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Studies on Biologically Significant Copper (II) / Manganese (II) / Uranyl (II) – Isoleucine Binary Complexes

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Abstract: The stability constants of Cu^{2+}, Mn^{2+} and UO_2^{2+} complexes with isoleucine were determined by paper electrophoretic technique. Present method is based upon the migration of a spot of the metal ions on a paper strip at different pH's of background electrolyte. A graph of pH against mobility gives information about the binary complexes and permit to calculate their stability constants. The first and second stability constants of [Cu(II) – isoleucine], [Mn(II) – isoleucine] and [UO_2(II) – isoleucine] complexes were found to be (8.41 ± 0.07; 6.43 ± 0.03), (3.87 ± 0.11; 2.61 ± 0.06) and (7.91 ± 0.13; 5.73 ± 0.04) for Cu(II), Mn(II) and UO_2(II) complexes, respectively at ionic strength 0.1 Mol/L and a temperature of 35 ^{0}C
Keywords: Paper ionophoresis, overall mobility, copper(II), manganese(II) and uranyl(II), stability constants, isoleucine.

Resumen. Las constantes de estabilidad de complejos de Cu^{2+}, Mn^{2+} and UO_2^{2+} con isoleucina fueron determinadas por la técnica de electroforesis en papel. El método se basa en la migración de la mancha de los iones metálicos en papel a diferentes pH en cierto electrolito. La gráfica del pH contra movilidad proporciona información sobre los compuestos binarios y permite el cálculo de las constantes de estabilidad. Las primeras y segundas constantes de estabilidad de los complejos [Cu(II) – isoleucina], [Mn(II) – isoleucina] y [UO_2(II) – isoleucina] fueron (8.41 ± 0.07; 6.43 ± 0.03), (3.87 ± 0.11; 2.61 ± 0.06) y (7.91 ± 0.13; 5.73 ± 0.04) para los complejos Cu(II), Mn(II) y UO_2(II), respectivamente, a fuerzas iónicas de 0.1 Mol/L y a 35 ^{0}C
Keywords: ionoforesis en papel, movilidad, cobre(II), manganeso (II), uranilo(II), constantes de estabilidad, isoleucina.

Introduction

Out of all the technique adopted for the study of metal ligand equilibria, we have opted for paper electrophoresis. Not much work, however is on record on the application of paper electrophoresis for examining complexation reactions. A theoretical treatment was adopted by Biernet [1] for the study of stepwise complex formation. Isoleucine is an essential, neutral, genetically coded amino acid. It is not synthesized in animals, hence it can be ingested. In plants and microorganism isoleucine biosynthesis include: acetolactate, acetohydroxy acid, isomeroreductase, dihydroxyacid dehydratase and valine aminotransferase. Isoleucine has several significant role in biological systems [2-9].

Metal ions play an important role in biological systems. Copper and manganese are essential metal while uranium is classified as toxic metal. Manganese deficiency in human being caused skeletal deformities due to impaired bone growth and reduced reproductive functions while excess causes serious and irreversible damage to the central nervours system and brain. Copper deficiency in human beings causes anemia while excess causes Jaundice and Wilson's diseases [10]. The studies in complexation reactions of bivalent copper, manganese and uranyl is of interest because of their nutrient properties and toxicity [11-25]. The electrophoretic technique usually has a number of drawbacks, temperature during electrophoresis, capillary flow on the paper, electroosmosis and adsorption affect the mobility of charged moieties [26]. The technique described here is almost free from these destroying factors.

Communications [27-30] from our laboratory described a new method for the study of binary and ternary complexes. The main objective of the work is to study the biologically significant isoleucine binary complexes with divalent essential metal cations and toxic metal oxocation. Divalent uranyl oxocation is chosen for the study alongwith copper(ii), manganese(ii) essential metal ions because of its toxic nature and medical applications. A search of the literature indicated that few reports are available on Cu(II) binary complexes with isoleucine and no previous report is available on Mn(II) and UO_2(II) complexes with isoleucine. Hence, attempts were made to establish the optimum conditions for metal(II) – isolecine binary complex formation. In addition, the present work describes an electrophoretic method for the determination of the stability constants of copper(II) / manganese(II) / uranyl(II) – isoleucine binary complexes.

