STUDIES ON BIOLOGICALLY SIGNIFICANT MERCURY (II), NICKEL (II) AND LEAD (II) BINARY COMPLEXES WITH α- AMINOBUTENOIC ACID IN SOLUTION

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ABSTRACT

Stability constants of binary Hg(II) - α-aminobutenoic acid, Ni(II) - α-aminobutenoic acid and Pb(II) - α-aminobutenoic acid have been determined by paper ionophoretic technique at 0.1 M ionic strength and a temperature of 35° C. The stability constants of Hg(II) - α-aminobutenoic acid, Ni(II) - α-aminobutenoic acid and Pb(II) - α-aminobutenoic acid, ML and ML_2 binary complexes were found to be $(7.81 \pm 0.03, 6.50 \pm 0.05)$, $(6.05 \pm 0.01, 5.15 \pm 0.06$ and $(4.20 \pm 0.02, 2.55 \pm 0.07)$ (logarithm of stability constant values), respectively.

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INTRODUCTION

For a mononuclear binary complex, if a central atom (central group) M (the 'metal' and a ligand L have been defined, then in the following expressions K_n is the stepwise formation constant, and β_n is the cumulative formation constant for the complex ML_n. They can both be referred to as stability constants (stepwise and cumulative) [1]

$$K_n = K(ML_{n-1} + L = ML_n)$$
$$\beta_n = K(M + nL = ML_n)$$

A significant development on the determination of stability constants of complexes was made by Jokl [2] in 1964. A theoretical treatment similar to that of Jokl was adopted by Biernat [3] for the study of stepwise complex formation. Banerjea [4] has classified nickel as beneficial and mercury as well as lead toxic metals, respectively. Beneficial metals are helpful for healthy life. Toxic metals are very harmful even at very low concentration. Nickel is an integral component of the enzymes urease, may be involved in the action of hydrogenase. Mercury is extremely harmful, even a concentration of 0.03 ppm in drinking water. Mercury deactivates sulphur containing enzymes with active – SH groups, affects brain cells and the central nervous system. Lead seriously affect the release of neurotransmitters which are vital for transmission of nerve impulses. Enzymic conversion of ATP to ADP by the enzyme ATP-ase releasing energy needed for life process is significantly inhibited by lead. Mercury, nickel and lead have several significant applications in biological systems [5-24]. 2-Amino – 3 – butenoic acid is a naturally occurring amino acids which do not occur in protein. It has several significant applications in biological systems [25-33].

Kiso [34] has done comprehensive study on paper electrophoretic migration of metal complexes. The electrophoretic technique usually suffers from numbers of defects, e.g. temperature rise during electrophoresis, capillary flow on paper, adsorption and molecular sizing affect the mobility of charged moieties [35]. The technique described here is almost free from these destroying factors.

Publications [36-40] from our laboratory have described a new method for the study of metal complexes. A search of the literature indicated few reports on Ni(II) - α-aminobutenoic acid Pb(II) - α-aminobutenoic acid complexes and no report on Hg(II) - α-aminobutenoic acid complexes. In view of this, attempts were made to establish the optimum conditions for metal(II) - α-aminobutenoic acid complex formation. In addition, the present paper describes a paper

electrophoretic method for the determination of the stability constants of Hg(II) / Ni(II) / Pb(II) - α-aminobutenoic acid binary complexes.

RESULTS

Chemical literature [41, 42] confirms that anionic species of amino acids are the sole ligating species for metal ions. The electrophoretic mobility of the metal spot against pH gives a curve with a number of plateaus as is shown in Figure 3. 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 $[CH_2 = CH – CH (NH_3^+) COOH]$ species of α-aminobutenoic acid is at a maximum and this species is not complexing. Beyond this range, metal ion spots have progressively decreasing mobility, complexation of metal ions should be taking place with anionic species of α-aminobutenoic acid whose concentration increases progressively with an increase of pH. Figure 3 shows three plateaus in mercury(II), nickel(II) and lead(II). Hence all three Hg(II), Ni(II) and Pb(II) form two complexes with the α-aminobutenoic acid anion. It is therefore assumed that the anionic species $[CH_2 = CH – CH (NH_2) COO^-]$ of α-aminobutenoic acid has complexed with the metal ions to form different complexes. Figure 3 reveals that Hg(II), Ni(II) and Pb(II) form their first complex movement towards the negative electrode. Hence, one $[CH_2 = CH – CH (NH_2) COO^-]$ must have combined with Hg(II), Ni(II) and Pb(II) ions to give $[Hg \{CH_2 = CH – CH (NH_2) COO\}]^+$ $[Ni \{CH_2 = CH – CH (NH_2) COO\}]^+$ and $[Pb \{CH_2 = CH – CH (NH_2) COO\}]^+$ complex cations, respectively. With a further increase of pH, mobility in all three 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 complex. Hence, two $[CH_2 = CH – CH (NH_2) COO^-]$ anions must have combined with Hg(II), Ni(II) and Pb(II) to give the $[Hg \{CH_2 = CH – CH (NH_2) COO\}_2]$, $[Ni \{CH_2 = CH – CH (NH_2) COO\}_2]$ and $[Pb\{CH_2 = CH – CH (NH_2) COO\}_2]$ complexes, respectively.

