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Metal Complexes in Biology and Medicine: The System Cadmium (II)/ Iron (II)/ Zinc (II)- α- Aminobutyric Acid

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ABSTRACT

Metal ions play an important role in biological systems. The importance of metal ions to essential functions of living systems and for wellbeing of living organisms is well known. Complexation reactions of α– aminobutyric acid with cadmium (II), iron (II) and zinc (II) have been studied in solution phase using paper ionophoretic technique. The present method is based upon the migration of a spot of metal ion on a paper strip at different pH values of background electrolyte containing 0.01M α -aminobutyric acid. A graph of pH against mobility was used to obtained information in the binary complexes and to calculate its stability constants. The logarithm stability constants of ML and ML_2 complexes of α -aminobutyric acid were found to be (4.01 ± 0.02; 2.72 ± 0.01), (3.73 ± 0.03; 2.65 ± 0.09), (4.45 ± 0.02; 2.80 ± 0.03) for cadmium (II), iron (II) and zinc (II) complexes, respectively at ionic strength 0.1 molL^{-1} and a temperature of 35º C. Metal based drugs bioactivity can be increase by metal chelation, which in turn increase their absorbance and stability. Metal complexes can offer their action such as anti-inflammatory, anti-diabetic, antimicrobial, anticancer and ant thyroid compounds.
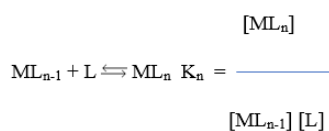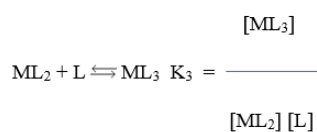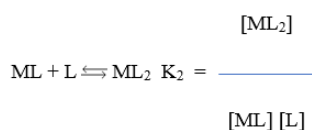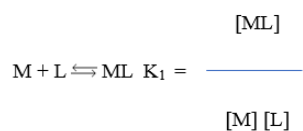
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Introduction

For the general case of complex ML_n, the stepwise formation or stability constant (K_a) are:

$$M + L \rightleftharpoons ML \quad K_1 = \frac{[ML]}{[M][L]}$$

$$ML + L \rightleftharpoons ML_2 \quad K_2 = \frac{[ML_2]}{[ML][L]}$$

$$ML_2 + L \rightleftharpoons ML_3 \quad K_3 = \frac{[ML_3]}{[ML_2][L]}$$

$$ML_{n-1} + L \rightleftharpoons ML_n \quad K_n = \frac{[ML_n]}{[ML_{n-1}][L]}$$

Where M and L are metal cation and ligand anion, respectively. For the calculation of total concentration of final complex product (ML_n), the overall formation constant is used.

$$\beta_n = \frac{[ML_n]}{[M][L]^n}$$

The overall formation constant is the product of stepwise formation constants

$$\beta_n = K_1 \cdot K_2 \cdot K_3 \cdot \ldots \ldots K_n$$

The inverse of information constant, the dissociation constant K_d is also some time useful

$$ML \rightleftharpoons M + L \quad K_d = \frac{[M][L]}{[ML]}$$

K_d has the same form as K_a for acids, which facilities comparisons between metal complexes and Bronsted acids. Out of all technique adopted for the study of metal ligand equilibria, we have opted for paper electrophoresis. Not much were, however, is on record on the application of paper electrophoresis for examining complexation reactions, metal complexes play an important role in various biological systems, hence the formation, stability, and reactivity of their complexes have been an active field of research [1].

Iron and zinc are classified as essential metals without which life cannot sustain. Cadmium is classified as toxic metal harmful even at low concentration. The average iron and zinc metal content of a healthy human (body weight 70 Kg) with plasma concentrations are 5.0 and 2.0 gram/man, respectively [2].

