Malaysian Journal of Chemistry, 2013, Vol. 15, No. 1, 077 – 084

Paper Electrophoretic Technique Studies of Mercury(II), Nickel(II) and Lead(II) Biologically Significant Binary Complexes with Proline in Solution

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Absrtact : A method involving use of paper electrophoresis is described for the study of equilibria in binary ligand [proline] complex systems in solution. This technique is based on the movement of the spot of metal ion in an electric field at various pH's of background electrolyte. A graph of pH versus mobility was used to obtain information on the binary complexes and to calculate stability constants. The stability constants of the ML and ML_2 binary complexes of [mercury(II) – proline], [nickel(II) – proline] and [lead(II) – proline] have been found to be (8.74 ± 0.04; 7.43 ± 0.03), (7.23 ± 0.01; 5.78 ± 0.03) and (4.93 ± 0.02; 3.25 ± 0.05) (logarithm stability constant values), respectively at ionic strength 0.1 M ($HClO_4$) and a temperature of 35° C.

Keywords: Electrophoretic method, Mercury(II) complexes, Nickel(II) complexes, Lead(II) complexes, Proline, Stability constant.

Received : 01.03.2013; Accepted : 22.10.2013

Introduction

Quantitative indication of the process of forming a complex comes from the evaluation of the stability constants which characterize the equilibria corresponding to the successive addition of ligands. That is, we can consider the steps

$$M + L \leftrightarrows ML$$

$$ML + L \leftrightarrows ML_2$$

$$\vdots \qquad\qquad \vdots$$

$$ML_{n-1} + L \leftrightarrows ML_n$$

These are characterized by equilibrium constants K_1, K_2 ….., K_n such that

$$K_1 = [ML] / [M][L]$$
$$K_2 = [ML_2] / [ML][L] \text{ and}$$
$$K_n = [ML_n] / [ML_{n-1}][L]$$

These constants K, are termed as stepwise formation constants. An alternative formulation is to consider the overall formulation reaction

$$M + nL \quad ML_n$$

Characterized by the n^{th} overall formation constant βn

$$\beta_n = [ML_n] / [M][L]^n = K_1 .. K_2 \ldots\ldots K_n$$

Metal complexes play an important role in various biological systems, hence the formation, stability and reactivity of these complexes have been an active field of research [1,2]. Nickel has classified as beneficial metal and lead as well as mercury as toxic metals in biological systems. The mercury, nickel and lead have significant biomedical applications but are toxic at higher concentration [3-6]. Proline or pyrrolidine – 2 – carboxylic acid ($C_5H_9NO_2$) is an amino acid found in protein. Proline is hygroscopic, colourless crystals with melting point 205º C. Proline has several significant applications in biological systems [7-9]. The paper electrophoretic technique usually suffers from a number of defects. Temperature changes during electrophoresis, capillary flow on paper, electro – osmosis and adsorption affect the mobility of charged moieties [10]. The present technique is free from these destroying factors and very convenient in use. It gives results in fair agreement with the accepted literature values.

Communications [11-12] from our laboratory described a new method for the study of metal complexes. A search of literature indicate few reports on Ni (II) – proline binary complexes and no report on Hg (II) / Pb (II) – proline binary complexes. In view of this, attempts were made to establish the optimum conditions for metal (II) – proline complex formation. In addition, present paper describes a paper electrophoretic method for the determination of the stability constants of these complexes.

Experimental Section

Instruments

Systronics (Naroda, India) paper electrophoresis equipment horizontal-cum-vertical type, model 604, has been used. The apparatus consisted of a PVC moulded double tank vessel. In our laboratory significant change in the instrument has been made. Two hollow rectangular plates covered with thin polythene sheets have been used through which thermostated water is run for controlling the temperature. The tanks were closed with a transparent PVC moulded lid. The whole assembly is tight, which prevent moisture changes, which may upset the equilibria in a paper strip. This assembly design thus keeps to a minimum the disturbing effects of evaporation from the unwanted liquid flow in the paper. Each electrolyte tank contains a separate electrode chamber. The auxillary unit is specially designed to operate either voltage mode or on current mode. 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. The electrophoresis cell showing sandwiched paper strips and water supply are shown in Figure 1.

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 [13, 14]. 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 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.

