Journal of Chromatography, 547 (1991) 554–559
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 243

Short Communication

Study of chelating tendency of sulphur-containing amino acids by electrophoresis

B. B. TIWARI*, R. K. P. SINGH, V. KUMAR and K. L. YADAVA
Electrochemical Laboratories, Department of Chemistry, University of Allahabad, Allahabad-211 002 (India)

(First received October 9th, 1990; revised manuscript received February 26th, 1991)

ABSTRACT

The stability constants of different complex species of some metal ions, *viz.*, Zn^{2+} and Cd^{2+} with methylcysteine, were determined electrophoretically at an ionic strength of 0.1 *M* perchloric acid and at 30°C. The stability constant of metal–nitrilotriacetate–methylcysteine complexes were found to be 3.72 and 3.54 (log K values) for Zn(II) and Cd(II) complexes, respectively.

INTRODUCTION

A significant development in the determination of stability constants of complexes was made by Jokl [1] in 1964. A theoretical treatment similar to that of Jokl was adopted by Biernat [2] for the study of stepwise complex formation [3]. We have previously described [4,5] a method for the study of mixed complexes. This work is an extension of that technique and reports observations on mixed systems, *viz.*, Zn^{2+}/Cd^{2+}–nitrilotriacetate–methylcysteine.

EXPERIMENTAL

Apparatus

A Systronics Model 604 electrophoresis system was been used. It has a built-in power supply (a.c.–d.c.) which is directly fed to paper electrophoretic tanks. In order to maintain the temperature constant, two hollow metallic plates coated with thin plastic paper on the outside were used for sandwiching paper strips, and thermostated water (35°C) was circulated through these plates.

pH measurements were made with an Elico Model L_{1-10} pH meter using a glass electrode.

0021-9673/91/$03.50

Chemicals

Zinc and cadmium perchlorate solutions were prepared from the nitrates (analytical-reagent grade) via the carbonates. The solutions were standardized and diluted to $5.0 \cdot 10^{-3}$ M.

Metal spots were detected with dithizone in carbon tetrachloride (for Zn^{2+}) and 1-(2-pyridylazo)-2-naphthol (PAN) (for Cd^{2+}). A saturated aqueous solution (0.9 ml) of silver nitrate was diluted with acetone to 20 ml. Glucose was detected by spraying with this solution and then with 2% ethanolic sodium hydroxide, when a black spot was formed.

Background electrolyte

The background electrolyte in the study of binary complexes was 0.1 *M* perchloric acid and $1.0 \cdot 10^{-2}$ *M* methylcysteine. For the study of the ternary system the background electrolyte was of 0.1 *M* perchloric acid, $1.0 \cdot 10^{-2}$ *M* nitrilotriacetate (NTA) and various amounts of 0.01 *M* methylcysteine. It was maintained at pH 8.5 by addition of sodium hydroxide for the study of mixed systems.

Stock solutions of 9.0 *M* perchloric acid, 2.0 *M* sodium hydroxide and 0.5 *M* methylcysteine were prepared from AnalaR chemicals (BDH, Poole, UK); 0.01 *M* nitrilotriacetic acid was prepared from the compound obtained from E. Merck (Darmstadt, Germany).

Procedure

The level of 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. The paper strips (Whatman No. 1, 30×1 cm^2) in triplicate were then spotted with metal ions 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 the strips lying in the two sides of the tank solutions. Then a 200 V potential difference was 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 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 movement by the potential gradient and expressed in cm^{2}V^{-1}.

RESULTS AND DISCUSSION

M(II)–methylcysteine binary system

The plot of the overall mobility of a metal spot against pH gives a curve with a number of plateaus, as shown in Fig. 1. The first, at the beginning, corresponds to a region in which metal ions are uncomplexed. A second plateau in each instance with positive mobility indicates the formation of 1:1 complex of a cationic nature. A further increase of pH results in a third plateau with zero mobility, which indicates the formation of an electrically neutral metal complex. The literature also assigns prominent liganding properties to unprotonated anionic species of methylcysteine, ruling out any such property to the zwitterion [6]. In view of the above observation, the