Heavy metals are defined as metals having a density higher than 5 g cm³. Cadmium has no known function as nutrients and seems to be more or less toxic to plants and microorganism. Concentrations of Cd ranged (0.04 nM – 0.32 nM) and (0.52 nM – 0.1 nM) in non – polluted and polluted soil, respectively [3]. Cd causes genotoxicity, teratogeny and cancer in some tissues and non malignent chronic toxicity in others (eg. Cancer in lung, nephrotoxicity in kidney) effect functions of neurotransmitters, hormones, and memory formation [4]. Cadmium has been shown to suppress iron uptake, induce phosphorus deficiency and reduce manganese transport in the growing plants [5].

Iron is important for electron transport in the respiratory chain and for various enzymatic reactions. Iron can harm biological systems since in redox – active form it catalyzes generation of highly reactive oxygen species. Both iron deficiency and overload impair cellular functions, the quantitation of iron in cells and extracellular fluids is of considerable interest [6]. Recent advances in genetics, molecular biology and biochemistry of iron metabolism have assisted in elucidating the molecular mechanism of iron homeostasis. Iron uptake and storage is tightly regulated by the feedback system of iron responsive element containing gene products and iron regulatory proteins that modulate the expression level of the genes involved in iron metabolism [7]. Zinc is an important trace element and essential for all living systems. Zinc is component in the metalloenzymes superoxide dismutase, alkaline phosphatase, alcohol dehydrogenase as well as RNA and DNA polymerase [8]. Zinc (II) complexes of α - amino acids and their derivatives with a Zn ($N_2 O_2$) coordination made were found to have in vitro insulinomimetic activity as estimated with the inhibition of free fatty acid release in isolated rat adipocytes treated with epinephrine. It was revealed that the insulinomimetic activities of zinc (II) complexes with overall stability constants (log β) less than 10.5 are higher than those of Zn SO_4 and VO SO_4 [9]. The studies in complexation reactions of bivalent cadmium, iron and zinc are of interest because of their nutrient properties and toxicity [10-18]. α – Aminobutyric acid is an isomer of the amino acid aminobutyric acid with chemical formula $C_4 H_9 NO_2$. It has two other isomers gamma – aminobutyric acid (GABA) and Beta – aminobutyric acid (BABA). It is a key intermediate in biosynthesis of ophthalmic acid or ophthalmate, which was first isolated from calf lens. GABA is one of the major inhibitory retinal neurotransmitters, and its localization has been established in nearly 40% of amacrine cells in all vertebrate's species examined. There are three classes of GABA (GABAa, GABAb, GABAc) and all have been shown in retina [19]. α – Aminobutyric acid has significant applications in biological systems [20,21]. Kiso has done comprehensive study on paper electrophoretic migration of metal complexes [22].

The paper electrophoretic technique usually suffers from a number of defects, capillary flow on paper, electro – osmosis, temperature during electrophoresis and adsorption affect the mobility of charged moieties [23]. The present technique is almost free from these destroying factors and very convenient in use. It gives results in fair agreement with accepted literature values. Communications from our laboratory described a new method for the study of metal complexes [24-26].

A search of literature indicated few reports on zinc (II) - α – aminobutyric acid and iron (II) - α – aminobutyric acid complexes but no report available on cadmium (II) - α – aminobutyric acid complexes. In view of this, attempts were made to establish the optimum conditions for metal (II) - α – aminobutyric acid complex formation. In addition, present paper describes on paper ionophoretic method for the determination of the nature and stability constants of Cd (II)/Fe (II)/Zn (II) - α – aminobutyric acid complexes.

