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STUDIES ON BIOLOGICALLY IMPORTANT CADMIUM (II) / IRON (II) / ZINC (II) – HOMOSERINE BINARY COMPLEXES IN SOLUTION

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ABSTRACT

In coordination compounds studies, knowledge of the magnitude of the stability constants of complexes is necessary for preliminary quantitative treatment. The present technique involving the use of paper electrophoresis is described for the study of equilibria in binary complex systems in solution. This method is based on the movement of a spot of metal ion in an electric field at various pH's of background electrolyte. A graph of pH versus mobility was used to obtain information on the binary complexes and to calculate its stability constants. The stability constants of the ML and ML_2 complex species of some metal ions, namely cadmium (II), iron (II) and zinc (II) with homoserine were determined in 0.1 mol / L perchloric acid and at a temperature of 35 ºC. The logarithm stability constants of the ML and ML_2 complexes of homoserine were found to be (3.91 ± 0.03, 2.58 ± 0.09), (3.57 ± 0.01, 2.45 ± 0.05) and (4.33 ± 0.01, 2.70 ± 0.07)(logarithm stability constant values) for the cadmium(II), iron(II) and zinc(II) complexes, respectively.

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INTRODUCTION

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]. Cd^{2+} has long biological half life (about 30 years in mammals) and slow excretion of Cd^{2+} contribute to Cd^{2+} accumulation in living cells, causing damage in the liver, kidneys, lungs and nervous system. Cd^{2+} directly inhibits succinate dehydrogenae and the respiratory chain in isolated liver mitochondria [2] cadmium has been reported to cause renal disturbances, lung insufficiency, bone lesions, cancer and hypertension in humans [3]. Iron atom is part of cytochromes human P 450 enzymes (P 450s) which play a pivotal role in drug metabolism [4]. Iron is a pro-oxidant that in the reductive intracellular environment catalyses hydroxyl radical formation through the fenton reaction [5]. Total close infusions of iron dextrane have been reported to exacerbate symptoms of rheumatoid arthritis [6]. Most organisms require iron as an essential element in a variety of metabolic and informational cellular pathways. More than 100 enzymes acting in a primary and secondary metabolism posses iron – containing co-factors such as iron – sulfur clusters or heme groups. The reversible Fe (II) / Fe(III) redox pair is best suited to catalyze a broad spectrum of redox reactions and to mediate electron chain transfer [7]. Zinc stabilizes the "in vitro" self – association of Myelin Basic Protein dissolved in a phosphate buffer [8]. Zinc has essential role in normal development and function of many key tissues, cells, effectors of immunity, gene expression, mitosis, apoptosis of lymphoid cells. Mild zinc deficiency can impair multiple mediators of host immunity ranging from the physical barrier of the skin to acquired cellular and humoral immunity [9]. Zinc is a trace mineral which is vital for the functioning of numerous cellular processes, is crucial for growth, and may play an important role in cancer etiology and outcome [10]. The cadmium (II), iron (II), and zinc (II) metal ions have significant biomedical applications but are toxic at higher concentrations [11-16].

Homoserine (also called isothreonine) is an α – amino acid with the chemical formula HO_2 CCH (NH_2) CH_2 CH_2OH. L-homoserine is not one of the common amino acids encoded by DNA. Homoserine is used by plants and bacteria to make methionine, threonine and isoleucine. Homoserine is similar to the amino acid serine except it has an extra methylene group. Homoserine is a naturally occurring amino acid which do not occur in protein. It is formed by reduction of aspartic acid via the intermediacy of aspartate semialdehyde. Homoserine has several significance in biological systems [17-20]. The present paper electrophoretic technique is free from any destroying factors and very convenient in use. It gives results in fair agreement with accepted literature values.

Publications [21, 22] from our laboratory described a new method for the study of metal complexes. A search of the literature indicated coordination of amino acids with metal ions [23, 24], but there are few reports available on

binary complexes of cadmium (II), iron (II) and zinc (II) with homoserine. In view of this, an attempt was made to establish the optimum condition for metal (II) – homoserine complex formation. In addition, the present paper describes a paper electrophoretic method for the determination of the stability constants of these complexes.

RESULTS

Chemical literature [25, 26] confirms that anionic species of amino acids are the sole ligating species for metal ions. Hence a metal ion spot on the paper strip shows a variation in composition of different ionic species of the amino acids in background electrolyte. So the mobility of metal ion spot would depend upon the pH of the background electrolyte.

The plot of overall electrophoretic mobility of metal spot against pH gives a curve with a number of plateaus shown in Figure 1.