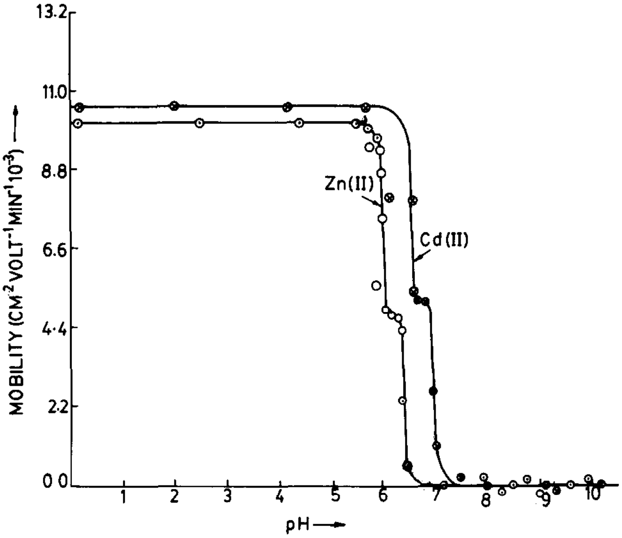

Fig. 1. Mobility curves for M–methylcysteine systems. ⊙, Zn(II)–methylcysteine; ⊗, Cd(II)–methylcysteine. Temperature, 35°C; ionic strength, 0.1.

complexation of a metal ion with the methylcysteine anion L^- may be represented by

$$M^{2+} + L^- \overset{K_1}{\rightleftharpoons} ML^+ \tag{1}$$

$$ML^+ + L^- \overset{K_2}{\rightleftharpoons} ML_2 \tag{2}$$

where M represents Zn or Cd and ML^+ and ML_2 are their complexes with methylcysteine.

The metal spot on the paper is thus a conglomeration of uncomplexed metal ions and 1:1 and 1:2 complexes. The overall mobility, U, is given by

$$U = \frac{u_0 + u_1K_1[L^-] + u_2K_1K_2[L^-]^2}{1 + K_1\,[L^-] + K_1K_2[L^-]^2} \tag{3}$$

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

For calculating 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 the 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 dissociation constants of methylcysteine ($k_1 = 10^{2.55}$, $k_2 = 10^{8.55}$) [7,8].

The concentration of liganding methylcysteine, L^-, is calculated with the equation

$$[L^-] = \frac{[L_T]}{1 + \frac{[H]}{k_2} + \frac{[H]^2}{k_1 k_2}} \tag{4}$$

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 plateaus of the mobility curve. These calculated values are given in Table I.

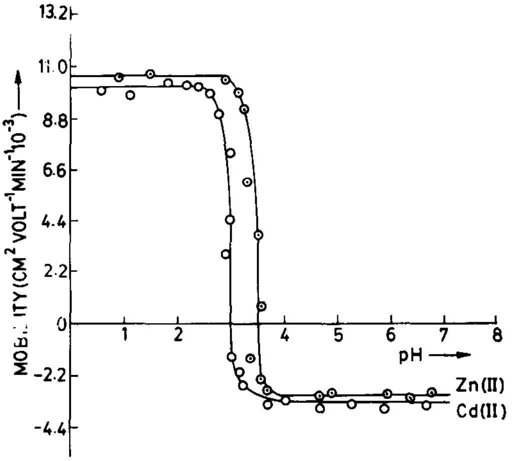

Fig. 2. Mobility curves for M–NTA systems. ○, Zn(II)–NTA; ⊙, Cd(II)–NTA. Temperature, 35°C; ionic strength, 0.1.

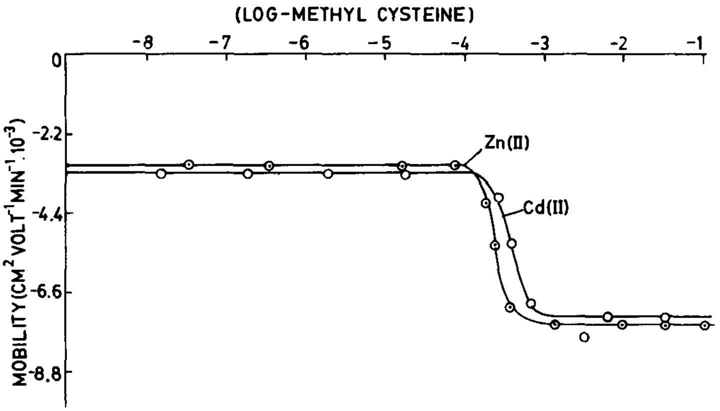

Fig. 3. Mobility curves for M–NTA–methylcysteine. ⊙, Zn(II)–NTA–methylcysteine; ○, Cd(II)–NTA–methylcysteine. Temperature, 35°C; ionic strength, 0.1.

TABLE I

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

Ionic strength = 0.1; temperature = 35°C; NTA anion = $N(CH_2COO)_3^{3-}$; methylcysteine anion = $CH_3SCH_2CH(NH_2)COO^-$.