Experimental
Apparatus
A systronic (Naroda, India) Model 604, electrophoresis was used. The apparatus consisted of a Poly (vinyl chloride) PVC moulded double tank vessel. In our laboratory significant change in the in the instrument has been made. Two hollow rectangular iron plates each weighing one kg, and covered with thin polythene sheets have been used through which thermostated water circulated for controlling the temperature. The tanks are closed with a transparent PVC moulded lid. The whole assembly is tight to prevent moisture change, which might upset the equilibria in the paper strip. This assembly design thus keeps to a minimum the disturbing effect of evaporation from the evaporation from the unwanted liquid flow in the paper strips. Each electrolyte tank contains a separate electrode chamber in which Pt-wire anode and cathode are placed, respectively. Applied voltage was from a stabilized source. Electrophoresis cell showing sandwiched paper strips is shown in Figure 1
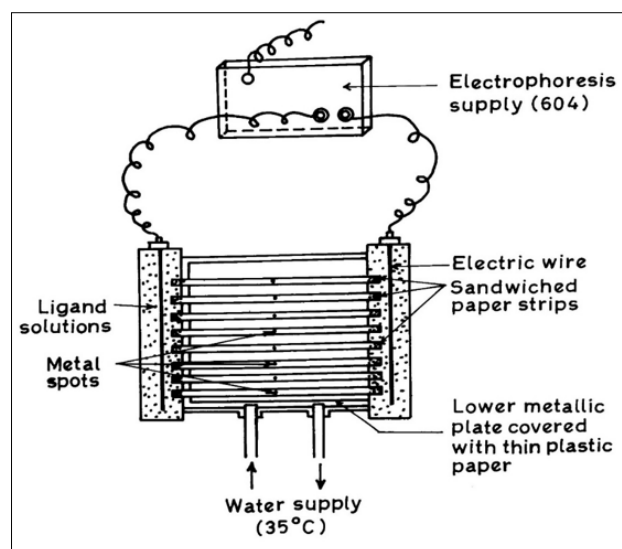

Figure 1: Electrophoresis Cell Showing Sandwiched Paper Strips

Whatman No 1 filter papers or chromatography were used for the purpose of electrophoresis. Elico (Hyderabad, India) Model L_{1-10} pH meter using 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
Preparation of Metal Solution
Cadmium (II), iron (II) and zinc (II) metal perchlorate solutions were prepared by the precipitation of metal carbonates from a 0.1 Mol L^{-1} solution of cadmium((II), iron (II) and zinc (II) nitrates with the solution of sodium carbonate (chemically pure grade, BDH, Poole, UK). The precipitation was washed with boiling

water and treated with calculated amounts of 1% perchloric acid. The metal contents of the filtrates were determined, and final concentration was kept 0.005 Mol L^{-1}.

Sodium Hydroxide Solution
Carbon dioxide free sodium hydroxide solution was prepared by dissolving 500 gms of sodium hydroxide in 500 mL of water in a flask. The flask was left overnight. The clear supernatant liquid was filtered rapidly using a high vacuum pump. A suitable volume of the filtrate was diluted, and the concentration determined by titrating against a standard oxalic acid solution. A solution (2.0 Mol L^{-1}) was obtained by suitable dilution. The concentration of stock solution was checked from time to time.

Detecting Reagents for Metal ions and Glucose
Metal spots were detected on the paper using dithizone in carbon tetrachloride for Zn (II). A 0.1 % solution of 1- (2 – pyridylazo) – 2 – naphthol (PAN) (E. Merc, Darmstadt, Germany) in ethanol was used for detecting the cadmium (II) and iron (II) metal ion. A 0.005 Mol L^{-1} glucose BDH, Analytical Reagent grade solutions was prepared in water and used as an electro osmotic indicator for the correction due to electro – osmosis. A saturated aqueous solution (o.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 solution of sodium hydroxide, when a black spot was formed. Paper strips showing the position of metal ions spot after electrophoresis is shown in Figure 2.
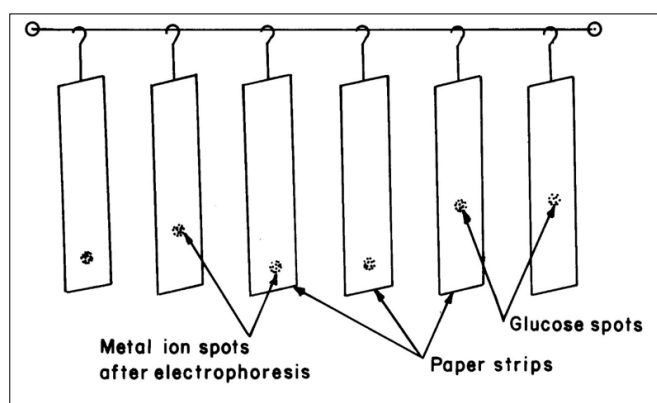

