Studies on Biologically Significant Mercury(II) , Nickel(II) and Lead(II) – Isoleucine Binary Complexes in Solution

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Abstract: The stability constants of Hg^{2+}, Ni^{2+} and Pb^{2+} complexes with isoleucine were determined by paper ionophoretic technique. Present method is based upon the migration of a spot of metal ions on a paper strip at different pHs 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 [Hg(II) – isoleucine], [Ni(II) – isoleucine] and [Pb(II) – isoleucine] complexes were found to be (8.80 ± 0.02, 7.52 ± 0.05), (7.27 ± 0.01, 5.86 ± 0.03 and (7.17 ± 0.05, 3.32 ± 0.09) for Hg(II), Ni(II) and Pb(II) complexes, respectively at 0.01 M ionic strength and a temperature of 35° C.

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Key words: paper electrophoretic technique; overall mobility; mercury(II) complexes; nickel(II) complexes; lead(II) complexes; isoleucine ; stability constant.

1. Introduction

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

$$M + L \leftrightarrows ML \qquad K_1 = \frac{[ML]}{[ML][L]}$$

$$ML + L \leftrightarrows ML_2 \qquad K_2 = \frac{[ML_2]}{[ML][L]}$$

$$ML_2 + L \leftrightarrows ML_3 \qquad K_3 = \frac{[ML_3]}{[ML_2][L]}$$

$$\text{---} \qquad\qquad \text{---}$$

$$ML_{n-1} + L \leftrightarrows ML_n \qquad K_1 = \frac{[ML_n]}{[ML_{n-1}][L]}$$

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

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

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

$$ML \leftrightarrows M + L \qquad K_d = \frac{[M][L]}{[ML]}$$

K_d has the same form as K_a for acids, which facilities comparisons between metal complexes and Brϕnsted acids.

A significant development on the determination of stability constants of complexes was made by Jokl (Jokl, 1964) [1]. 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 (Sherman, 1987) [2]. Banerjea (Banerjea, 1995) [3] has classified nickel as beneficial and mercury as well as lead as toxic metals, respectively. The average nickel content of healthy human body weight (70 kg) is 0.01 g/man. The amount of elements nickel, lead and mercury in human diet (mg/day) are (0.30-0.50), (0.06-0.50) and (0.004 – 0.020), respectively. Deficiency of nickel in body effect the function of liver. Mercury is extremely harmful, even in concentration of 0.03 ppm in drinking water is not permissible. Mercury enters natural waters through industrial discharge (such as of Chlor – alkali industry) whereby bacterial action it is converted into very soluble and water soluble methylmercury ion, Hg $(CH_3)^+$, which is taken by the fish and through food chain enters higher animal and man. Lead destroys sulphur containing proteins and enzymes, causes damage to DNA, RNA, brain and central nervous system functions. Lead also inhibits several steps in the formation of hemoglobin. Mercury(II), Nickel(II) and Lead(II) have significant biomedical applications but are toxic at higher concentration (Sutton, 2007; Das, 2007; Kaneko, 2007 ; Sumi, 2008; Karthikeyan, 2008; Lee, 2009; Shokrollahi, 2009; Khan, 2010; Chang, 2010; Saeed, 2010; Na'aliya, 2010; Chandra, 2009; Patil, 2009; Chattopadhyay, 2010; Bulut, 2009; Prakash, 2011; Adeyemi, 2009; Wen-Xing, 2010; Sheta, 2009; Herranz, 2010; Ou-Yang, 2010; Imanpoor, 2010; Vanysek, 2008; Hassan, 2008) [4-27].

Isoleucine is an essential amino acid, which means that humans cannot synthesize it, so it must be part of our diet. It is generally coded amino acid, its codons are AUU, AUC and AUA. Isoleucine promotes muscle recovery, needed for the formation of hemoglobin regulation of blood sugar level and blood clot formation. Deficiency of Isoleucine is only found in people deficient in dietary protein but symptoms may include headaches, dizziness, fatigue, depression, confusion as well as irritability, symptoms of deficiency may mimic the symptoms of hypoglycemia. Isoleucine has several significant applications in biological systems (Papp, 2010; Li, 2009; Mitrophanov, 2008; Irvine, 2008; Sato, 2011; Kashiwada, 2007; Kawabe, 2006; Takors, 2007; Dimitrov, 2007) [28-36]. The usual drawbacks of paper electrophoretic technique like variation in temperature during electrophoresis, capillary flow on paper, electro-osmosis and adsorption affecting the mobility of charged moieties, are quite well known (Shaw, 1969)[37].

