 elec-

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trophoresis 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

Zinc and cadmium perchlorate solutions were prepared as described elsewhere [6]. Metal spots were detected on the paper using dithizone in carbon tetrachloride (BDH, Poole, UK) for Zn(II) and 1-(2-pyridylazo)-2-naphthol (PAN) (Merck, Darmstadt, Germany) for Cd(II). A saturated aqueous solution (0.9 ml) of silver nitrate was diluted with acetone to 20 ml. Glucose, as a black spot, was detected by spraying with this solution and then with 2% ethanolic sodium hydroxide.

Background electrolyte

The background electrolytes used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M penicillamine. For the study of ternary system the background electrolytes used were 0.1 M perchloric acid, 0.01 M NTA and various amounts of 0.01 M penicillamine. The ternary system was maintained at pH 8.5 by the addition of sodium hydroxide.

Stock solutions of 9.0 M perchloric acid, 2.0 M sodium hydroxide and 0.5 M penicillamine were prepared from analytical-reagent grade chemicals (BDH). A 0.01 M NTA solution was prepared from the compound obtained from Merck.

Procedure

The hollow base plate in the instrument was made horizontal with a spirit level. A 150-ml volume of background electrolyte was placed in each tank of the electrophoretic apparatus. Paper strips (Whatman No. 1, 30 x 1 cm) in triplicate were then spotted with metal ion solutions and glucose in the centre with a micropipette and were subsequently placed on the base plate and sandwiched under the upper hollow

metallic plate with the ends of strips lying in the two sides of the tank solutions. A 200-V potential difference was then applied between the tank solutions and electrophoresis was carried out for 60 min. Subsequently the strips were removed and the spots were detected. The averages of triplicate strips were noted for calculations and the movement of the glucose spot was used as a correction factor. It was found that the variation in the movement was about 5%. The mobilities were calculated by dividing the distance by the potential gradient and are expressed in $\text{cm}^2 \text{V}^{-1} \text{min}^{-1}$.

RESULTS AND DISCUSSION

M(II)-penicillamine binary system

The plot of the ionophoretic mobility of a metal spot against pH is a curve with a number of plateaux, as shown in Fig. 1. The first corresponds to a region in which metal ions are uncomplexed. The second plateau in each instance with zero mobility indicates the formation of 1:1 neutral complexes. With a further increase in pH the mobility decreases, giving rise to a third plateau lying in negative region, indicating

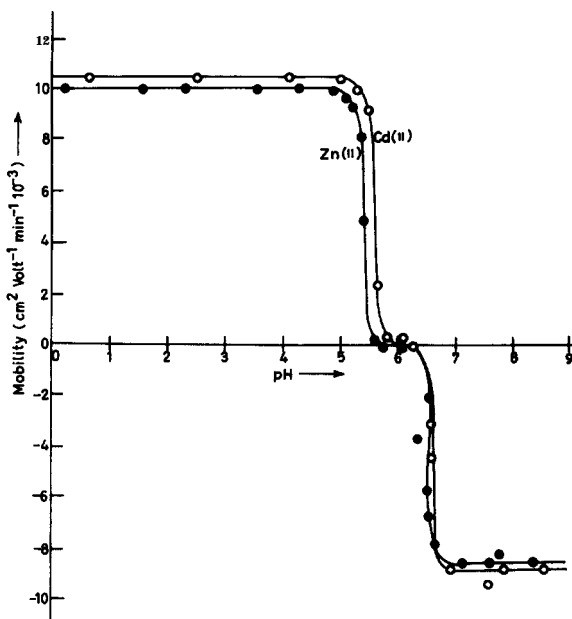
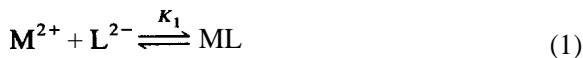


Fig. 1. Mobility curves for the M-penicillamine systems. ● = Zn(II)-penicillamine; ○ = Cd(II)-penicillamine.

an anionic nature of the metal complexes. The predominant liganding properties of **unprotonated** anionic species of penicillamine have also been reported ruling out any such property of a zwitterion [8]. In view of these observations, the complexation of the metal ions with the **penicillamine anion** [L^{2-}] can be represented as



The metal spot on the paper is thus a combination of uncomplexed metal ions, a 1:1 complex and a 1:2 complex. The overall mobility of these is given by

$$U = \frac{u_0 + u_1 K_1 [L^{2-}] + u_2 K_1 K_2 [L^{2-}]^2}{1 + K_1 [L^{2-}] + K_1 K_2 [L^{2-}]^2} \quad (3)$$