Figure 2: Paper Strips Showing Position of Metal Ion Spots After Electrophoresis

Background Electrolyte
The background electrolytes used in the study of binary complexes were 0.1 Mol L^{-1} perchloric acid and 0.1 Mol L^{-1} α – aminobutyric acid. The system was maintained at various pH by the addition of sodium hydroxide. Stock solution of 5.0 Mol L^{-1} perchloric acid (SDS, Analytical Reagent grade), 2.0 Mol L^{-1} sodium hydroxide (Analytical Reagent grade) and 0.5 Mol L^{-1} α – aminobutyric acid was prepared. Each solution was standardized using the appropriate method.

Procedure
Whatman No.1 filter paper 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 mounted on insulated plate. Each of the two-electrolyte vessels were filled with 150 mL of background electrolyte containing 0.1 Mol L^{-1} perchloric acid and 0.01 Mol L^{-1} α – aminobutyric acid. 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° C) was circulated in the plates to keep the temperature constant. The lid was then placed on the instrument to make it airtight. It was left for 10 minutes to insure wetting the strips. Subsequently a direct 200 Volts potential was applied between the electrodes. Electrophoresis was carried 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 center 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 on metal ions were recorded at various pH values of the background electrolyte obtained by adding sodium hydroxide solution. The ionic strength being maintained at 0.1 Mol L^{-1}. The observed mobility of migrants was calculated by using the formula.

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

After applying the correction factor the observed mobility is given as

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

where U = mobility of metal ion / complex ion, d = mean of duplicate distance travelled by metal ion / complex ion; d_G = mean duplicate distance travelled by glucose spot; x = field strength; t = time for electrophoresis. The mobility of metal / complex ion spots on the paper strips were thus calculated and are reported with different pH values Figure 3.
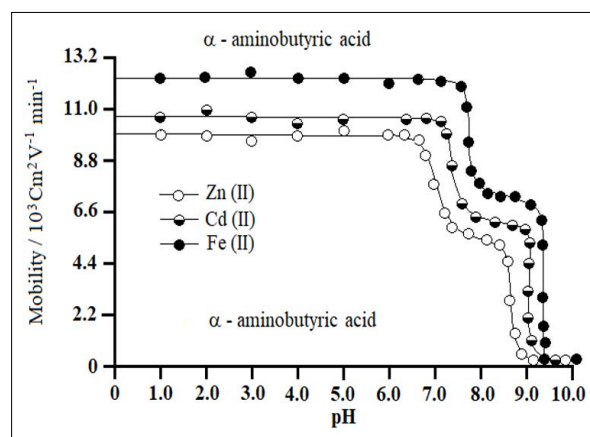

Figure 3: Mobility Curves for Metal (II) - α – Aminobutyric Acid Complexes

Background electrolytes = 0.1 Mol L^{-1} perchloric acid and 0.01 Mol L^{-1} α – aminobutyric acid. Concentration of metal ions = 0.005 Mol L^{-1}. Variation in the pH was made by the addition of sodium hydroxide.

The protonation constants of pure α – aminobutyric acid was determined by the same paper electrophoresis technique. The two paper strips were spotted with α – aminobutyric acid along with two glucoses using 0.1 Mol L^{-1} perchloric acid only in a background electrolyte. The electrophoresis was carried for 60 minutes as for metal ion. The electrophoresis speed was calculated.

The speed of α – aminobutyric acid spots are reported with various pH values. The individual speeds of duplicate spots were found to be fairly equal.