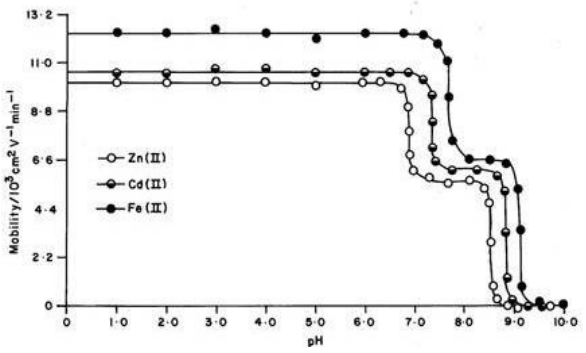


***Figure 1**: Mobility curves for the metal(II) – homoserine systems. Background electrolytes: 0.1 M perchloric acid and 0.01 M homoserine. The paper strips were spotted with 0.1 µL of sample solution and glucose (for making osmotic correction).*

A plateau is obviously an indication of a pH range where speed is practically constant. The every plateau indicates the formation of certain complex species. In the low pH region protonated species of homoserine in non-complexing. Beyond this region, metal ion spots have progressively decreasing velocity, and hence complexation of metal ionic species of ligand homoserine, whose concentration increases progressively with increase of pH, Figure 1 shows that first plateau corresponds to a region in which metal ions are uncomplexed. The second plateau in each case with positive mobility indicating the formation of 1:1 complex of cationic nature. The mobility decreases and resulting in a third plateau which obviously corresponds to overwhelmingly formation of 1:2 complex of neutral nature. The present studies give clear evidence of the complexation of anionic species of homoserine with Cd^{2+}, Fe^{2+} and Zn^{2+} metal ions forming 1:1 and 1:2 binary metal complexes. In view of the above observation, the complexation of metal ion with homoserine anion may be represented as:

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

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

where M^{2+} is Cd^{2+}, Fe^{2+} and Zn^{2+} metal cation; $[L^{-}]$ is the α-aminobutyric acid anion; K_1 and K_2 first and second stability constants of pure homoserine , 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 and the overall mobility U is given by equation (3) [27].

$$U = \frac{\Sigma\, u_{x\cdot p} \cdot \beta_{x\cdot p}[HpL]^{x}}{\Sigma\, \beta_{x\cdot p}[HpL]^{x}} \qquad (3)$$

where $[HpL]^x$ is the concentration of general complex species; β_{xp} is the overall mobility constant of the complex; u_{xp} is the speed of the general complex $[M(HpL)^x]$ present in the combination. On taking into consideration different equilibria, the above equation is transformed into the following form:

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

The dissociation constants of pure homoserine [$k_1 = 10^{2.27}$, $k_2 = 10^{9.28}$] were determined by same electrophoretic technique. The mode of dissociation of pure homoserine can be represented as:

$$[CH_2(OH)\,CH_2\,CH\,(NH_3^+)\,COOH]$$

$$-H^+ \downarrow\uparrow \; k_1$$

$$[CH_2(OH)\,CH_2\,CH\,(NH_3^+)\,COO^-]$$

$$-H^+ \downarrow\uparrow \; k_2$$

$$[CH_2(OH)\,CH_2\,CH\,(NH_2)\,COO^-]$$

For calculating first stability constant, K_1, the region between the first and second plateau is relevant. 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 $K_1 = 1/[CH_2(OH)CH_2CH(NH_2)COO^-]$. Using the dissociation constants of pure homoserine, the concentration of homoserine anion, $[L^-]$, is determined for the pH, from which K_1 can be calculated. The concentration of chelating homoserine anion, $[L^-]$, is calculated with the help of equation (4).

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

where $[L_T]$ = total concentration of ligand homoserine (0.01 mol L^{-1}). k_1 and k_2 = first and second dissociation constants of pure homoserine, respectively. The second stability constant K_2, of second 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.

