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Studies on Complexation in Solution with a Paper Electrophoretic Technique [The System Copper(II)/Cobalt(II)−Methionine−Penicillamine][†]

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Complexation reactions of methionine and penicillamine with copper(II) and cobalt(II) have been studied in the solution phase using a paper ionophoretic technique. This method is based on the movement of a spot of metal ion in an electric field with the complexants added in the background electrolyte at pH 8.5. The concentration of primary ligand methionine was kept constant, while that of the secondary ligand penicillamine was varied. A plot of log[penicillamine] versus mobility was used to obtain information on the mixed complexes and to calculate stability constants. The stability constants of the complexes copper(II)−methionine−penicillamine and cobalt(II)−methionine−penicillamine have been found to be 3.65 ± 0.03 and 3.05 ± 0.07 (logarithm stability constant values), respectively, at a temperature of 35 °C and ionic strength of 0.1 M.

Introduction

Mixed metal complexes play an important role in various biological systems, hence the formation, stability, and reactivity of these complexes have been an active field of research.[1] Due to growing interest in the use of sulfur-containing compounds in analytical as well as structural studies of metal complexes, sulfur-containing amino acids, methionine and penicillamine, are taken in the present course of study. The methionine and penicillamine have several significant applications in biological systems.[2−10]

Copper and cobalt are classified as essential metals without which life cannot be sustained. After iron and zinc, copper is the third most abundant transition element in the human body, and it plays an important series of biological functions as it is involved in electron transport; in oxidase systems like amine oxidase, ascorbate oxidase, galactose oxidase, and lysyl oxidase; in oxygenases like tyrosinases; in the transport of oxygen (hemocyanines); in the dismutation of the superoxide radical (superoxide dismutase); and even in iron metabolism (ceruloplasmin). Two hereditary diseases—Wilson’s disease and Menke’s syndrome concerning copper metabolism—are well-known.[11] Cobalt is an essential metal in many coenzymes and enzymes. Dysfunction in enzymatic processes, e.g., megalocytie anemia, may therefore result from cobalt deficiency. The copper(II) and cobalt(II) metal ions have significant biomedical applications but are toxic at higher concentration.[12−21]

Kiso[22] has done a comprehensive study on paper electrophoretic migration of metal complexes. The present electrophoretic technique is almost free from a number of defects such as temperature during electrophoresis, capillary flow on paper, electroosmosis, and adsorption. The technique is very convenient in use. It gives results in fair agreement with accepted literature values. Publications[23−26] from our laboratory described a new method for the study of mixed complexes. A search of the literature indicated coordination of amino acids with metal ions,[27−31] but there are few reports available on binary and no reports available on mixed complexes of copper(II) and cobalt(II) with methionine and penicillamine. In view of this, an attempt was made to establish the optimum conditions for metal(II)−methionine and metal(II)−methionine−penicillamine complex formation. In addition, the present paper describes a paper electrophoretic method for the determination of the stability constants of these complexes.

Experimental Section

Instruments. Systronics (Naroda, India) paper electrophoresis equipment horizontal−cum vertical type, model 604, has been used. The apparatus consisted of a PVC molded double tank vessel. In our laboratory, a significant change to the instrument has been made. Two hollow rectangular plates covered with thin polythene sheets have been used through which thermostatted water is run for controlling the temperature. The tanks were closed with a transparent PVC molded lid. The whole assembly is tight, which prevents moisture changes, which may upset the equilibria in a paper strip. This assembly design thus keeps to a minimum the disturbing effects of evaporation from the unwanted liquid flow in the paper. Each electrolyte tank contains a separate electrode chamber. The auxiliary unit is specially designed to operate either in voltage mode or in current mode.

An Elico (Hyderabad, India) model $L_{1\text{-}10}$ having glass and calomel electrodes assembly and working on 220 V/50 Hz established a.c. mains was employed for pH measurements.

