Metal Complexes in Biology and Medicine: The System

Mercury(II) / Nickel(II) / Lead(II) – Norvaline

ABSTRACT

In coordinate compounds, knowledge of stability constants of the complexes is necessary for preliminary quantitative treatment. Metal ions are fundamental elements for the maintenance of life spans of the human, animals and plants and play an important role in biological systems. A recent 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 movement of a spot of metal ion in an electric field at various pHs of background electrolyte. A graph of pH versus mobility give the information for binary complexes and to its calculate stability constants. The logarithm stability constants of the ML and ML_2 complexes of norvaline were found to be (8.61 ± 0.03; 7.05 ± 0.07), (6.93 ± 0.05; 5.47 ± 0.03) and (4.57 ± 0.02; 3.00 ± 0.05) for the mercury(II), nickel(II) and lead(II) complexes, respectively at ionic strength 0.1 M perchloric acid and a temperature of 35 ° C.

Keywords: Electrophoretic technique, mercury(II) complexes, nickel(II) complexes, lead(II) complexes, norvaline, overall mobility , stability constant.

1. INTRODUCTION

Recent advances in inorganic chemistry have made possible formation of number of transitional metal complexes with organic ligands of interest, which can be used as

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

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

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

$$ML_2 + L \leftrightarrows ML_3 \quad K_3 = \frac{[ML3]}{[ML_2][L]}$$

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

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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

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

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

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

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

Metal complexes play an important role in various biological systems [1] and in different field of chemistry [2]. The complexation data involving essential metal ions and bioactive ligand norvaline give insight into many physicochemical processes in living systems. Banerjea has classified nickel as beneficial metal and lead as well as mercury as tonic metal in the biological systems [3]. The mercury (II), nickel (II) and lead (II) have significant biomedical applications

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but are toxic at higher concentration [4-27]. Norvaline an amino acid $C_5H_{11}NO_2$ isomeric with valine and usually made synthetically. It is not found in proteins. Norvaline has several significant applications in biological systems [28-35].

Kiso [36] has done a comprehensive study on paper electrophoretic migration of metal complexes. The present electrophoretic technique is almost free from a number of defects such as temperature during electrophoresis, capillary flow on paper, electroosmosis and adsorption. The technique is very convenient in use. It gives results in fair agreement with accepted literature values. Communications [37, 38] from our laboratory described a new method for the study of metal complexes. The present work is extension of this technique and reports observations on binary systems, viz.: Hg(II) / Ni(II) / Pb(II) – norvaline.

2. EXPERIMENTAL SECTION

Instruments

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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.

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. The electrophoresis cell showing sandwiched paper strips and water supply are shown in Figure 1.

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Chemicals

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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 [39, 40]. 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 of 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 electroosmosis. 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 the positions of the metal ion spots after electrophoresis are shown in Figure 2.

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Background electrolyte

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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 norvaline (BDH, Poole, UK) solutions were

prepared. The background electrolyte used in the study of binary complexes were 0.1 M perchloric acid and 0.01 M norvaline. The system was maintained at various pH by the addition of sodium hydroxide.

Procedure

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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 norvaline. 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 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 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 on 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.

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

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 of duplicate distance travelled by glucose spot; x = field strength; t = time for electrophoresis.

The protonation constants of pure norvaline were determined by same paper electrophoresis technique. The two paper strips were spotted with pure norvaline along with two with 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. The speed of the metal ion / norvaline spots are reported with pH values. The individual speeds of the duplicate spots were found to be fairly equal