Experimental

Apparatus

A Systronic (Naroda, India) Model 604, electrophoresis system was used. The apparatus consisted of a PVC molded double tanks vessel. In our laboratory significant change in the instrument has been made. Two hollow rectangular plates covered with thin polyethylene sheets have been used through which thermostated water is run for controlling the temperature. The tanks were closed with a transparent PVC molded lid. The whole assembly is tight which prevent moisture changes, which may upset the equilibria in a paper strip. The assembly design thus keeps to a minimum the disturbing effect of evaporation from the unwanted liquid flow in the paper. Each electrolyte tank contains a separate electrode chamber in which the anode and cathode are placed, respectively.

Elico (Hyderabad, India) Model L_{1-10} having a glass and calomel electrode assembly and working on 220 V/50 Hz established a.c. mains, were employed for the pH measurements. Electrophoresis cell showing sandwiched paper strips is shown in Figure 1.

Chemicals

Solutions of copper(II), manganese(II), and uranyl(II) metal perchlorate were prepared by preliminary precipitation of the metal carbonate from a 0.1 M solution of sodium carbonate (analytical reagent grade; BDH, Poole, UK). The precipitate was thoroughly washed with boiling water and treated with a suitable amount of 1% perchloric acid. The resulting mixture was heated to boiling on a water bath and then filtered. The solutions were standardized and diluted to 5.0 x 10^{-3}M with doubly distilled water.

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

The background electrolyte used in the study of complexes was 0.1 M perchloric acid with 0.01 M isoleucine. Stock solution of 5.0 M perchloric acid was prepared by its 70% solution (SDS, AnalaR grade), 0.5 M isoleucine (BDH, Poole, UK) and 2.0 M sodium hydroxide (Analytical – Reagent grade) solutions were prepared. Each solution was standardized using appropriate methods.

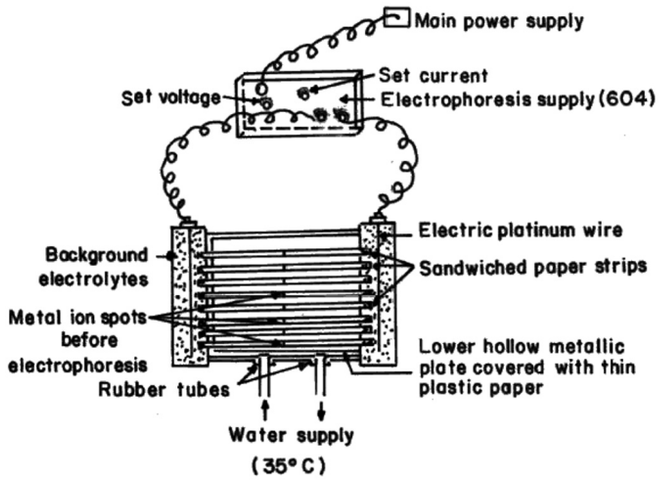

Fig. 1. Electrophoresis cell showing sandwiched paper strips.

Procedure

Binary complexes

Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. For recording the observation of a particular metal ion, two paper strips were spotted with the metal ion solution along with additional two spotted with glucose using 1.0 µL pipette and then mounted on the insulated plate. Each of the two electrolyte vessels were filled with 150 mL of background electrolyte containing 0.1 M perchloric acid and 0.01 M isoleucine. The paper becomes moistened with the background electrolyte solutions due to diffusion. The second insulated plate was placed on paper strips and then thermostated water (35ºC) was circulated into the plates to keep the temperature constant. The lid was then placed on the instrument to make it air tight. It was left for 10 minutes to insure wetting of strips. Subsequently a direct 220 V potential was applied between the electrodes. Electrophoresis was carried out for 60 minutes after which the strips were removed from the tank and dried. The metal ion and glucose spots were detected by specific reagents. Paper strips showing metal ion/complex ion and glucose spots after electrophoresis are shown in Figure 2. The leading and tailing edge were measured from marked center point and the mean taken. The distance moved by glucose spot was subtracted (in case of migration toward anode) to obtain correct path length. Migration towards anode and cathode were designated by negative and positive signs respectively.