Chemicals. Solutions of copper(II) and cobalt(II) metal perchlorate were prepared by preliminary precipitation of metal carbonates from 0.1 M solution of sodium carbonate (chemically pure grade, BDH, Poole, UK), which were washed with boiling water and treated with calculated amounts of 1 % analytical reagent grade perchloric acid. These were boiled on a water bath and filtered. The metal contents of the filtrates were determined, and the final concentration was kept at $5.0 \cdot 10^{-3}$ M.

A 0.1 % solution of 1-(2-pyridylazo)-2-naphthol (PAN) (E. Merck, Darmstadt, Germany) in ethanol was used for detecting the metal ions. A 0.005 M glucose (BDH, AnalaR) solution was prepared in water and used as an electro-osmotic indicator for the correction due to electro-osmosis. A saturated aqueous

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solution (0.9 mL) of silver nitrate was diluted with acetone to 20 mL. Glucose was detected by spraying with this silver nitrate solution and then with 2 % ethanolic sodium hydroxide, when a black spot was formed.

Background Electrolytes. Stock solutions of 5.0 M perchloric acid (SDS, AnalaR grade), 2.0 M sodium hydroxide (AnalaR grade), 0.5 M methionine, and 0.5 M penicillamine (BDH) were prepared. Each solution was standardized using the appropriate method. The background electrolytes (BGEs) used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M methionine. For the study of the mixed system, the BGEs used consisted of 0.1 M perchloric acid, 0.01 M methionine, and various amounts of 0.01 M penicillamine. The mixed system was maintained at pH 8.5 by the addition of sodium hydroxide.

Procedure. Binary Complexes. Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. For recording the observation of particular metal ions, two paper strips were spotted with the metal ion solution along with an additional two spotted with glucose using a 1.0 μL pipet and then mounted on the insulated plate. Each of the two electrolyte vessels was filled with 150 mL of background electrolyte containing 0.1 M perchloric acid and 0.01 M methionine. The paper becomes moistened with the background electrolyte solutions due to diffusion. The second insulated plate was placed on paper strips, and then thermostatted 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 min to ensure wetting of the strips. Subsequently, a direct 220 V potential was applied between the electrodes. Electrophoresis was carried out for 1 h, after which the paper strips were removed from the tanks with a glass rod and dried. The metal ion and glucose spots were detected by specific reagents. The leading and tailing edges were measured from a marked center point, and the mean was taken. The distance moved by the glucose was subtracted (in the case of migration toward the anode) to obtain the correct path length. Migration toward the anode and cathode was designated by negative and positive signs, respectively. The potential gradient through the strips was found to be 7.5 $\mathrm{V \cdot cm^{-1}}$. Mobility was calculated by dividing the movement by the potential gradient and time. The observation was repeated for different pH values of the background electrolyte. A plot of mobility against pH is shown in Figure 1.

The protonation constants of pure methionine were determined by using the same paper electrophoretic technique. The two paper strips were spotted with pure methionine along with an additional two spotted with glucose using 0.1 M perchloric acid only as background electrolyte. The electrophoresis was carried out for 1 h as for the metal ions. The electrophoretic mobility was recorded. The mode of dissociation of pure methionine is shown in Figure 2.

Mixed Complexes. Paper strips in duplicate were marked with metal ions along with an additional two marked with glucose. After soaking the paper strips with the BGE, electrophoresis was carried out for 1 h at 220 V potential differences as in the case of binary complexes. For subsequent observations, penicillamine solution, maintained at pH 8.5 with NaOH, was recorded. The mobility of metal ions were recorded versus log[penicillamine] and is shown in Figure 3.