3. RESULTS

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_3 CH_2 CH_2 CH (NH_3^+) COOH] species of norvaline is at a maximum and this species is non-complexing. Beyond this range, metal ion spots have progressively decreasing mobility, complexation of metal ions should be taking place with anionic species of norvaline whose concentration increases progressively with an increase of pH. Figure 3 shows three plateaus in Hg(II), Ni(II) and Pb(II), hence all three Hg(II), Ni(II) and Pb(II) form two complexes with the norvaline anion. It is therefore assumed that the one anionic species of norvaline [CH_3 CH_2 CH_2 $CH(NH_2)$ COO^-] must have combined with Hg (II), Ni (II) and Pb (II) to form [Hg {CH_3 CH_2 CH_2 $CH(NH_2)$ COO}]$^+$, [Ni {CH_3 CH_2 CH_2 $CH(NH_2)$ COO}]$^+$ and [Pb {CH_3 CH_2 CH_2 $CH(NH_2)$ 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 anionic species of norvaline [CH_3 CH_2 CH_2 $CH(NH_2)$ COO^-] must have combined with Hg (II), Ni(II) and Pb(II) to give the [Hg {CH_3 CH_2 CH_2 $CH(NH_2)$ $COO\}_2$], [Ni {CH_3 CH_2 CH_2 $CH(NH_2)$ $COO\}_2$] and [Pb{CH_3 CH_2 CH_2 $CH(NH_2)$ $COO\}_2$] complexes, respectively.

Further increase of pH has no effect on the mobility of metal ions, which indicates no further interaction between metal ions and ligand. In general, the complexation of metal ions with norvaline anion may be represented as:

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

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

where M^{2+} is Hg^{2+} Ni^{2+} and Pb^{2+} metal ions; $[L^{-}]$ is the norvaline 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 and the overall mobility U is given by equation (3) [41].

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

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 $u_{x \cdot p}$ is the speed of the metal complex formed by the combination of x anions containing, p, protons each. β's are taking the

overall stability constant of the different metal complexes formed in the interaction. On taking into consideration different equilibrium above equation (3) transformed into following useful form of equation (4)

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

where u_0, u_1 and u_2 are mobilities of uncomplexed metal ion, 1:1 metal complex and 1:2 metal complex, respectively. The dissociation constants of pure norvaline ($k_1 = 10^{2.31}$; $k_2 = 10^{9.65}$) [42] were determined by same paper electrophoretic technique. The mode of dissociation of pure norvaline can be represented as:

$$[CH_3\ CH_2\ CH_2\ CH\ (NH_3^+)\ COOH]$$

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

$$[CH_3\ CH_2\ CH_2\ CH\ (NH_3^+)\ COO^-]$$

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

$$[CH_3\ CH_2\ CH_2\ CH\ (NH_2)\ COO^-]$$

Using dissociation constants of pure norvaline the concentration of norvaline anion $[L^-]$ is determined for the pH value(s) of interest, from which K_1 can be calculated. The concentration of complexing norvaline anion $[L^-]$ is calculated with the help of equation (5)

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

where $[L_T]$ is total concentration of ligand norvaline (0.01 M); k_1 and k_2 are first and second dissociation constants of pure norvaline, 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 uncomplex u_0, and that of first complex, u_1 at a pH value where $K_1 = 1/[L^-]$.

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

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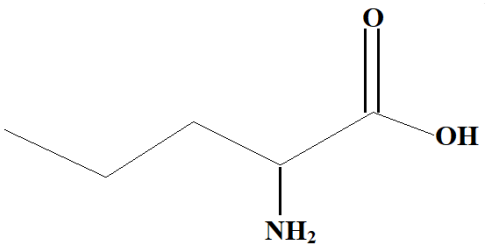
4. DISCUSSION

It is observed from Table 1 that stability constants follow the order:

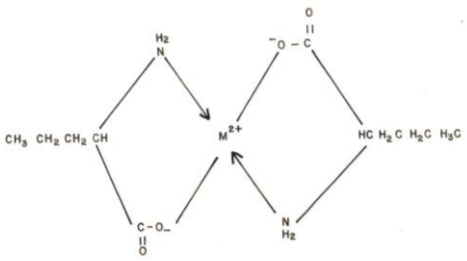
mercury (II) > nickel(II) > lead(II)

The high stability constant values of the mercury (II) – norvaline complexes indicate strong bonding between the mercury (II) cation and the norvaline anion, while the low stability constant values of lead (II) – norvaline complexes indicate weak bonding between the lead (II) cation and norvaline anion. The higher stability of mercury (II) complexes may be ascribed to the greater affinity of mercury (II) for the oxygen donor ligands. The molecular structure of norvaline is given as:



It is also observed from Table 1 that the second stability constant values are found to be lower in comparison to 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 [43]