Publications (Tewari, 2001, 1995, 1996, 2005, 2009, 2010)[38-43] from our laboratory described new method for the study of metal complexes. A search of literature indicated very few report on Ni(II) – isoleucine complexes and no report on Hg(II) – isoleucine and Pb(II) – isoleucine complexes. In view of this attempt were made to establish the optimum conditions for metal(II) – isoleucine complex formation. In addition, present paper describes a paper electrophoretic method for the determination of stability constants of these complexes.

2. Experimental Section

2.1 Instruments

Systronics (Naroda, India) paper electrophoresis equipment horizontalcum-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 auxiliary unit is specially designed to operate either voltage mode or on current mode.

An Elico (Hyderabad, India) model L_{1-10} having glass and calomel electrodes assembly and working on 220 V 50 Hz established a.c mains, was used for pH measurements. Electrophoresis cell showing sandwiched paper strips and water supply is shown in Figure 1.

2.2 Chemicals

Mercury(II), nickel(II) and lead(II) perchlorate solutions were prepared by preliminary precipitation of metal carbonates from a 0.1 M solution of sodium carbonate (AnalaR grade, BDH, Poole, UK). The precipitates were thoroughly washed with boiling water and treated with calculated amounts of 1 % perchloric acid. The resulting mixture was heated to boiling on a water bath and then filtered. The metal content of the filtrates were determined and final concentration was kept at 0.005 M (Kolthoff, 1957; Vogel, 1978) [44, 45]. The position of the Ni^{2+} spots

on the paper at the end of the experiment was detected using ammonical dimethylglyoxime (DMG), that of Pb^{2+} detected by 0.1% solution 1 – (2 – pyridylazo) – 2 – naphthol (PAN) (Merck, Darmstadt, Germany) in ethanol, that of Hg^{2+} detected using hydrogen sulphide in water. The 0.005 M glucose (BDH, AnalaR) solution was prepared in water and used as an 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 solution of sodium hydroxide, when a black spot was formed. Paper strips showing position of metal ions spot after electrophoresis is shown in Figure 2.

2.3 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 isoleucine (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 isoleucine. The system was maintained at various pH by the addition of sodium hydroxide.

2.4 Procedure

Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. For recording observation of 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 was filled with 150 mL of BGE solutions containing 0.1 M perchloric acid and 0.01 M isoleucine. The paper become 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 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 the strips. Subsequently a direct 240 V potential was applied between 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. The leading and tailing edges were measured from marked center point and the mean 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 observation of metal ions were recorded at various pH values of the background electrolyte, 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:

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

where U = mobility of metal ion / complex ions; d = mean of duplicate distance travelled by metal ion / complex ion; d_G = mean of duplicate distance travelled by glucose spot; x = field strength (7.5 V/cm); t = time for electrophoresis.

The dissociation constants of pure isoleucine were determined by the same paper electrophoresis technique. The two paper strips were spotted with pure isoleucine along with two glucose using 0.1 M perchloric acid only in a background electrolyte. The electrophoresis was carried for 60 minutes as for metal ions. The electrophoretic speed was calculated and the speed of the metal ion / isoleucine spots are reported with pH values. The individual speeds of the duplicate spots were found to be fairly equal.

3. Results

As is evident from Figure 3, the plot of overall electrophoretic mobility of the metal ion spot against the pH gives a curve with two plateaus in each case. The first plateau corresponds to a region in which the metal ions are uncomplexed. It is obvious that protonated ionic species of isoleucine, which exists at low pH range are non-complexing [CH_3 - CH_2 – CH (CH_3) – CH (NH_3^+) - COOH]. Figure 3 secret that mercury(II), nickel(II) and lead(II) ions form their first complex movements toward negative electrode. Hence, one isoleucine anionic species [CH_3 - CH_2 – CH (CH_3) – CH (NH_2) - COO^-] must have combined with mercury(II), nickel(II) and lead(II) to give 1:1, [Hg{CH_3 CH_2 – CH (CH_3) CH (NH_2) COO}]$^+$, [Ni {CH_3 CH_2 – CH (CH_3) – CH (NH_2) COO}]$^+$ and [Pb {CH_3 CH_2 – CH (CH_3) - CH (NH_2) COO]$^+$ complex cations, respectively.