Results and Discussion

Metal(II)−Methionine Binary System. The ionophoretic mobility of the metal spot against pH gives a curve with a number of plateaus as is shown in Figure 1. A constant speed over a range of pH is possible only when a particular complex species is overwhelmingly formed. Thus, every plateau is indicative of formation of a certain complex species. The first one corresponds to a region in which metal ions are uncomplexed. In this region of low pH, the concentration of the [$(CH_3)_2C(SCH_3)CH(NH_3^+)COOH$] species of methionine is at a maximum, and this species is noncomplexing. Beyond this range, metal ion spots have progressively decreasing mobility, and complexation of metal ions should be taking place with anionic species of methionine whose concentration increases progressively with an increase of pH. Figure 1 shows three plateaus in both Cu(II) and Co(II), hence both Cu(II) and Co(II) form two complexes with the methionine anion. The chemical literature also assigns a prominent chelating property to the zwitterions.[32] It is therefore assumed that the anionic species [$(CH_3)_2C(SCH_3)CH(NH_2)COO^-$] of methionine has complexed with the metal ions to form different complexes. Figure 1 discloses that Cu(II) and Co(II) ions form their first complex movement toward the negative electrode. Hence, one [$(CH_3)_2C(SCH_3)CH(NH_2)COO^-$] must have combined with Cu(II) and Co(II) ions to give [$Cu\{(CH_3)_2C(SCH_3)CH(NH_2)COO\}]^+$ and [$Co\{(CH_3)_2C(SCH_3)CH(NH_2)COO\}]^+$ complex cations, respectively. With a further increase of pH, mobility in both the metal ions decreases giving rise to a third plateau with zero mobility that indicates its neutral nature. The third plateau in each case is due to a (1:2) metal−ligand

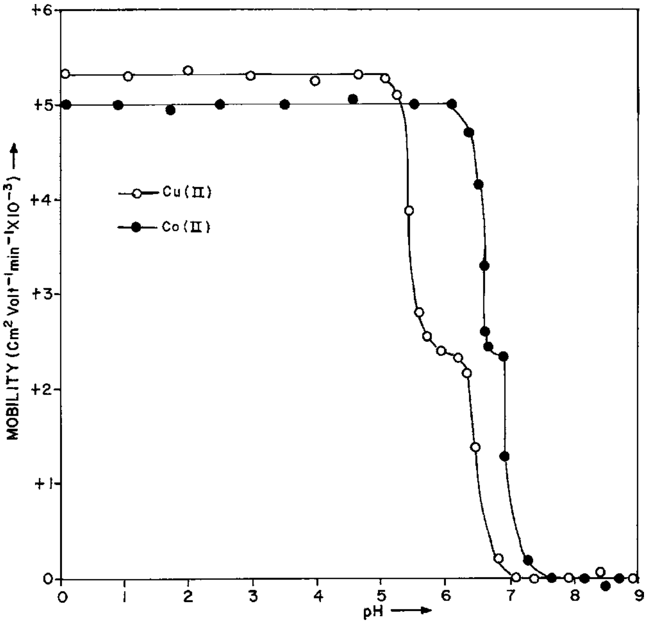

Figure 1. Mobility curves for the metal(II)−methionine systems. ○, Cu(II)−methionine; ●, Co(II)−methionine. Background electrolytes: 0.1 M perchloric acid and 0.01 M methionine. The paper strips were spotted with 0.1 μL of sample solution and glucose (for making osmotic correction).

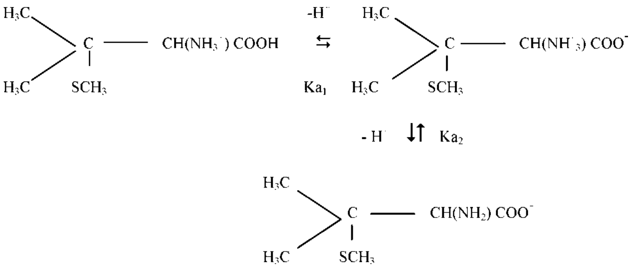

Figure 2. Mode of dissociation of pure methionine. Ionic strength: 0.01 M. Temperature: 35 °C.

complex. Hence, two [$(CH_3)_2C(SCH_3)CH(NH_2)COO^-$] anions must have combined with copper(II) and cobalt(II) to give the [$Cu\{(CH_3)_2C(SCH_3)CH(NH_2)COO\}_2$] and [$Co\{(CH_3)_2C(SCH_3)CH(NH_2)COO\}_2$] complexes, respectively.