Metal ion	Calculated values of stability constants[a]				Literature values of stability constants[a]			
	$\mathrm{Log}K^{\mathrm{M}}_{1_{\mathrm{ML}}}$	$\mathrm{Log}K^{\mathrm{M}}_{2_{\mathrm{ML}_2}}$	$\mathrm{Log}K^{\mathrm{M}}_{\mathrm{M-NTA}}$	$\mathrm{Log}K^{\mathrm{M-NTA}}_{\mathrm{M-NTA-L}}$	$\mathrm{Log}K^{\mathrm{M}}_{1_{\mathrm{ML}}}$	$\mathrm{Log}K^{\mathrm{M}}_{2_{\mathrm{ML}_2}}$	$\mathrm{Log}K^{\mathrm{M}}_{\mathrm{M-NTA}}$	$\mathrm{Log}K^{\mathrm{M-NTA}}_{\mathrm{M-NTA-L}}$
Zn(II)	4.55	4.15	10.66	3.72	4.46 [7] 4.46 [9]	4.06 [7] 4.06 [9]	10.66 [7] 10.00 [8]	–
Cd(II)	3.95	3.55	9.78	3.54	3.77 [7] 3.77 [9]	3.32 [7] 3.32 [9]	9.78 [7] 9.40 [8]	–

[a] $K^{\mathrm{M}}_{1_{\mathrm{ML}}} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]}$; $\mathrm{Log}K^{\mathrm{M}}_{2_{\mathrm{ML}_2}} = \frac{[\mathrm{ML}_2]}{[\mathrm{ML}][\mathrm{L}]}$; $K^{\mathrm{M}}_{\mathrm{M-NTA}} = \frac{[\mathrm{M-NTA}]}{[\mathrm{M}][\mathrm{NTA}]}$; $K^{\mathrm{M-NTA}}_{\mathrm{M-NTA-L}} = \frac{[\mathrm{M-NTA-L}]}{[\mathrm{M-NTA}][\mathrm{L}]}$.

Metal(II)–NTA binary system

The overall mobilities of the metal ion spots were plotted against the pH of the background electrolyte containing NTA (Fig. 2). As the mobility of the second plateau is in the negative range, the complex formed with a bivalent metal ion should have a 1:1 composition with a net negative charge. The stability constants of these complexes were calculated in the same manner as for complexes with other amino acids. These values are recorded in Table I.

Metal–NTA–methylcysteine system

The study of this system was done at pH 8.5 for a purpose. It is observed from the mobility curves for M–methylcysteine and M–NTA binary systems that binary complexes are formed at pH <8.5. Therefore, it would be preferable to study the transformation of the M–NTA complex into the M–NTA–methylcysteine complex at pH 8.5 in order to avoid any side interactions.

On the plot of mobility *versus* log (concentration of added amino acid) a curve is obtained, as shown in Fig. 3. The first constant value of the mobility obviously corresponds to the mobility of the M–NTA complex, whereas the second constant mobility corresponds to the mobility of a second complex. This new complex cannot be a 1:1 or 1:2 metal–amino acid complex as the mobilities of such complex species are different from that of the new complex. The interaction may be represented by

$$\mathrm{ML'} + \mathrm{L} \overset{K'}{\rightleftharpoons} \mathrm{ML'L} \tag{5}$$

Under these conditions, the overall mobility can be given by the expression

$$U = \frac{u_0 + u_1 K'[\mathrm{L}]}{1 + K'[\mathrm{L}]} \tag{6}$$

where u_0 and u_1 are the mobilities of M–NTA and mixed ML'L complexes, respectively. These mobilities pertain to the two-plateau region of the curve; using again the principle of average mobility, K' can be determined to be equal to $1/[\mathrm{L}^-]$. All these values of K' are given in Table I.

REFERENCES

1 V. Jokl, *J. Chromatogr.*, 14 (1964) 71.
2 J. Biernat, *Rocz. Chem.*, 38 (1964) 343.
3 R. K. P. Singh, J. K. Sirkar, J. R. Yadava, P. C. Yadava and K. L. Yadava, *Electrochim. Acta*, 26 (1981) 395.
4 P. C. Yadava, A. K. Ghose, K. L. Yadava and A. K. Dey, *J. Chromatogr.*, 119 (1976) 563.
5 J. R. Yadava, J. K. Sirkar and K. L. Yadava, *Electrochim. Acta*, 26 (1982) 391.
6 J. R. Blackburn and M. M. Jones, *J. Inorg. Nucl. Chem.*, 35 (1973) 1605.
7 A. E. Martell and R. M. Smith, *Critical Stability Constants, Vol. 1, Amino Acids*, Plenum Press, New York, London, 1977, p. 49.
8 D. D. Perrin, *Stability Constants of Metal Ion Complexes, Part B, Organic Ligands* (*IUPAC Chemical Series*, No. 22), Pergamon Press, Oxford, 1979.
9 L. G. Sillen and A. E. Martell, *Stability Constants of Metal Ion Complexes* (*Special Publication*, No. 17), Chemical Society London, 1964.