The third plateau in each case is in 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 give 1:2, [Hg{CH_3 CH_2 CH (CH_3) CH (NH_2) $COO\}_2$], [Ni {CH_3 CH_2 CH (CH_3) CH (NH_2) $COO\}_2$] and [Pb {CH_3 CH_2 CH (CH_3) CH (NH_2) $COO\}_2$] neutral complexes, respectively.

The chemical literature also assigns a prominent chelating property to the zwitterions (Blackburn, 1973) [46]. 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 gives clear evidence of the complexation of the anionic species of isoleucine 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 isoleucine anion may be represented as:

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

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

where M^{2+} represents the Hg^{2+}, Ni^{2+} and Pb^{2+} metal ions, [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 the uncomplexed metal ions; 1:1 complex, and 1:2 complex. The spot is moving under the influence of the electric field, and the overall mobility is given by the equation of Jokl (Jokl, 1964) [47]

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

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:

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

where u_0, u_1 and u_2 are the mobilities of uncomplexed metal ions, 1:1 and 1:2 metal complexes, respectively. The dissociation constants of pure isoleucine ($k_1 = 10^{2.25}$, $k_2 = 10^{9.62}$) was determined by some paper electrophoretic technique. The mode of dissociation of pure isoleucine is shown in Figure 4. Using the dissociation constants of isoleucine, the concentration of the pure isoleucine anion [L^-] is determined for the pH values) of interest from which K_1 can be calculated. The concentration of complexing isoleucine anion [L^-] is calculated with the help of the equation.

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

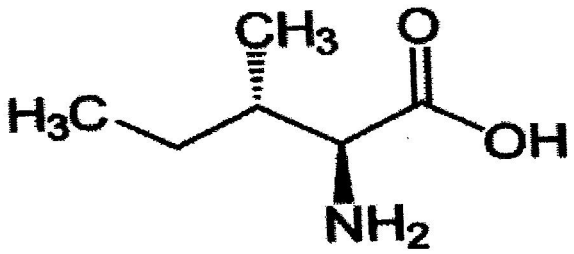
where $[L_T]$ is the total concentration of the isoleucine ligand, (0.01 M); k_1 and k_2 are the first and second dissociation constants of pure isoleucine, respectively.

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

4. Discussion

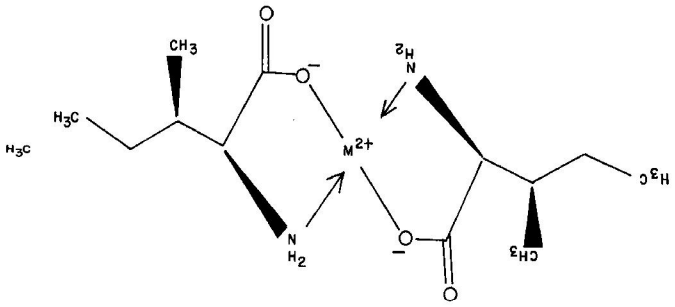
The precision of the method is limited to that of paper electrophoresis, and uncertainty in the result is ± 5%. Hence, it cannot replace the most reliable methods, even though it is new approach deserving further development. The stability constants of metal complexes can be very easily calculated by this technique, therefore the present method is advantageous over other methods (viz., polarography, potentiometry, solubility, etc. reported in chemical literature. The molecular structure of isoleucine is as follows:



It is observed from Table 1 that value of first and second stability constants of ML^+ and ML_2 complexes follow the order:

mercury(II) > nickel(II) > lead(II).

The proposed structure for the ML_2 complexes may be given as:



The values of second stability constant are found to be lower in comparison to first stability constant in each system, this may be due to the decrease in coordinating tendency of ligand with the higher state of aggregation. It is also clear from Table 1 that mercury(II) -isoleucine and lead(II) – isoleucine complexes are found to have highest and lowest stability constant values, respectively. Therefore it is inferred that Hg^{2+} cations has greater affinity with oxygen donor ligand, while Pb^{2+} cation has lesser affinity with oxygen donor ligands.

Table 1: Stability constants of binary complexes of mercury(II), nickel(II) and lead(II) with α-aminobutenoic acid.