Background electrolyte (BGE)

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

Procedure

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. The hollow base plate in the instrument was made horizontal using a spirit level and 150 – ml volume of BGEs containing 0.1 M perchloric acid and 0.01 M proline was placed in each of the two tanks of the electrophoretic apparatus. The papers become moistened with the BGEs 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 15 minutes to insure wetting of strips. Subsequently 200 Volts potential was applied between the tank solutions to initiate electrophoresis. The electrophoresis was carried for 60 minutes after which the paper strips were taken out by means of glass rod, dried on a horizontal platform and the spots detected. The dried paper strips with metal ion and glucose spots are shown in Figure 2. The observations were repeated for different pH values of BGE (variation in pH was made by addition of sodium hydroxide solution). The differences in the distances in the duplicates were noted for the calculation. The distance travelled toward the anode was assumed to be negative and that toward cathode positive. The actual distance of the sample plot was measured after taking into account the distance travelled by the reference glucose spot. Ionophoretic observations on metal ions were recorded at various pH values of the BGEs obtained by adding sodium hydroxide solution, the ionic strength being maintained at 0.1 M. The observed mobility of migrant was calculated by using the formula.

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

After applying the correction factor the observed mobility is given as

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

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 scheme for paper electrophoresis set up is shown in Figure 3. The mobility of metal / complex ion spots on the paper strips were thus calculated and are reported with different pH values (Figure 4).

Results

The electrophoretic mobility of the metal spot against pH gives a curve with a number of plateaus as is shown in Figure 4. 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 in the beginning corresponds to a region in which metal ions are uncomplexed. In lies in a low pH region where the concentration of the protonated species of proline is obviously maximum, hence it is concluded that this protonated species of proline is non-complexing. The mobility of uncomplexed Hg (II), Ni (II) and Pb (II), metal ions are 12.32, 10.34 and 12.65 cm^2 $volt^{-1}$ min^{-1} x 10^{-3}, respectively. Beyond this range metal ion spots have progressively decreasing mobility and hence complexation of the metal ion should be taking place with other ionic species of proline, the concentration of which increases progressively with increase of pH.

Figure 4 reveals a second plateau with positive mobility indicating the formation of 1:1 complex of cationic nature. The mobilities of 1:1 complex of Hg (II), Ni (II) and Pb (II) are 6.16, 5.28 and 6.38 cm^2 $volt^{-1}$ min^{-1} x 10^{-3}, respectively. Further increase of pH gives rise to a third plateau with zero mobility indicating the formation of 1:2 complexes of neutral nature.

Further increase of pH has no effect on the mobility of metal ions, which indicates no further interaction between metal ions and ligands. It is significant that these studies give clear evidence of the complexation of anionic species of proline with metal ions forming two binary complexes of 1:1 and 1:2 composition. In general, the complexation of metal ions with proline anion may be represented as:

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

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

Where M^{2+} is Hg^{2+} Ni^{2+} and Pb^{2+} metal ions; $[L^-]$ is the proline 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 complex. The spot is moving under the influence of electric field and the overall mobility is given by equation of Jokl [15].

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

Where $[HpL]^x$ is the concentration of general complex species; β_{xp} is the overall mobility constant of the complex; $u_{x \cdot p}$ 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 following useful form:

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

where u_0, u_1 and u_2 are mobilities of uncomplexed , 1:1 and 1:2 metal complexes, respectively. Equation (4) has been used for calculating stability constants of the complex of metal ions with proline.

The dissociation constant of pure proline (k_1 = $10^{1.90}$; k_2 = $10^{10.03}$) were obtained by same paper electrophoretic technique. The mode of dissociation of pure proline can e represented as:

$$\overline{[CH_2\ CH_2\ CH_2\ CH\ (NH_2^+)\ COOH]}$$

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

$$\overline{[CH_2\ CH_2\ CH_2\ CH\ (NH_2^+)\ COO]}$$

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

$$\overline{[CH_2\ CH_2\ CH_2\ CH\ (NH)\ COO^-]}$$

With the help of dissociation constants of pure proline, the concentration of the ligating proline $[L^-]$ is calculated.

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

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

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

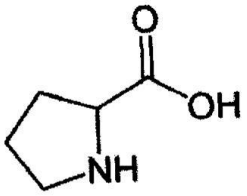
Discussion

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

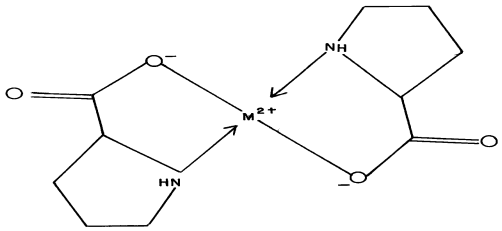
mercury(II) > nickel(II) > lead(II)

The second stability constant values are found to be lower in comparison with the first stability constant in each case. This may be due to the decrease in coordinating tendency of the ligand with higher state of aggregation [16]. It is also clear from Table 1 that mercury(II) – proline and lead(II) – proline have maximum and minimum stability constant values. Therefore it can be inferred that mercury(II) and lead(II) cations have greater and lesser electron affinity with oxygen donor ligand.