Results and Discussion

The plot of overall electrophoretic mobility of metal spot against pH is shown in Figure 3. The first plateau in the beginning corresponds to a region in region in which metal ions are uncomplexed. It is obvious that protonated ionic species of the α – aminobutyric acid, which exist in low pH range are non – complexing, $[CH_2 (OH) CH_2 CH (NH_3^+) COOH]$. Figure 3 discloses that cadmium (II), iron (II) and zinc (II) metal ions from their first complex movements towards negative electrode, hence one α – aminobutyric acid anionic species $[CH_2 (OH) CH_2 CH (NH_2^+) COO^-]$ must have combined with cadmium (II), iron (II) and zinc (II) to give 1:1, $[Cd\{CH_2 (OH) CH_2 CH (NH_2) COO\}]^+$;$[Fe\{CH_2 (OH) CH_2 CH (NH_2) COO\}]^+$ and $[Zn\{CH_2 (OH) CH_2 CH (NH_2) COO\}]^+$Complex cations, respectively. The second plateau lies in the positive region indicating the cationic nature of 1:1 metal ligand complex. The third plateau in each case is in zero region of metal ligand complex. Hence two anionic species of α – aminobutyric acid $[CH_2 (OH) CH_2 CH (NH_2) COO^-]$ must have combined with metal ions to give 1:2, $[Cd \{CH_2 (OH) CH_2 CH (NH_2) COO\}_2]$; $[Fe\{CH_2 (OH) CH_2 CH (NH_2) COO\}_2]$ and $[Zn\{CH_2 (OH) CH_2 CH (NH_2) COO\}_2]$ neutral 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.

Chemical literature also assigns prominent chelating properties of the unprotonated anionic species of α – aminobutyric acid ruling out any such property to zwitterions [27]. In general, the complexation of metal ions with α – aminobutyric acid anion [L⁻] may be represented as

$$M^{2+} + L^- \xrightleftharpoons{K_1} ML^+ \quad (1)$$
$$ML^+ + L^- \xrightleftharpoons{K_2} ML_2 \quad (2)$$
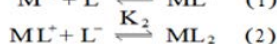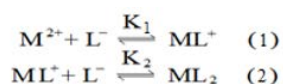
Wherein M^{2+} is Cd^{2+}, Fe^{2+} and Zn^{2+} metal ions; [L⁻] is the ligand α – aminobutyric acid anion, K_1 and K_2 are the first and second stability constants, respectively, ML^+ and ML_2 = 1:1 and 1:2 metal complexes, respectively. The metal spot on the paper is thus a combination of uncomplexed metal ions, 1:1 and 1:2 metal complexes. The spot is moving under the influence of electric field and overall mobility is given by equation of Jokl [28]

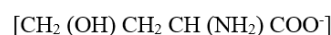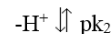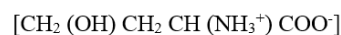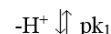$$U = \frac{\Sigma U_{xp} \beta_{xp} [HpL]^x}{\Sigma \beta_{xp} [HpL]^x} \quad (3)$$

Wherein $[Hp L]^x$ 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 $[M (H_p L)^x]$ present in the conglomeration. On taking into consideration different equilibria, the above equation is transformed into the following useful form

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

Wherein u_0, u_1 and u_2 are mobilities of uncomplexed metal ion, 1:1 metal complex and 1:2 metal complex, respectively [28].

The protonation constants of pure α – aminobutyric acid (pk_1 = 2.30; pk_2 = 9.63) was obtained by the same paper ionophoretic technique the mode of dissociation of pure α – aminobutyric acid represented as

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

$$-H^+ \Updownarrow pk_1$$

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

$$-H^+ \Updownarrow pk_2$$

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

For calculating first stability constant K_1, the region between the first and second plateau is pertinent. The overall mobility U will be equal to the arithmetic mean of mobility of the uncomplexed metal ion u_o and that of the first complex u_1 at a pH where

$$K1 = 1/ [CH2 (OH) CH2 CH (NH2) COO^-].$$

The first stability constant K_1 can be calculated with the help of concentration of α – aminobutyric acid anion and protonation constant of pure α – aminobutyric acid. The concentration of chelating amino acid (α – aminobutyric acid) species [L⁻] is calculated with the help of equation.

$$[L^-] = \frac{[L_T]}{1 + [H] / k_2 + [H]^2 / k_1 k_2} \quad (5)$$

Where $[L_T]$ = is the total concentration of ligand (α – aminobutyric acid) 0.01Mol L⁻¹. The second stability constant K_2 of 1:2 metal ligand complex can be calculated by taking into consideration the region between the second and third plateau of the mobility curve. The calculated values of first and second stability constants are given in Table 1.