Further, increase of pH has no effect on the mobility of metal ions. In view of the above observations, the complexation of metal ions with α-aminobutenoic acid anion $[L^-]$ may be represented as:

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

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

where M^{2+} represents the Hg^{2+}, Ni^{2+} and Pb^{2+} metal ions, $[L^-]$ is the α-aminobutenoic acid anion, and 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 [43].

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

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:

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

where u_0, u_1 and u_2 are the mobilities of uncomplexed metal ions, 1:1 and 1:2 metal complexes, respectively.

For calculating first stability constant, K_1 the region between first and second plateau is pertinent. The overall mobility U 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 where $K_1 = 1/[CH_2 = CH – CH (NH_2) COO^-]$.

With the help of dissociation constants of pure α-aminobutenoic acid [$k_1 = 10^{2.45}$, $k_2 = 10^{9.25}$ (paper electrophoretically obtained value], the concentration of α-aminobutenoic acid anion is determined at the pH of average mobility from which K_1 can be calculated.

The concentration of chelating α-aminobutenoic acid species [L^-] is calculated with the help of equation.

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

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

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

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	7.81 ± 0.03
	ML_2	K_2	6.50 ± 0.05
Nickel(II)	ML^+	K_1	6.05 ± 0.01 (5.21 [47])
	ML_2	K_2	5.15 ± 0.06 (4.46 [47])
Lead(II)	ML^+	K_1	4.20 ± 0.02 (4.70 [47])
	ML_2	K_2	2.55 ± 0.07 (4.50 [47])

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

DISCUSSION

It is observed from the Table 1 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 researchers.

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 new approach worth further development.

It is observed from Table 1 that stability constants of ML and ML_2 complexes follow the order :

mercury(II)　>　nickel(II)　>　lead(II).

The molecular structure of α-aminobutenoic acid is given as:
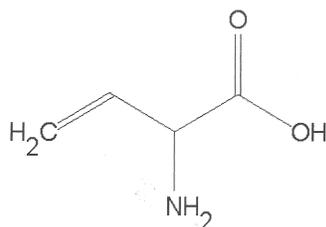
The values of second stability constant of ML_2 complexes are found to be lower in comparison to first stability constant of ML complexes in each case this may be due to the decrease in coordinating tendency of ligand with the higher state of aggregation. In other words, the metal progressively lesson its tendency of linkage with a ligand on progressive filling of vacant orbitals [44]. Higher stability constant values of mercury(II) - α-aminobutenoic acid indicate greater affinity of mercury(II) with oxygen donor ligands, while lower stability constant values of lead(II) - α-aminobutenoic acid indicate lesser affinity of lead(II) with oxygen donor ligands. The probable structure for general ML_2 complex may be given as:
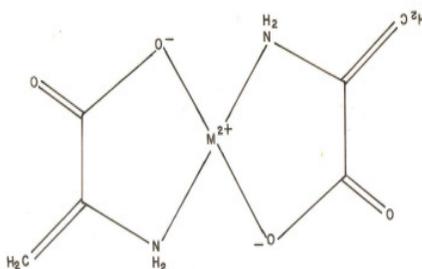
EXPERIMENTAL

Instruments

Electrophoresis equipment from Systronic (Naroda, India), model 604 was used. The equipment has a built – in power supply (a.c – d.c) that is fed directly to the paper electrophoresis tank. The potential in each experiment was kept at 240 V and electrophoresis was carried out for 60 minutes. An Elico (India) model L_{1-10} with 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.
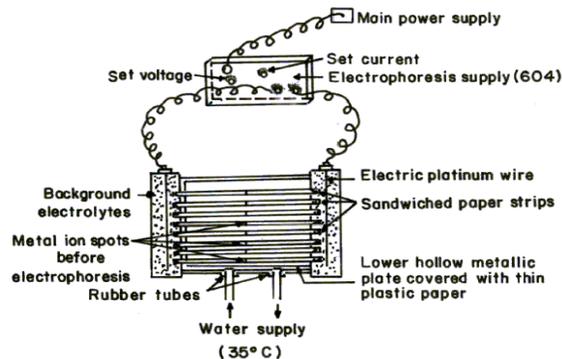
Fig. 1 Electrophoresis cell showing sandwiched paper strips.