The molecular structure of pure proline is given below:



The stability constants of metal complexes can be very easily calculated by this technique, therefore present method has significant advantages over other methods (viz: polarographic, potentiometric, solubility etc.) reported in Chemical Literature for the determination of stability constants of metal complexes. According to standard deviation (statistics) the precision of the method is limited to that of paper electrophoresis, and uncertainty in the result is ± 2 %. Hence, it cannot replace the most reliable methods, even though it is a new approach deserving further development. It is also observed from the Table 1 that calculated values of stability constants at a temperature of 35º C are higher in comparison to literature values of stability constants at 25º C. The proposed structure for the ML_2 complexes may be given as:



The first and second stability constants of mercury (II), nickel (II) and uranyl (II) complexes with hydroxyproline are found to be [(8.65 ± 0.02, 7.14 ± 0.05); (7.02 ± 0.04, 5.56 ± 0.07); (4.71 ± 0.01, 3.11 ± 0.03)], respectively at ionic strength 0.1 M and a temperature of 35º C [17]. The stability constants of metal (II) – proline complexes are found to be higher than metal (II) – hydroxyproline complexes. The lower stability constant values in case of metal (II) – hydroxyproline may be due to presence of a hydroxyl group in the ring of hydroxyproline. High molecular weight of hydroxyproline in comparison to proline may be another factor for the lower stability constant values of metal (II) – hydroxyproline complexes.

Conclusions

The following conclusions can be shown from the present study:

1. Mercury (II), nickel (II) and lead (II) have significance in biological systems but as such they are toxic, and proline may be used to reduce the level of these metal ions in the biological systems.
2. Mercury (II) – proline complexes is found to have maximum stability constant among the three ML_2 complexes studied.
3. The biologically significant mercury (II), nickel (II) and lead (II) complexes with proline can be prepared on large scale at a particular pH of the background electrolyte solution.
4. The present novel electrophoretic technique is very helpful in finding that complex system is formed or not, and if formed, its stability constant can also be determined.

References

1. S. E. Sherman and S. J. Lippard, *Chem. Rev.* **87**, 1153 (1987).

2. P. K. Bhattacharya, *J. Sci. Ind. Res*. **40**, 382
3. (1981).
4. M. A. K. Khan and F. Wang, *Environ. Toxicol. Chem*. **28(8),** 1567 (2009).
5. A. Prakash, M. P. Gangwar and K. K. Singh, *J. Dev. Biol. Tissue Eng*. **3(2),** 13 (2011).
6. K. Grubel, A. L. Fuller, B. M. Chamberst, et al., *Inorg. Chem*. **49(3),** 1071 (2010).
7. E. A. Silveira, J. H. F. Lizardo, L. P. Souza, et al., *Braz. J. Med. Biol. Res*. **43(5)**, 492 (2010).
8. L. Szabados and A. Savoure, *Trends Plant Sci*. **15(2)**, 89, (2009).
9. C. E. Umebese, T. O. Olatimilehim, and T. A. Ogunsusi, *Am. J. Agri. Biol. Sci*., **4(3),** 224 (2009).
10. R. J. Collingham and M. Griffin, *Amino Acids* **36(4)**, 659 (2009).
11. D. J. Shaw, *Electrophoresis*, Academic Press, London, 1969.
12. B. B. Tewari, *J. Mex. Chem. Soc*. **52(3)**, 219 (2008).
13. B. B. Tewari, *J. Chem. Eng. Data* **55(5)**, 1779 (2010).
14. I. M. Kolthoff and R. Belcher, *Volumetric Analysis*, Vol. **3**, Interscience Publisher Inc., New York, 1957.
15. A. I. Vogel, *Text Book of Quantitative Inorganic Analysis*, including Elementary Instrumental Analysis, 4th Edition, Longman's, London, 1978.
16. V. Jokl, *J. Chromatogr*. **13**, 451, (1964).
17. J. D. Joshi, *Indian J. Chem*. **21**, 446, (1982).
18. B. B. Tewari, *Bolivan J. Chem*. **28(1),** 6 (2011).
19. A. E. Martell and R. M. Smith, *Critical Stability Constants, Vol. **1***, Amino Acids, Plenum Press, New York, London, 1974, p. 69.
20. D. D. Perrin, *Stability Constants of Metal Ion Complexes*, Part B, Organic Ligands, Pergamon Press, Oxford, UK, IUPAC Series No. **22**, 1979, p. 311.