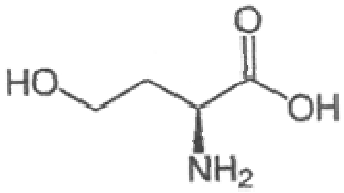
Table 1: *Stability constants of binary complexes of Cadmium(II), Iron(II) and Zinc(II) with homoserine*[a]

Metal Ions	Complexes	Stability Constants	Stability Constant Values
Cd^{2+}	ML^+	K_1	3.91 ± 0.03 (3.69 [30]) (3.69 [31])
	ML_2	K_2	2.58 ± 0.09
Fe^{2+}	ML^+	K_1	3.57 ± 0.01
	ML_2	K_2	2.45 ± 0.05
Zn^{2+}	ML^+	K_1	4.33 ± 0.01 (4.45 [30]) (4.54 [31])
	ML_2	K_2	2.70 ± 0.07 (3.52 [30]) (3.52 [31])

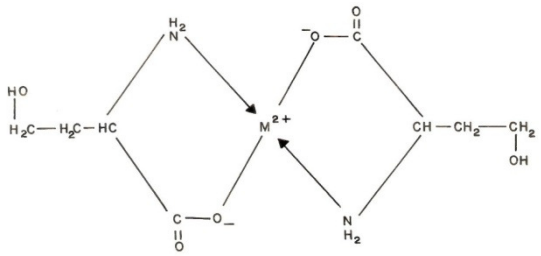
[a] Ionic strength = 0.1 M; temperature = 35 ºC; M = metal cations; L = ligand (homoserine); homoserine anion: [$CH_2(OH)\,CH_2CH\,(NH_2)COO^-$];
[b] Literature values are given in the bracket at 25 ºC.

DISCUSSION

It is observed from Table 1 that stability constants are approximately similar to literature values obtained from different sources is mainly due to the difference in temperature, ionic strength and experimental used by different workers. The present technique is limited to charged species and 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 few approach worth developing. The molecular structure of homeserine amino acid is given below:



It is clear from Table 1 that the first and second stability constants follow the order zinc (II) > cadmium (II) > iron (II). The second stability constant values are found to be lower in comparison with the first stability constant in each case. This may be due to the decrease in coordinating tendency of the ligand with higher state of aggregation [28]. The height stability constant values of the zinc (II) – homoserine complexes indicate strong bonding between the zinc (II) cation and homoserine anion, while the low stability constant values of the iron (II) – homoserine complexes indicate weak bonding between the iron (II) cation and homoserine anion. The higher stability of zinc (II) complexes may be ascribed to the greater affinity to zinc cation for the oxygen donor ligands. The stability of metal (II) complexes may also be depend on electronic configuration and position of metal cations in the periodic table, because ligand anion is similar in each case. The probable structure for metal (II) – homoserine (ML_2) complexes is shown in scheme 1.



CONLUSIONS

The following conclusions can be drawn from present study.

1. Cadmium (II), iron (II) and zinc (II) are important for biological systems but as such they are toxic, the homoserine may be used to reduce the level of these metal ions in the biological systems.
2. The present electrophoretic technique is very helpful in finding that complex system is formed or not, and if formed its stability constants can also be determined.
3. Zinc (II) – homoserine and iron (II) homoserine binary complexes are found to have maximum and minimum stability constant values, respectively.
4. The second stability constants of binary complexes are found to have low values in comparison to first stability constant values in each case.
5. Stability constants of metal complexes can be very easily calculated by this technique, so the present technique has significant advantages over the other physiochemical methods reported in chemical literature for the determination of stability constants of metal complexes.

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EXPERIMENTAL

Instruments

Systronics (Naroda, India) paper electrophoresis equipment horizontal-cum-vertical type, model 604, has been used. The apparatus consisted of a PVC moulded double tank vessel. In our laboratory significant change in the instrument has been made. Two hollow rectangular plates covered with thin polythene sheets have been used through which thermostated water is run for controlling the temperature. The tanks were closed with a transparent PVC moulded lid. The whole assembly is tight, which prevent 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 Whatman no. 1 filter papers for chromatography were used for the purpose of electrophoresis.

Elico (Hyderabad, India), Model $L_{1\text{-}10}$ pH meter using working a glass and calomel electrodes assembly working on 220 V/50 Hz established a.c. mains, was used for the pH measurements, pH meter was calibrated with buffer solution of pH 7.0.

Chemicals:

Metal solutions

Solutions of cadmium(II), iron(II) and zinc(II) 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 5.0×10^{-3} M [29].

Detecting reagents for metal ions and electro-osmotic indicator

Metal spots were detected on the paper using dithizone in carbon tetrachloride for zinc(II) and 0.1 % solution of 1 – (2 – pyridylazo) – 2 – naphthol (PAN) (E. Merck, Darmstadt, Germany) in ethanol was used for detecting the cadmium(II) and iron(II) metal ions. A 0.005 M glucose (BDH, AnalaR) solution were 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

Stock solution of 5.0 M perchloric acid was prepared from its 70 % solution (SDS, AnalaR grade). 2.0 M sodium hydroxide and 0.5 M homoserine (BDH, Poole, UK) solutions were prepared. The background electrolyte used in the study of binary complexes were 0.1 M perchloric acid and 0.1 M homoserine. The system was maintained at various pH by the addition of sodium hydroxide.