Chemicals

Mercury(II), nickel(II) and lead(II) and 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 [45, 46]. 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.
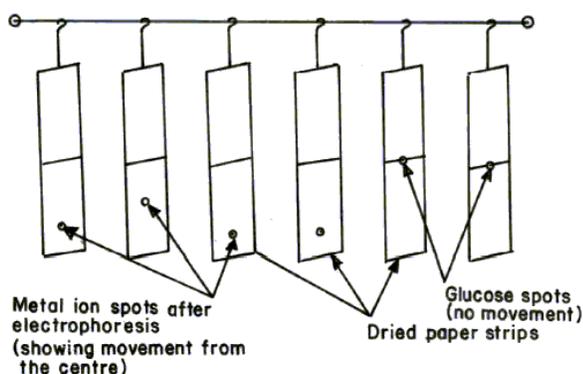

Fig. 2 Paper strips showing position of metal ion spots after electrophoresis

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 α-aminobutenoic acid (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 α-aminobutenoic acid. 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 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 background electrolyte solutions containing 0.1 M perchloric acid and 0.01 M α-aminobutenoic acid. 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 speed of the metal ions / complex ions are reported with pH values. A plot of mobility against pH curve for metal (II) - α-aminobutenoic acid is shown in Figure 3. The scheme for paper electrophoresis set up is shown in Figure 4.
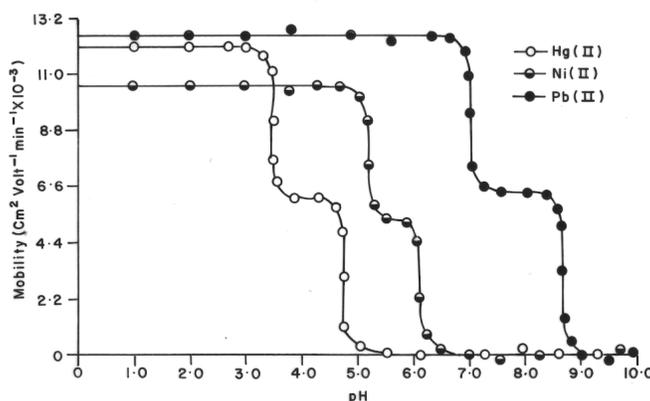
Figure 3. *Mobility curve for the metal(II) - α-aminobutenoic acid systems.*
= Hg(II) - α-aminobutenoic acid,   *Ni(II) - α-aminobutenoic acid*

= Pb(II) - α-aminobutenoic acid. Background electrolyte = 0.1 M perchloric acid and 0.01 M α-aminobutenoic acid. 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).

CONCLUDING REMARKS

The following conclusions can be drawn from the present study.

Mercury(II), nickel(II) and lead(II) are significant but since they are toxic, α-aminobutenoic acid may be used to reduce the level of these metal ions in biological systems. Mercury(II) - α-aminobutenoic acid and Lead(II) - α-aminobutenoic acid complexes were found to have higher and lower stability constant values, respectively. The ML_2 complexes are found to have low stability constant values and are less stable in comparison with the ML complexes in each system. Biologically important mercury(II), nickel(II) and lead(II) complexes with α-aminobutenoic acid can be prepared on a large scale at a particular pH of background electrolyte. Paper electrophoretic technique is very helpful in determining whether a complex system is formed or not and if formed its stability constants can also be determined.

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                        ┌─────────────────────────┐
                        │    Main power supply     │
                        └─────────────────────────┘
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                                    ▼
                    ┌─────────────────────────────┐
                    │  Electrophoresis supply 604  │
                    └─────────────────────────────┘
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        ┌──────────────────────────────────────────────────────┐
        │  Paper strips were spotted with metal ion solutions    │
        │       and mounted on an insulated metallic plate       │
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            ┌──────────────────────────────────────────┐
            │  Both electrolytic vessels were filled     │
            │  with 150 mL Background electrolyte (BGE)  │
            └──────────────────────────────────────────┘
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        ┌──────────────────────────────────────────────────────┐
        │  Second insulated metallic plate was placed on         │
        │                moistened paper strips                  │
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        │  In order to keep the temperature constant             │
        │  thermostated water (35° C) was circulated in both     │
        │                  insulated plates                      │
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        │  Left the experiment for 10 minutes to ensure the      │
        │  wetting of paper strips. A 200 V potential was        │
        │  applied between the electrodes. Electrophoresis was   │
        │  run for 60 minutes                                    │
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        ┌──────────────────────────────────────────────────────┐
        │  Paper strips were removed after electrophoresis       │
        │  by glass rod and dried. Metal spots were detected     │
        │  by specific reagents                                  │
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        │  Corrected movement of metal spots were measured       │
        │  and mobility was calculated                           │
        └──────────────────────────────────────────────────────┘
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Figure 4

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