Table 1: Stability Constants of Binary Complexes of Binary Complexes of Cadmium (II) Iron (II) and Zinc (II) with α – Aminobutyric Acid

Metal ions	Stability constants	Complexes	Logarithm stability calculated	Constant values literature
Cadmium (II)	K_1 K_2	ML+ ML2	4.01 ± 0.02 2.72 ± 0.01	- -
Iron (II)	K_1 K_2	ML+ ML2	3.73 ± 0.03 2.65 ± 0.09	3.37 [29] -
Zinc (II)	K_1 K_2	ML+ ML2	4.45 ± 0.02 2.80 ± 0.03	4.54 ± 0.04 [29] 4.11 [29]

Iron strength = 0.1 Mol L⁻¹; temperature 35º C; α – aminobutyric acid anion: $[CH_3 - CH_2 CH (NH_2) COO^-]$; M= metal cations $[Cd^{2+}, Fe^{2+}, Zn^{2+}]$; L = Ligand (α – aminobutyric acid); concentration of metal cation = 0.005 Mol L⁻¹; pH of solution 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 the Table 1 that first and second stability constants of cadmium (II), iron (II) and zinc (II)- α – aminobutyric acid complexes follow the order Log K_1 > Log K_2, the corresponding second stability constant values are found to be lower for all complexes. It is therefore inferred that coordinating tendency of a ligand decreases with the higher state of aggregation.

It is also clear from the Table 1 that first and second stability constants follow the order zinc (II) > cadmium (II) > iron (II). It is observed from Table 1 that the presented stability constant values are similar to literature values. The slight deviation in values obtained from different sources is mainly due to the difference in temperature and ionic strength used by different researchers.

The stability constants of metal complexes can be very easily calculated by this technique, therefore present method have significant advantages over other methods (viz: polarography, potentiometry, solubility etc.) reported in chemical literature for the determination of stability constants of metal complexes. According to standard deviation (statistics) the precision of method limited to that of paper electrophoresis, and uncertainty in the result is ± 5%. Hence, it cannot immediately replace the most reliable methods, even though it is new approach deserving further development.

The high stability constant values of zinc (II) - α − aminobutyric acid complexes indicating strong bonding between zinc (II) cation and α − aminobutyric acid anion. Whilst low stability constant values of iron (II)- α − aminobutyric acid complexes indicate weak bonding between iron (II) cation and α − aminobutyric acid anion. The higher stability of zinc (II) complexes may be due to its greater affinity for oxygen donor ligands.

The proposed structure for metal (II) - α − aminobutyric acid, ML_2, binary complexes may be given as follows
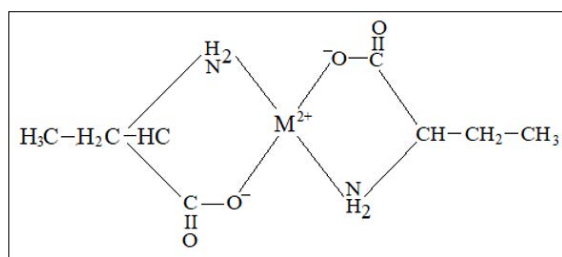
Concluding Remarks
The following conclusions can be drawn from the present studies:
- The present paper ionophoretic technique is very helpful in finding that complex systems is formed or not, if formed its stability constant can also be determined.
- The cadmium (II), iron (II) and zinc (II) are significant for biological systems but as such they are toxic, the α − aminobutyric acid may be used to reduce the level of these metal ions in the biological systems.
- The ML2 complexes are found to have low stability constant values and are less stable in comparison to ML complexes.
- The stability of metal (II) - α − aminobutyric acid complexes follow the order zinc (II) > cadmium (II) > iron (II).
- Zinc (II) metal ion have shown greater affinity with oxygen donor ligands in comparison to cadmium (II) and iron (II).
- Stability constants of metal complexes can be very easily calculated by this technique, so the present paper electrophoretic 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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