A further increase of pH has no effect on the mobility of metal ions, which indicates no further interaction between the metal ions and ligands. It is significant that these studies give clear evidence of the complexation of the anionic species of these amino acids with metal ions forming two varieties of binary complexes of 1:1 and 1:2 in composition. In general, the complexation of metal ions with methionine anion may be represented as

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

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

where M^{2+} is the Cu^{2+} and Co^{2+} ions; [L^-] is the methionine anion; and K_1 and K_2 are the first and second stability constants, respectively.

The metal spot on the paper is thus a conglomeration of uncomplexed metal ions, 1:1 complex, and 1:2 complex. For the spot moving under the influence of an electric field, the overall mobility "U" is given by eq 3

$$U = \frac{u_0 + u_1K_1[L^-] + u_2 + K_1K_2[L^-]^2}{1 + K_1[L^-] + K_1K_2[L^-]^2} \tag{3}$$

where u_0, u_1, and u_2 are the mobilities of the uncomplexed metal ions, 1:1, and 1:2 metal complexes, respectively. The acidity constants of pure methionine ($Ka_1 = 2.25$; $Ka_2 = 9.00$)[33] were determined by the same paper ionophoretic technique. The mode of dissociation of pure methionine is shown in Figure 2.

Using the acidity constants of methionine, the concentration of the pure methionine anion [L^-] is determined for the pH value(s) of interest from which K_1 can be calculated. The concentration of the complexing methionine anion [L^-] is calculated with the help of the equation

$$[L^-] = \frac{[L_T]}{1 + [H]/Ka_2 + [H]^2/Ka_1 \cdot Ka_2} \tag{4}$$

where [L_T] is the total concentration of the methionine ligand (0.01 M) and Ka_1 and Ka_2 are the first and second acidity constants of pure methionine, respectively.

For calculating the first stability constant, K_1, the region between the first and second plateau is pertinent. The overall mobility will be equal to the arithmetic mean of the mobility of uncomplexed metal ion, u_0, and that of first complexes, u_1, at a pH value where $K_1 = 1/[(CH_3)_2C(SCH_3)CH(NH_2)COO^-]$.

The second stability constant, K_2, of the 1:2 complex can be calculated by taking into consideration the region between the second and third plateau of the mobility curve. The calculated values of K_1 and K_2 are given in Table 1.

It is clear from Table 1 that the stability constant values of the Cu(II)−methionine complex are greater than the Co(II)−methionine complex. Therefore, it is to be thought that the Cu(II)−methionine complex is more stable in comparison to the Co(II)−methionine complex.

The acidity constants of pure penicillamine ($Ka_1 = 1.90$; $Ka_2 = 7.85$; $Ka_3 = 10.55$) were determined by the paper electrophoretic technique. The stability constants of Cu(II)−penicillamine and Co(II)−penicillamine binary complexes were also determined under similar experimental conditions, as the metal(II)−methionine binary systems. The calculated values of K_1 and K_2 are given in Table 2.

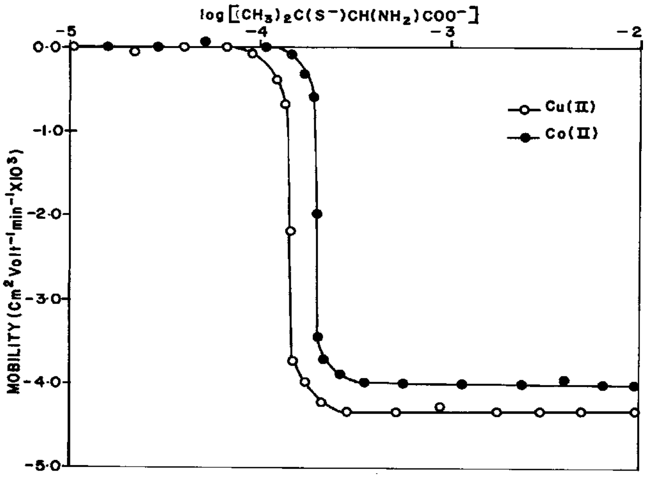

Figure 3. Mobility curves for the metal(II)−methionine−penicillamine systems. ○, Cu(II)−methionine−penicillamine; ●, Co(II)−methionine−penicillamine. Background electrolytes: 0.1 M perchloric acid and 0.01 M methionine and various amounts of 0.01 M penicillamine. pH: 8.5 (maintained by addition of sodium hydroxide). The paper strips were spotted with 0.1 μL of sample solution and glucose (for making osmotic correction).