It is clear from the Table 1 that calculated stability constant values are approximately similar to literature values. The slight divergence in the values obtained from different sources is mainly due to the difference in temperature and ionic strength used by different sources. According to standard deviation (statistics) the precision of the method is 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 proposed structure of ML_2 complex is given as follows:



The recent parallel studies on metal complexes in biology and medicine have been reported in chemical literature. Lead toxicity is an important environmental disease and its effects on the human body are devastating. A review on lead toxicity has been reported by Wani et al. [44]. Collin et al. [45] has described a review on bioaccumulation of lead and its effects on human. Acute and chronic symptoms of lead poisoning include kidney, brain, reproductive organ and CNS/PNS damage. The major of nickel in the evolution of life on planet earth has been investigated by Fontecilla-Camps [46]. Ni-rich volcanic emissions have resulted in alterations of the biological carbon cycle caused by high archaeal production of greenhouse CH_4 gas and the ensuring global temperature elevation. Wang et al. [47] has studied application of nickel (II) complexes to the efficient synthesis of α – or ß – amino acids. Nonproteininorganic α – or ß – amino acids are widely utilized for biological, biochemical, pharmaceutical and asymmetric chemical investigations.

Synthesis of amino acid Schiff base nickel (II) complexes as potential anticancer drugs in vitro has been investigated by Li et al. [48]. Carter et al. [49] has investigated urease, the first enzyme to be crystallized, contains a dinuclear nickel metallocentre that catalyzes the decomposition of urea to produce ammonia, a reaction of great agricultural and medical importance. A paper on comprehensive review concerning Hg – Se reciprocal action as a potential mechanism of

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protective action of Se against Hg toxicity as well as a potential detoxification mechanism has presented by Kurus et al. [50]. Rafati – Rahimzadeh et al. [51] has investigated current approaches of the management of mercury poisoning. Besides supportive therapy, British anti lewisite, dimercaprol (BAL), 2 -3 – dimercaptosuccinic acid (DMSA, succimer) and dimercaptopropanessuffoxid acid (DMPS) are currently use as chelating agent in mercury poisoning. Interesting phenomenon for the co-incubation of Me Hg with Secys (selenocysteine), promoted the uptake of Me Hg in Hep G_2 cells, but reduced the cytotoxicity of Me Hg has been explained by Wang et al. [52]. Excretion of mercury with $Secys_2$ has been studied by ICP – MS – based technique. Gopal et al. [53] has presented a comprehensive survey on the expediated anti – COVID -19 options enabled by metal complexes.

A study to investigate the differences in cadmium and mercury concentrations between children with autism spectrum disorder (ASD) and controls was conducted by Ramazani et al. [54]. Polis et al. [55] has investigated L – norvaline, a new therapeutic agent against Alzheimer's disease. Norvaline and norleucine may have been more abundant protein components during early stage of cell evolution has been reported by Alvarez – Carreno et al. [56]. Gilinsky et al. [57] has presented data which indicated that L – norvaline is a potential antihypertensive agent and deserves to be clinically investigated. Norvaline has several medical applications in biological systems [58 – 60].

Scheme -I : In general, the calculation of first (K_1) and second (K_2) stepwise stability constants of binary metal complexes can be explained in following steps :

K_1

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$$M + L \rightleftharpoons ML$$

$$K_1 = \frac{[ML]}{[M]\,[L]}$$

$$[ML] = K_1\,[M]\,[L]$$

$$K_2$$

$$ML + L \rightleftharpoons ML_2$$

$$K_2 = \frac{[ML_2]}{[M]\,[L]}$$

$$[ML_2] = K_2\,[ML]\,[L]$$

Where M = metal cation; L = Ligand anionic species; K_1 and K2 are first and second stability constants, respectively.

$$U = \sum u_i\, f_i$$

Where U = overall mobility; u = mobility of particular species; f = mole fraction of particular species.