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

For the calculation of the first stability constant (K_1), the region between the first and second plateaux is pertinent. The overall mobility U will be equal to the arithmetic mean of the mobility of uncomplexed metal ion, u_0 , and that of first complex, u_1 , at a pH where $K_1 = 1/[L^{2-}]$. With the help of the dissociation constants of penicillamine ($k_1 = 10^{1.66}$, $k_2 = 10^{2.67}$ and $k_3 = 10^{9.49}$) and are presented in Table I. The complexation may be represented as

penicillamine, [L^{2-}], is calculated by

$$[L^{2-}] = \frac{[L_T]}{1 + [H]/k_1 + [H]^2/k_1 k_2 + [H]^3/k_1 k_2 k_3} \quad (4)$$

where $[L_T]$ = total concentration.

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

M(ZZ)-NTA binary system

Fig. 2 shows the overall mobilities of the metal spots in the presence of NTA at different pH. The mobility at the last plateau is negative, showing the anionic nature of both the **Zn(II)** and **Cd(II)** complexes. The stability constants (K_3) of these complexes were calculated using the same method as described for the **M-penicillamine** system (the dissociation constants for NTA are $k_1 = 10^{1.66}$, $k_2 = 10^{2.67}$ and $k_3 = 10^{9.49}$) and are presented in Table I. The complexation may be represented as



M-NTA-penicillamine ternary system

This system was studied at pH 8.5. It is observed from the mobility curves of the

TABLE I

STABILITY CONSTANTS OF BINARY AND TERNARY COMPLEXES OF Zn(II) AND Cd(II)

Ionic strength = 0.1; temperature = 35°C; NTA anion = $N(CH_2COO)_3^{3-}$; penicillamine anion = $(CH_3)_2C(S)CH(NH_2)COO^-$.

Values	Metal ion	Stability constant ^a			
		Log K_{1ML}^M	Log $K_{2ML_2}^M$	Log K_{3M-NTA}^M	Log $K_{4M-NTA-L}^{M-NTA}$
Calculated (this work)	Zn(II)	9.80	17.11	8.88	5.36
	Cd(II)	9.35	16.70	8.05	5.18
Literature values	Zn(II)	9.48 [9]	19.42 [9]	10.66 [9]	—
		9.42 [10]	19.44 [10]	10.00 [10]	—
		10.00 [11]	18.98	[11] —	—
	Cd(II)	9.51 [11]	18.51	[11] —	—
		10.89 [9]	—	9.78 [9]	—
		11.48 [11]	18.50 [11]	9.48 [11]	—

^a $K_{1ML}^M = [ML]/[M][L]$; $K_{2ML_2}^M = [ML_2]/[ML][L]$; $K_{3M-NTA}^M = [M-NTA]/[M][NTA]$; $K_{4M-NTA-L}^{M-NTA} = [M-NTA-L]/[M-NTA][L]$; M = metal cation; L = ligand (penicillamine); NTA = nitrilotriacetate.

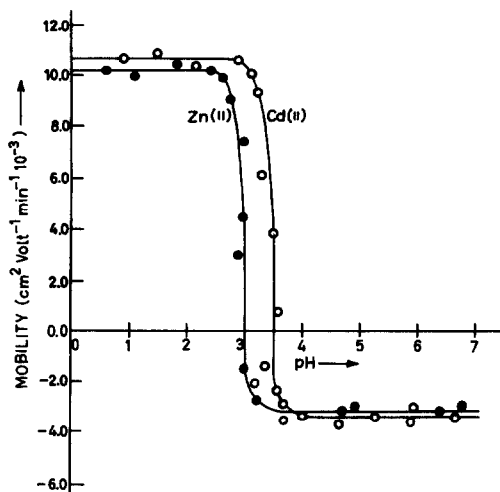


Fig. 2. Mobility curves for the M-NTA systems. ● = Zn(II)-NTA; ○ = Cd(II)-NTA.