Procedure

Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. For recording observation of particular metal ion, two 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 hydroxyproline. The paper became moistened with the background electrolyte solutions due to diffusion. The second insulated plate was placed on paper strips and then thermostated water (35 ºC) was circulated in 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 200 Volts potential was applied between the electrodes. Electrophoresis was carried out for 60 minutes after which these strips were removed from the tank and dried. The metal ion and glucose spots were detected by specific reagents. The leading and tailing edge were measured from the marked centre point and the mean were taken. The distance moved by glucose 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 observations of metal ions were 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.

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

After applying the correction factor the observed mobility is given as

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

When 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 scheme for paper electrophoresis set up is shown in Figure 2.

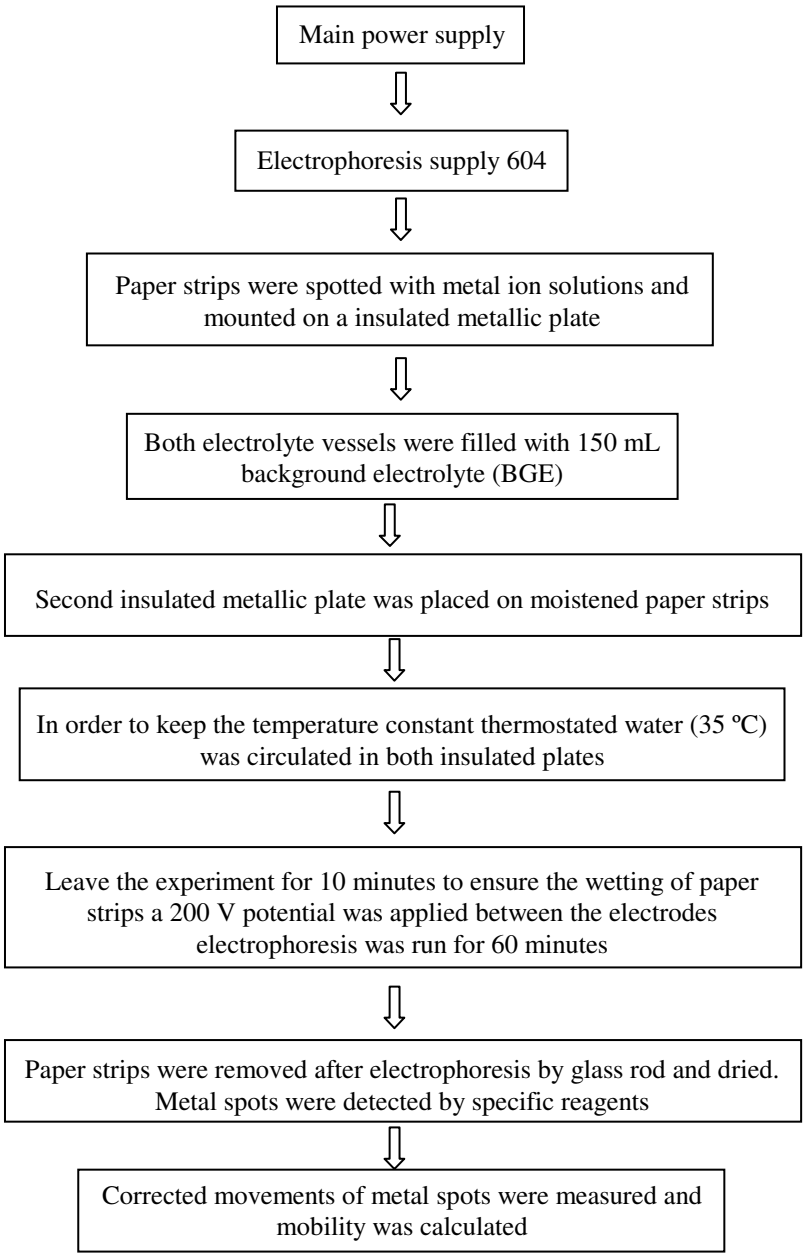

Figure 2*: The scheme for paper electrophoresis set*

The protonation constants of pure homoserine were determined by same paper electrophoretic technique. The two paper strips were spotted with pure homoserine along with two other spotted with glucose using 0.1 M perchloric acid only in 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 / amino acid are reported with pH values. The individual speeds of the duplicate spots were found to be fairly equal.

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