$$U = u_M f_M + u_{ML} f_{ML} + u_{ML2}\, f_{ML2}$$

$$\text{Mole fraction} = \frac{\text{Number of moles}}{\text{total mole}}$$

$$\text{Total mole} = [M] + [ML] + [ML_2]$$

$$= [M] + K_1\,[M]\,[L] + K_2\,[ML]\,[L]$$

$$= [M] + K_1\,[M]\,[L] + K_1 K_2\,[M]\,[L]^2$$

$$= [M]\,[1 + K_1\,[L] + K_1 K_2\,[L]^2]$$

On considering $[1 + K_1 [L] + K_1 K_2 [L]^2] = J$

$= MJ$

$$f_M = \frac{[M]}{[M]. J} = \frac{1}{J}$$

$$f_{ML} = \frac{K_1 [M][L]}{[M] J} = \frac{K_1 [L]}{J}$$

$$f_{ML2} = \frac{K_1 K_2 [M] [L]^2}{[M] J} = \frac{K_1 K_2 [L]^2}{J}$$

$$U = \frac{u_M}{J} \frac{1 + u_{ML} K_1 [L]}{J} + \frac{u_{ML2} K_1 K_2 [L]^2}{J}$$

$$U = \frac{u_M + u_{ML} K_1 [L] + u_{ML2} K_1 K_2 [L]^2}{J}$$

On putting the value of J in above equation

$$U = \frac{U_M + u_{ML} K_1 [L] + u_{ML2} K_1 K_2 [L]^2}{1 + K_1 [L] + K_1 K_2 [L]^2}$$

For the calculation of first stability constant (K_1), first and second plateau is considered then

$$U = \frac{U_M + u_{ML2} K_1 [L]}{1 + K_1 [L]}$$

when $K_1 [L] = 1$

then $K_1 = \frac{1}{[L]}$

$U = \frac{u_M + u_{ML}}{2}$ (half mobility)

Therefore, first stability constant $K_1 = 1/[L]$. [L] is concentration of ligating species at half of the mobility of first and second plateaus of mobility curve.

For the calculation of second stability constant, (K_2), second and third plateau is considered then

$$U = \frac{U_{ML} K_1 [L] + u_{ML2} K_1 K_2 [L]^2}{K_1 [L] + K_1 K_2 [L]^2}$$

$$= \frac{K_1 [L] [u_{ML} + u_{ML2} K_2 [L]}{K_1 [L] [1 + K_2 [L]]}$$

when $K_2 [L] = 1$

then $K_1 = \frac{1}{[L]}$

$U = \frac{u_{ML} + u_{ML2}}{2}$ (half mobility)

Therefore, second stability constant $K_2 = 1/[1]$. [1] is the concentration of ligating species at half of the mobilities of second and third plateaus of mobility curve.

Scheme – II: In general, calculation of **overall stability constants** (β) of binary metal complexes can be explained in following steps:

β

$$ML_2 + 2L' \rightleftharpoons ML'_2 + 2L$$

Where M = metal cations; L = primary ligand; L' = secondary ligand; β = overall stability constant

$$\beta = \frac{[ML'_2]\,[L]^2}{[ML_2]\,[L']^2}$$

$$[ML'_2] = \beta\,[ML_2]\,\frac{[L']^2}{[L]^2}$$

$$U = \sum u_i f_i$$

Where U = overall mobility; u = mobility of particular species; f = mole fraction of particular species.

$$U = u_{ML2}\, f_{ML2} + u_{ML'2}\, f_{ML'2}$$

Where U = overall mobility; u = mobility particular complex species; f = mole fraction of particular complex species.

$$\text{Mole fraction} = \frac{\text{Number of moles}}{\text{Total moles}}$$

$$\text{Total mole fraction} = f_{ML2} + f_{ML'2}$$

$$\text{Total mole} = [ML_2] + [ML'_2]$$

$$= [ML_2] + \beta\,[ML_2]\,\frac{[L']^2}{[L]^2}$$

$$= [ML_2] \cdot \{1 + \beta\,\frac{\beta\,[L']^2}{[L]^2}\}$$

On considering $\{1 + \beta\,\frac{[L']^2}{\quad}\}$ is equal to J

$$[L]^2$$

$$= [ML_2] \cdot J$$

$$\text{Now } f_{ML2} = \frac{[ML_2]}{[ML_2] \cdot J} = \frac{1}{J}$$

$$f_{ML2} = \frac{[ML'_2]}{[ML_2] \cdot J}$$

$$= \frac{\beta\,[ML_2]\,\frac{[L']^2}{[L]^2}}{[ML_2] \cdot J}$$

$$= \frac{\beta\,\frac{[L']^2}{[L]^2}}{J}$$

$$\text{Now } U = u_1 \; {}_{1/J} + u_2 \,.\, \frac{\beta\,\frac{[L']^2}{[L]^2}}{J}$$