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

Metal ions	Complexes	Stability Constant	Logarithm Stability Constant value *
Mercury(II)	ML^+	K_1	8.74 ± 0.04
	ML_2	K_2	7.43 ± 0.03
Nickel(II)	ML^+	K_1	7.23 ± 0.01 (5.95) [18] (6.15) [19]
	ML_2	K_2	5.78 ± 0.03 (4.95) [18] (5.13) [19]
Lead(II)	ML^+	K_1	4.93 ± 0.02
	ML_2	K_2	3.25 ± 0.05

Note.
Ionic strength = 0.1 M; temperature = 35º C; M = metal cations (Hg^{2+}, Ni^{2+} and Pb^{2+}); L = Ligand (proline);

proline anion = $[\overbrace{CH_2\,CH_2\,CH_2\,CH}\,(NH)\,COO^-]$
*Literature values are given in parentheses at 25ºC.

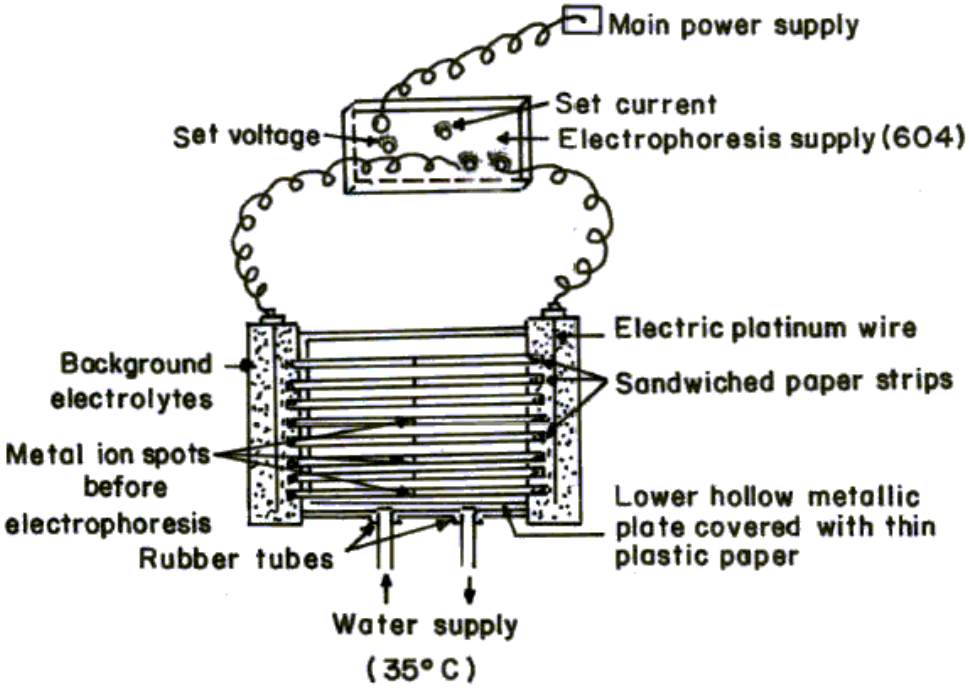


Fig. 1 Electrophoresis cell showing sandwiched paper strips.