Electrophoretic observation of metal ions was recorded at various pH values of the background electrolyte obtained by adding NaOH solution, the ionic strength being maintained at 0.1 M, the observed mobility of migrant was calculated by using the formula:

$$U = \frac{d}{x \times t}$$

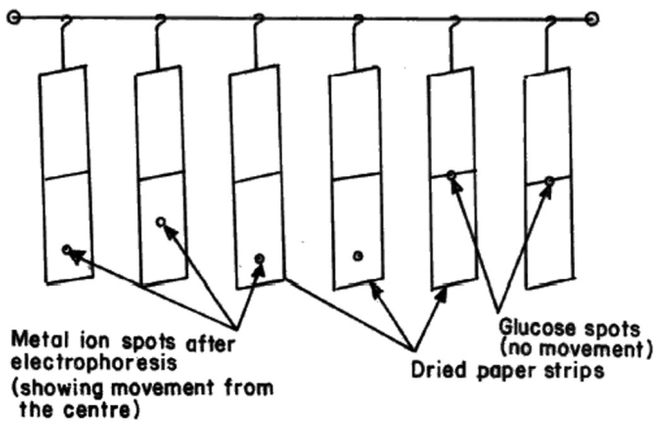

Fig. 2. Paper strips showing position of metal ion spots after electrophoresis.

after applying the correction factor the observed mobility is given as:

$$U = \frac{d \pm d_G}{x \times t}$$

where U = mobility of metal ion/complex ion; d = mean of duplicate distance travelled by metal ion / complex ion; d_G = mean of duplicate distance travelled by glucose spot; x = field strength; t = time for electrophoresis.

The protonation constants of pure isoleucine were determined by the same paper electrophoretic technique. The two paper strips were spotted with pure isoleucine along with two other spotted glucose using 0.1 M perchloric acid only as a background electrolyte. The electrophoresis was carried out for 60 minutes as for metal ions. The electrophoretic speed was calculated.

The speeds of the metal ion/complexion are reported with pH values. The individual speeds of the duplicate spots were found to be fairly equal. A plot of mobility against pH is shown in Figure 3.

Results

The plot of overall electrophoretic mobility of the metal spot against pH is shown in the Figure 3. The first plateau in the beginning corresponds to a region in which metal ions are uncomplexed. It is obvious that protonated ionic species of the isoleucine, which exist in low pH ranges are non-complexing [CH_3 CH_2 CH (CH_3) CH (NH_3^+) COOH]. Figure 3 disclose that Cu^{2+}, Mn^{2+} and UO_2^{2+} ions form their first complex movements towards negative electrode. Hence, one isoleucine anionic species [CH_3 CH_2 CH (CH_3) CH (NH_2) COO^-] must have combined with Cu^{2+}, Mn^{2+} and UO_2^{2+} to gives 1:1, [Cu {CH_3 CH_2 CH (CH_3) CH (NH_2) COO}]$^+$, [Mn {CH_3 CH_2 CH (CH_3) CH (NH_2) COO}]$^+$ and [UO_2 {CH_3 CH_2 CH (CH3) CH (NH_2) COO}]$^+$ complex cations, respectively. The third plateau in each case in the zero region showing neutral nature of metal – ligand complex. Hence, two anionic species of isoleucine [CH_3 CH_2 CH(CH_3) CH(NH_2) COO^-] must have combined with metal ions to gives 1:2, [Cu {CH_3 CH_2 CH(CH_3) CH(NH_2) COO}$_2$], [Mn {CH_3 CH_2 CH(CH_3) CH (NH_2) COO}$_2$] and [UO_2 {CH_3 CH_2 CH (CH_3) CH (NH_2) COO}$_2$] complexes, respectively. Further increase of pH has no effect on the mobility of metal ions, which indicates no further interaction between metal ions and ligands. In general the complexation of metal ions with isoleucine anion may be given as:

$$M^{2+} + L^- \overset{K_1}{\leftrightarrows} ML^+ \quad (1)$$

$$ML^+ + L^- \overset{K_2}{\leftrightarrows} ML_2 \quad (2)$$

Where M^{2+} is Cu^{2+}, Mn^{2+} metal ions and UO_2^{2+} oxocation; [L^-] is the isoleucine anion; K_1 and K_2 are the first and second stability constants, respectively. The metal spot on the paper is thus a combination of uncomplexed metal ions, 1:1 and 1:2 metal complexes. The spot is moving under the influence of electric field, if non-protonated and protonated species are considered, the overall mobility can be given by expression

$$U = \frac{\begin{array}{c} U_{0,0} \bullet \beta_{0,0} + U_{1,0} \bullet \beta_{1,0} [L] + U_{2,0} \bullet \beta_{2,0} [L]^2 + \text{———} \\ + U_{1,1} \bullet \beta_{1,1} [HL] + U_{2,1} \bullet \beta_{2,1} [HL]^2 + \text{———} \\ + U_{2,1} \bullet \beta_{2,1} [H_2L] + U_{2,2} \bullet \beta_{2,2} [H_2L]^2 + \text{———} \end{array}}{\begin{array}{c} \beta_{0,0} + \beta_{1,0} [L] + \beta_{2,0} [L]^2 + \beta_{1,1} [HL] \\ + \beta_{2,1} [HL]^2 + \beta_{1,2} [H_2L] \\ + \beta_{2,2} [H_2L]^2 + \text{———} \end{array}} \quad (3)$$

Where $U_{0,0}$ is the speed of uncomplexed metal ion, $U_{1,0}$ is the speed of complex formed by the combination of one unprotonated anionic ligand with metal ion and $U_{x \times p}$ is the speed of the metal complex formed by the combination of X anions containing, p,protons each. β's are the overall stability constant of the different metal complexes formed in the interaction. On taking into consideration different equlibria above equation transformed into following useful form:

$$U = \frac{u_0 + u_1 K_1 [L^-] + u_2 K_1 K_2 [L^-]^2}{1 + K_1 [L^-] + K_1 K_2 [L^-]^2} \quad (4)$$

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 dissociation constant of pure isoleucine (ka_1 = 2.25, ka_2 = 9.62) [31] were determined by same paper electropho-

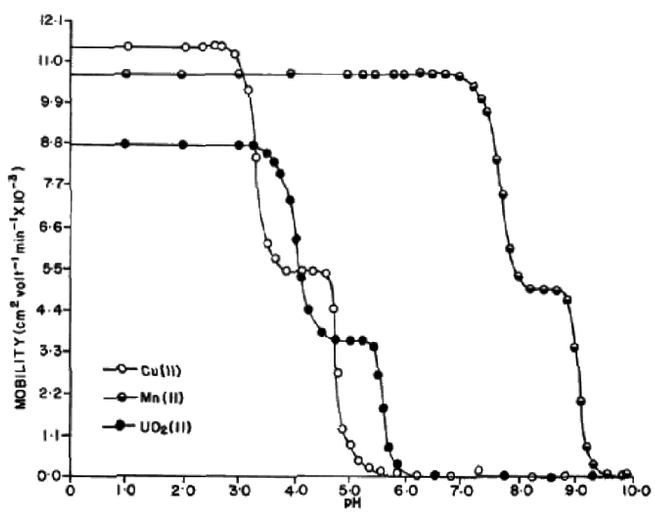

Fig. 3. Mobility curve for the metal(II) - isoleucin system –○– = Cu(II) - Isoleucine, –●– = Mn(II) - Isoleucine, –⊖– = UO_2(II) - Isoleucine. Concentration of Cu(II), Mn(II) and UO_2(II) = 0.005M. The paper strips were spotted with 01 µl of sample solutions and glucose (for making osmotic corrections).