$$U = \frac{u_1 + u_2 \,.\, \beta\,\frac{[L']^2}{[L]^2}}{J}$$

On putting the value of J in above equation

$$U = \frac{u_1 + u_2 . \beta [L']^2 / [L]^2}{1 + \beta [L']^2 / [L]^2}$$

when $\beta \frac{[L']^2}{[L]^2} = 1$

$$\beta = \frac{[L]^2}{[L']^2}$$

$$U = \frac{u_1 + u_2}{2} \quad (\text{half mobility})$$

Therefore, overall stability constant $\beta = [L]^2 / [L']^2$. The [L] and [L'] are the primary and secondary ligating species at half of the mobility of first and second plateaus of the mobility curve.

5. CONCLUDING REMARKS

Several conclusions can be drawn from the present study.

1. Mercury (II), nickel (II) and lead (II) are significant for biological systems but as such they are toxic at higher concentration.
2. Norvaline may be used to reduce the level of these metal ions in biological systems.
3. Mercury (II) – norvaline and lead (II) – norvaline complexes are found to have highest and lowest stability constant values, respectively.

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A good research that contribute to stability of heavy metals in biological systems. Recommendation for future study is required. Thanks

4. The ML_2 binary complexes are found to have lower stability constant values in comparison to ML complexes in each system.

5. The present modified electrophoretic technique is helpful in finding if complexes are formed or not, and if formed their stability constants can also be determined.

6. Biologically important mercury (II), nickel (II) and lead (II) complexes with norvaline can be prepared on large scale at a particular pH of the background electrolyte solution.

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Table 1. Stability constants of binary complexes of mercury(II), nickel(II) and lead(II) with norvaline.

Metal ion	**Complexes**	**Stability Constant**	**Logarithm Stability Constant value ***
Mercury(II)	ML^+	K_1	8.61 ± 0.03
	ML_2	K_2	7.05 ± 0.07
Nickel(II)	ML^+	K_1	6.93 ± 0.05 (5.42 [61]) (5.68 [62])
	ML_2	K_2	5.47 ± 0.03 (4.45 [61]) (4.42 [62])
Lead(II)	ML^+	K_1	4.57 ± 0.02
	ML_2	K_2	3.00 ± 0.05

Ionic strength = 0.1 M; temperature = 35 ° C; M = metal cations (Hg^{2+}, Ni^{2+}, Pb^{2+}); ligand = norvaline; norvaline anion = [CH_3 CH_2 CH_2 CH (NH_2) COO^-]

*Literature values are given in parentheses.

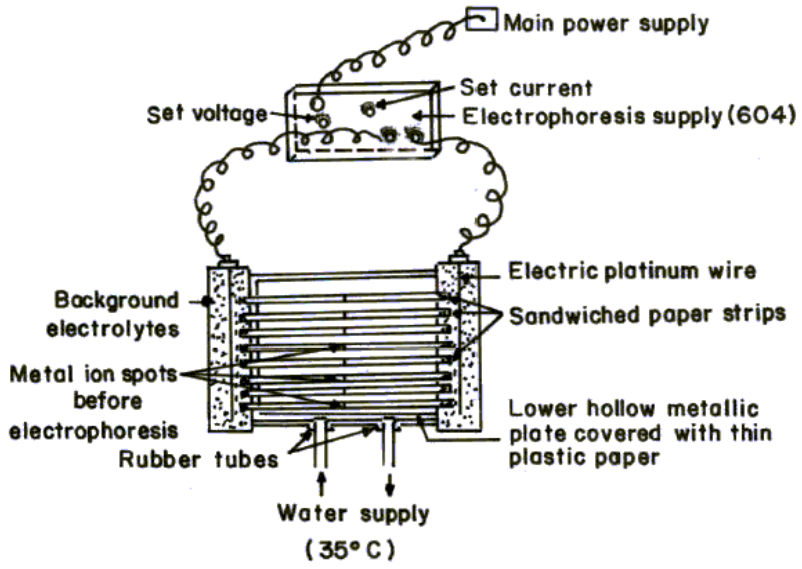

Fig. 1 Electrophoresis cell showing sandwiched paper strips.