It is clear from Tables 1 and 2 that the stability constant values of the Cu(II)/Co(II)−penicillamine binary complexes are approximately double those of the Cu(II)/Co(II)−methionine binary complexes. Therefore, Cu(II)/Co(II)−penicillamine binary complexes are considered to be more stable than Cu(II)/Co(II)−methionine binary complexes. This may be due to coordination of the double negatively charged anionic ligand of penicillamine [L^{2-}] with metal cations to form more stronger bonds than the single negatively charged anionic ligand of methionine [L^-].

Metal(II)−Methionine−Penicillamine Mixed System. Mixed complexes are formed due to attachment of two different ligating species to a central metal ion. As follows from the mobility curve of the metal(II)−methionine complexes that are formed at pH < 8.5. It was therefore considered necessary to study the transformation of metal(II)−methionine binary complexes into metal(II)−methionine−penicillamine mixed complexes at pH 8.5 to avoid any side interaction.

The plot of mobility against log of concentration of added penicillamine gives a curve (Figure 3) containing two plateaus, one in the beginning and the other at the end. The mobility in the range of the first plateau corresponds with the mobility of the metal(II)−methionine complex. Further, the mobility in the last plateau does not coincide with the mobility of 1:1 or 1:2 metal(II)−methionine complexes. It is thought that the mobility in the last plateau is due to coordination of the penicillamine anion to the 1:1 metal(II)−methionine complex resulting in the formation of a 1:1:1 metal(II)−methionine−penicillamine mixed complex as

$$[ML]^+ + [L']^{2-} \overset{K_3}{\rightleftarrows} [MLL']^- \tag{5}$$

where L′ is the penicillamine anion and K_3 is the stability constant of the mixed complex.

There is no third plateau, and no further interaction with the penicillamine anion is possible. 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

$$U = u_0f_{[M-L]} + u_1f_{[M-L-L']} \tag{6}$$

where u_0, u_1 and $f_{[M-L]}$, $f_{[M-L-L']}$ are the mobilities and mole fractions of the [metal(II)−methionine] and [metal(II)−methio-

Table 1. Stability Constants of Binary Complexes of Copper(II) and Cobalt(II) with Methionine and Mixed Complexes of Copper(II) and Cobalt(II) with Methionine and Penicillamine[a]

metal ions	complexes	stability constants	log stability constant values[b]
Cu^{2+}	$[Cu\{(H_3C)_2C(SCH_3)CH(NH_2)COO\}]^+$ (1:1, binary)	K_1	6.40 ± 0.01 [7.87][34]
	$[Cu\{(H_3C)_2C(SCH_3)CH(NH_2)COO\}_2]$ (1:2, binary)	K_2	4.50 ± 0.03 [6.85 ± 0.03][34]
	$[Cu\{(H_3C)_2C(SCH_3)CH(NH_2)COO\}\{(H_3C)_2C(S)CH(NH_2)COO\}]^-$ (1:1:1, mixed)	K_3	3.65 ± 0.03
Co^{2+}	$[Co\{(H_3C)_2C(SCH_3)CH(NH_2)COO\}]^+$ (1:1, binary)	K_1	4.40 ± 0.03 [4.12][35]
	$[Co\{(H_3C)_2C(SCH_3)CH(NH_2)COO\}_2]$ (1:2, binary)	K_2	4.20 ± 0.05 [3.44][35]
	$[Co\{(H_3C)_2C(SCH_3)CH(NH_2)COO\}\{(H_3C)_2C(S)CH(NH_2)COO\}]^-$ (1:1:1, mixed)	K_3	3.05 ± 0.07

[a] Ionic strength = 0.1 M; temperature = 35 °C; $[(H_3C)_2C(SCH_3)CH(NH_2)COO^-]$ = methionine anion (primary ligand); $[(H_3C)_2C(S^-)CH(NH_2)COO^-]$ = penicillamine anion (secondary ligand). [b] Literature values are given in brackets.