Table. stability constants of binary complexes of Cu^{2+}, Mn^{2+} and UO_2^{2+} with isoleucine

Metal ions	Complexes	Stability constants	Logarithm stability constants values	
			Present study	Literature value
Cu^{++}	ML^+	K_1	8.41 ± 0.07	8.40 [31] 8.40 [33]
	ML_2	K_2	6.43 ± 0.03	7.00 [31] 7.00 [33]
Mn^{++}	ML^+	K_1	3.87 ± 0.11	-
	ML_2	K_2	2.61 ± 0.06	-
UO_2^{++}	ML^+	K_1	7.91 ± 0.13	-
	ML_2	K_2	5.73 ± 0.04	-

Ionic strength = 0.1 Mol/L temperature = 35ºc; Isoleucine anion= [CH_3 CH_2 CH (CH_3) CH(NH_2) COO^-]; M = metal cations Cu^{2+}, Mn^{2+} and oxocation UO_2^{2+}; L = ligand (isoleucine).

retic technique. The mode of dissociation of isoleucine can be represented as:

$$[CH_3 - CH_2 - CH\ (CH_3) - CH(NH_3^+)\ COOH]$$
$$-H^+\uparrow\downarrow\ ka_1$$
$$[CH_3 - CH_2 - CH\ (CH_3) - CH(NH_3^+)\ COO^-]$$
$$-H^+\uparrow\downarrow\ ka_2$$
$$[CH_3 - CH_2 - CH\ (CH_3) - CH\ (NH_2)\ COO^-]$$

For calculating first stability constant, K_1, the region between first and second plateau is relevant. The overall mobility will be equal to the arithmetic mean of the mobility of uncomplexed metal ion, u_0, and that of the first complex, u_1, at a pH value where $K_1 = 1/CH_3\ CH_2\ CH(CH_3)\ CH(NH_2)\ COO^-]$. The concentration of complexing isoleucine anion $[L^-]$ is calculated with the help of equation

$$[L^-] = \frac{[L_T]}{1 + \frac{[H]}{Ka_2} + \frac{[H]^2}{Ka_1 \bullet Ka_2}} \quad (5)$$

Where $[L_T]$ is the total concentration of ligand isoleucine [0.01 M], ka_1 and ka_2 are the first and second dissociation constants of the pure isoleucine, respectively.

The second stability constant, K_2 of 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 the Table.

Discussion

It is clear from the Table that first and second stability constants follow the order

copper(II) > uranyl(II) > manganese(II)

The values of the second stability constants are found to be lower in comparison to first stability constant in each case, this may be due to the decrease in coordinating tendency of ligand with higher state of aggregation [32].Maximum stability constant values of copper(II) – isoleucine indicate strong bonding between copper(II) cation and isoleucine anion. Whilst lowest stability constant value of manganese(II) – isoleucine complex indicate weak bonding between manganese(II) cation and isoleucine anion. The higher stability of copper(ii) complexes may be ascribed to its greater affinity for the oxygen donor ligand . The stability constants of metal complexes can be very easily calculated by this technique, therefore present method has significant advantages over other methods (viz; polarography, potentiometry, solubility etc.) reported in chemical literature for the determination of stability constants of metal complexes.

It is observed from the Table that calculated stability constants values are similar to literature values. The slight divergence in the values obtained from different sources is mainly due to the difference in temperature, ionic strength and experimental conditions used by different workers. The precision of the method is limited to that of paper electrophoretic technique. Nevertheless in view of the uncertainty ± 5% attending to the measurement of mobility of the metal spots, the results reported here are fairly reliable.

Conclusion

It may be concluded from these studies that copper(ii) manganese(ii) cations and uranyl(ii) oxocation are important for biological systems but as such they are toxic at higher concentration . The isoleucine may be used to reduce the level of these ions in the biological systems. It can also be con-

cluded from present studies that copper(ii) – isoleucine and manganese(ii) – isoleucine complexes have highest and lowest stability constants values, respectively among the two cations and one oxocation studied. The simple modified electrophoretic technique has thus proved to be helpful in deciding whether a complex system is formed or not and if it is formed its stability constants can also be determined.

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