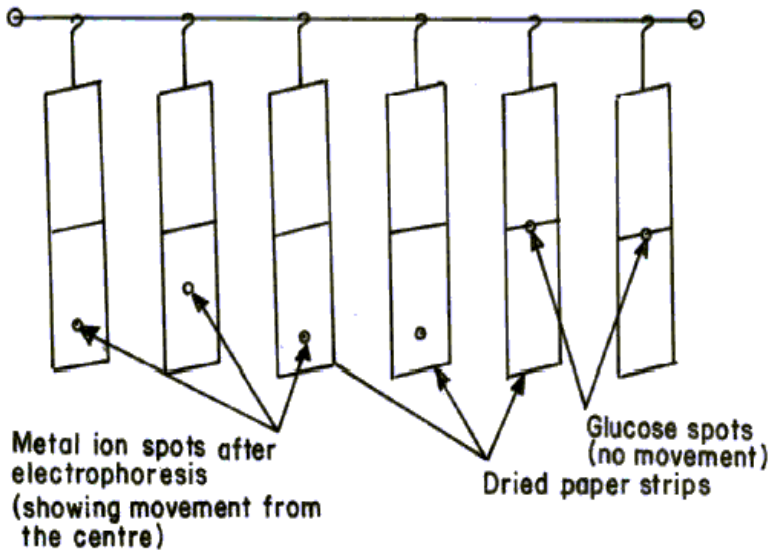

Fig. 2 Paper strips showing position of metal ion spots after electrophoresis

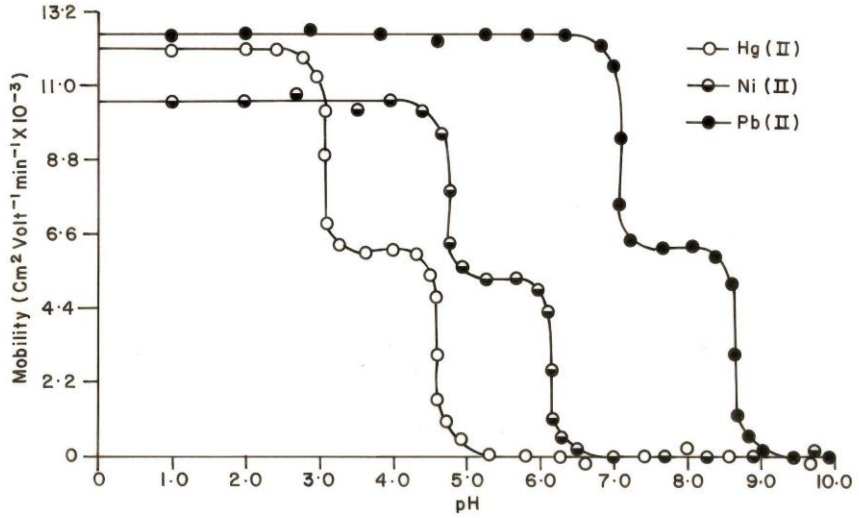

Fig. 3 Mobility curves for the metal (II) – norvaline systems. —○— = Hg (II) – norvaline; = —●— nickel(II) – norvaline —●— = Pb (II) – norvaline. Background electrolyte = 0.1 M perchloric acid and 0.01 M norvaline. pH was maintained by addition of sodium hydroxide. Concentration of Hg^{2+}, Ni^{2+} and Pb^{2+}= 0.005 M. Ionic strength = 0.1 M. Temperature = 35 °C. The paper strips were spotted with 0.1 µl of sample solutions and glucose (for making osmotic corrections).