Table 2. Stability Constants of Binary Complexes of Copper(II) and Cobalt(II) with Penicillamine[a]

metal ions	complexes	stability constants	log stability constant values[b]
Cu^{2+}	$[Cu\{(CH_3)_2C(S)CH(NH_2)COO\}]$ (1:1, binary)	K_1	12.61 [16.50][35] [9.50][36]
	$[Cu\{(CH_3)_2C(S)CH(NH_2)COO\}_2]^{2-}$ (1:2, binary)	K_2	9.70 [5.20][35] [16.90][36]
Co^{2+}	$[Co\{(CH_3)_2C(S)CH(NH_2)COO\}]$ (1:1, binary)	K_1	8.90 [9.20][36]
	$[Co\{(CH_3)_2C(S)CH(NH_2)COO\}_2]^{2-}$ (1:2, binary)	K_2	6.17 [18.18][36]

[a] Ionic strength = 0.1 M; temperature = 35 °C; penicillamine anion = $[(CH_3)_2C(S^-)CH(NH_2)COO^-]$. [b] Literature values are given in brackets.

nine−penicillamine] complexes, respectively. On adding the values of mole fractions, eq 6 becomes

$$U = \frac{u_0 + u_1K_3[(CH_3)_2C(S^-)CH(NH_2)COO^-]}{1 + K_3[(CH_3)_2C(S^-)CH(NH_2)COO^-]} \qquad (7)$$

The concentration of the penicillamine anion is determined from Figure 3 at the mean of overall mobility of the first and second plateau of the mobility curves. At pH 8.5, the penicillamine ligand has been completely disassociated and is present as the double negatively charged anionic species [L^{2-}]. The mixed stability constant (K_3) is calculated with the help of eq 8.

$$K_3 = \frac{[(CH_3)_2C(SCH_3)CH(NH_2)COO^-]^2}{[(CH_3)_2C(S^-)CH(NH_2)COO^-]^2} \qquad (8)$$

The calculated values of K_3 are given in Table 1. It is observed from Tables 1 and 2 that the order of stability constants, viz., copper(II) > cobalt(II), is same for metal(II)−methionine, metal(II)−penicillamine binary, and metal(II)−methionine−penicillamine ternary complexes, but the corresponding stability constant values are lower in the mixed complexes. Therefore, the mixed complexes are considered to be less stable than binary complexes of metal cations with methioine and penicillamine. It is further inferred that the coordinating tendency of a ligand decreases with higher state of aggregation.

It is also observed from Tables 1 and 2 that the stability constant values are approximately similar to literature values. The slight deviation in the values obtained from different sources is mainly due to the difference in temperature, ionic strength, and experimental conditions used by different workers. No comparison can be made for the stability constant values of mixed ligand complexes because they are reported for the first time.

The stability constants of metal complexes can be very easily calculated by this technique. The present technique is limited to charged species, and the precision of the method is not as high as other physicochemical methods. However, uncertainty in the results is ± 5 %. It is not felt that it can replace the most reliable methods, although it is a new approach worth developing.

Concluding Remarks

The simple electrophoretic technique presented has thus proved helpful in deciding whether a mixed complex system is formed or not, and if it is formed its stability constants can be calculated. It can be concluded from these studies that sulfur-containing amino acids methionine and penicillamine may be used to reduce the level of copper(II) and cobalt(II) in biological systems. The stability constants of metal complexes can be very easily calculated by this technique, so the present electrophoretic technique has significant advantages over other methods reported in the chemical literature for the determination of stability constants of metal complexes.

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