M-penicillamine and M-NTA binary systems that binary complexes are formed at $\text{pH} < 8.5$. Therefore, it was considered necessary to study the transformation of the $[\text{M-NTA}]^-$ binary complex into the $[\text{M-NTA-penicillamine}]^{3-}$ mixed complex at $\text{pH} 8.5$ to avoid any side interaction. The plot of mobility against the logarithm of the concentration of added penicillamine gives a curve containing two plateaux (Fig. 3). It is inferred that the moiety in the last

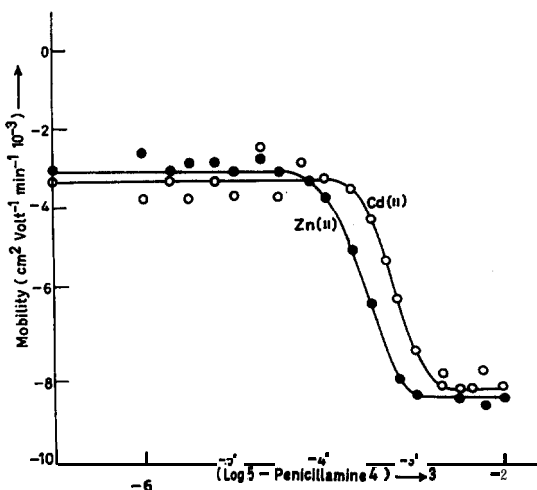
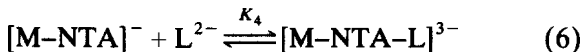


Fig. 3. Mobility curves for the M-NTA-penicillamine systems. ● = Zn(II)-NTA-penicillamine; ○ = Cd(II)-NTA-penicillamine.

plateau is due to coordination of penicillamine anion to the $[\text{M-NTA}]^-$ moiety (1:1), resulting in the formation of a 1:1:1 $[\text{M-NTA-penicillamine}]^{3-}$ mixed complex:



where L is penicillamine.

In this electrophoretic study, the transformation of a simple complex into a mixed complex takes place, hence the overall mobility (U) of this complex is given by

$$U = u_0 f_{[\text{M-NTA}]} + u_1 f_{[\text{M-NTA-L}]} \quad (7)$$

where u_0 , u_1 and $f_{[\text{M-NTA}]}$, $f_{[\text{M-NTA-L}]}$ are the mobilities and mole fractions of the $[\text{M-NTA}]^-$ and $[\text{M-NTA-L}]^{3-}$ complexes, respectively.

On adding values of the mole fractions, the above equation becomes

$$U = (u_0 + u_1 K_4 [\text{L}^{2-}]) / (1 + K_4 [\text{L}^{2-}]) \quad (8)$$

where u_0 and u_1 are the mobilities in the region of the two plateaux of the curve.

The concentration of penicillamine anion at $\text{pH} 8.5$ was calculated. K_4 is obviously equal to $1/[\text{L}^{2-}]$. All these values of K_4 (stability constants of mixed complexes) are given in Table I.

It can be concluded from these studies that penicillamine and NTA can be used to reduce the levels of Zn(II) and Cd(II) in biological systems.

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REFERENCES

- 1 C.P. Jocelyn, *Biochemistry of the SH Group*, Academic Press, London, New York, 1972.
- 2 R.G. Moore and P.J.R. Williams, *Coord. Chem. Rev.*, 18 (1976) 125.
- 3 A.J. Fee, *Struct. Bonding (Berlin)*, 23 (1975) 11-52.
- 4 R.J. Sorensen, *J. Med. Chem.*, 19 (1976) 135.
- 5 B.B. Tiwari, R.K.P. Singh, Kamaluddin and K.L. Yadava, *Trans. Soc. Adv. Electrochem. Sci. Technol.*, 25 (1990) 124.
- 6 B.B. Tiwari, R.K.P. Singh and K.L. Yadava, *J. Chromatogr.*, 542 (1991) 537.

- 7 B.B. Tiwari, R.K.P. Singh and K.L. Yadava, *Bull. Soc. Chim. Fr.*, 128 (1991) 141.
- 8 J.R. **Blackburn** and M.M. Jones, *J. Inorg. Nucl. Chem.*, 35 (1973) 1605.
- 9 A.E. **Martell** and R.M. Smith, *Critical Stability Constants, Amino Acids*, Vol. 1, Plenum Press, New York, London, 1977, p. 48.
- 10 D.D. Perrin, *Stability Constants of Metal Ion Complexes, Part B, Organic Ligands (IUPAC Chemical Series, No. 22)*, Pergamon Press, Oxford, 1979.
- 11 L.G. **Sillen** and A.E. **Martell**, *Stability Constants of Metal Ion Complexes (Special Supplement No. 17)*, Chemical Society, London, 1964.