Metal ions	Complexes	Stability constants	Logarithm stability constant values*
Mercury(II)	ML^+	K_1	8.80 ± 0.02
	ML_2	K_2	7.52 ± 0.03
Nickel(II)	ML^+	K_1	7.27 ± 0.01 {5.40 (Martell, 1974) [48] }
	ML_2	K_2	5.86 ± 0.03 {4.30 (Martell, 1974) [48] }
Lead(II)	ML^+	K_1	7.17 ± 0.05
	ML_2	K_2	3.32 ± 0.09

Ionic strength = 0.1 M; temperature = 35 ° C; M = metal cations (Hg^{2+}, Ni^{2+}, Pb^{2+}); L = ligand (isoleucine) ; isoleucine anion = [CH_3 CH_2 CH (CH_3) CH (NH_2) COO^-].
*Literature values are given in parenthesis.

5. Conclusions

The following conclusions can be drawn from the present study:

Mercury (II), nickel (II) and lead (II) 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 biological systems. Mercury (II) – isoleucine and lead (II) – isoleucine complexes are found to have highest and lowest stability constant, respectively. ML_2 complexes are found to have low stability constant value and less stable in comparison to ML complexes in each system. Biologically important mercury (II) – isoleucine, nickel(II) – isoleucine and lead(II) – isoleucine can be prepared on large scale at particular pH of the background electrolyte. 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.

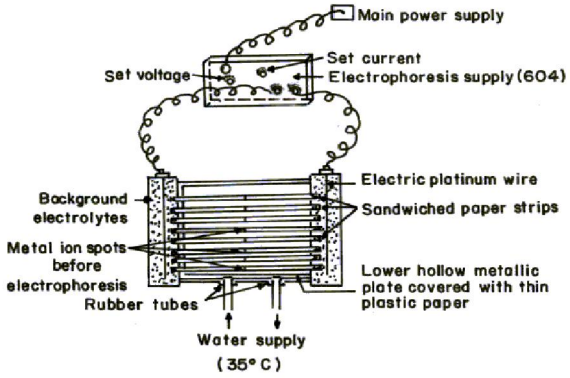


Fig. 1 Electrophoresis cell showing sandwiched paper strips.

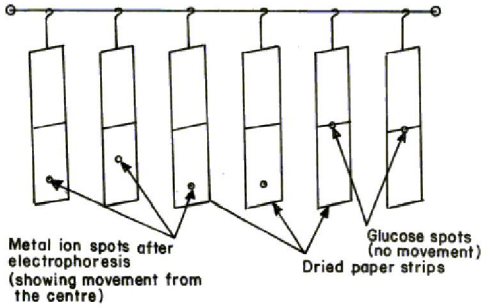
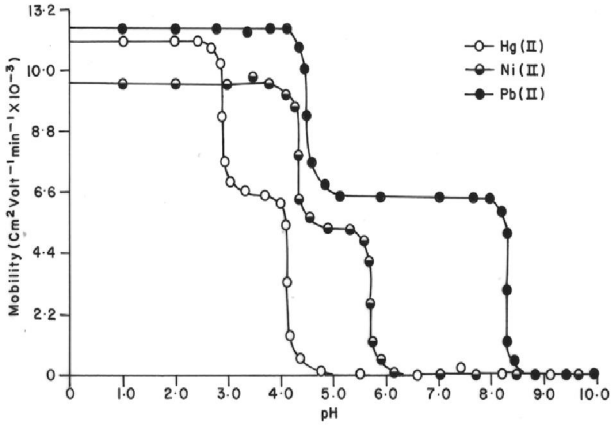


Fig. 2 Paper strips showing position of metal ion spots after electrophoresis



Figur

Pb(II) – isoleucine. Background electrolyte – 0.1 M perchloric acid and 0.01 M isoleucine. pHs were maintained by addition of sodium hydroxide. The paper strips were spotted with 0.1 µL of sample solutions and glucose (for making osmotic corrections).

$$[CH_3\ CH_2\ CH\ (CH_3)\ CH\ (NH_3^+)\ COOH]$$
$$k_1 \quad \downarrow\uparrow \quad -H^+$$
$$[CH_3\ CH_2\ CH\ (CH_3)\ CH\ (NH_3^+)\ COO^-]$$
$$k_2 \quad \downarrow\uparrow \quad -H^+$$
$$[CH_3\ CH_2\ CH\ (CH_3)\ CH\ (NH_2)\ COO^-]$$

Figure 4. Mode of dissociation of pure isoleucine. Ionic strength = 0.01 M. temperature = 35°C.

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