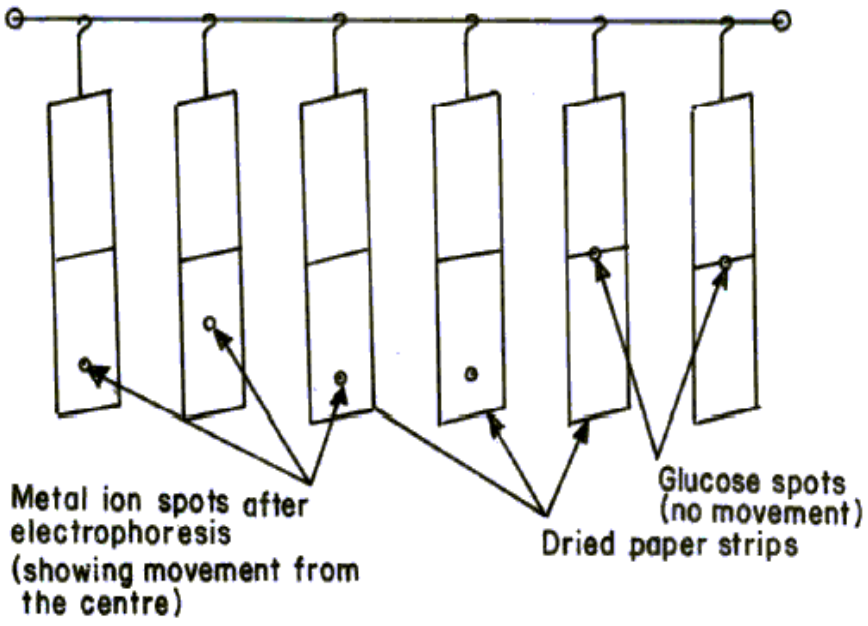


Fig. 2 Paper strips showing position of metal ion spots after electrophoresis

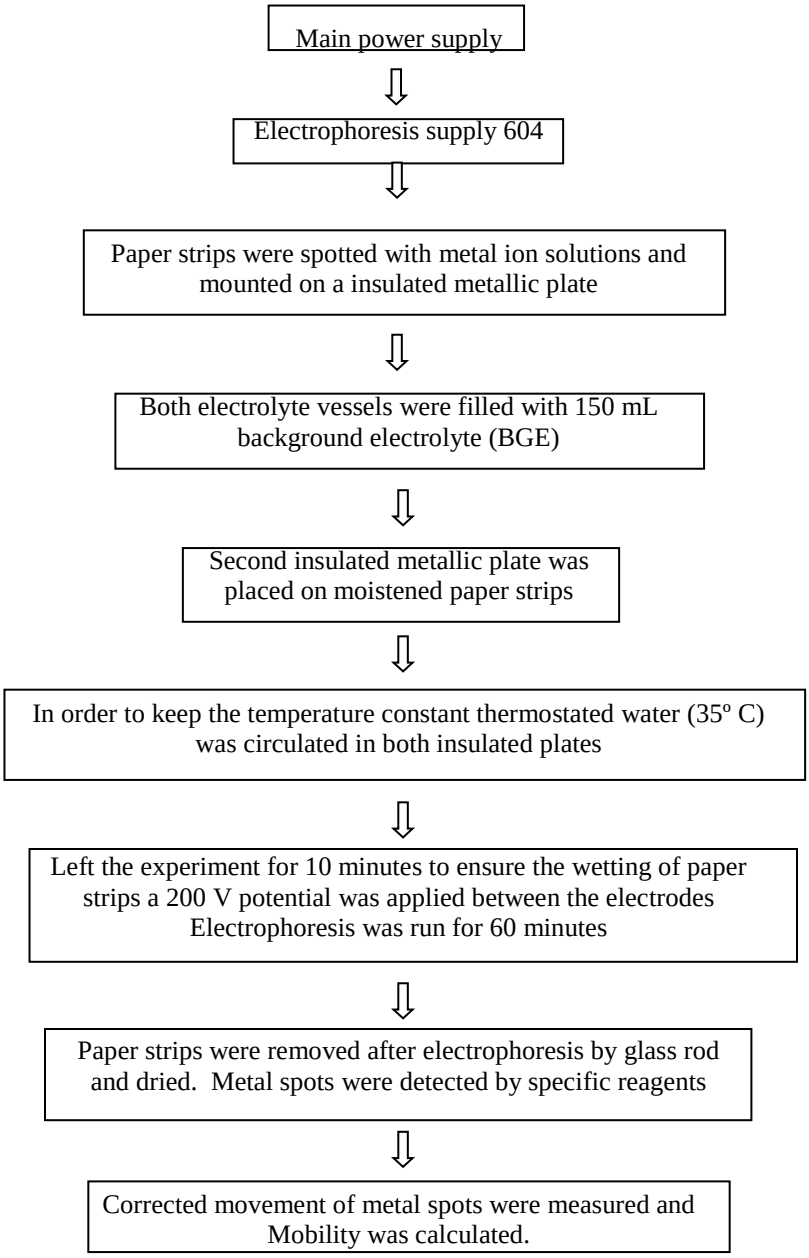


Fig. 3 : The scheme for paper electrophoresis set-up

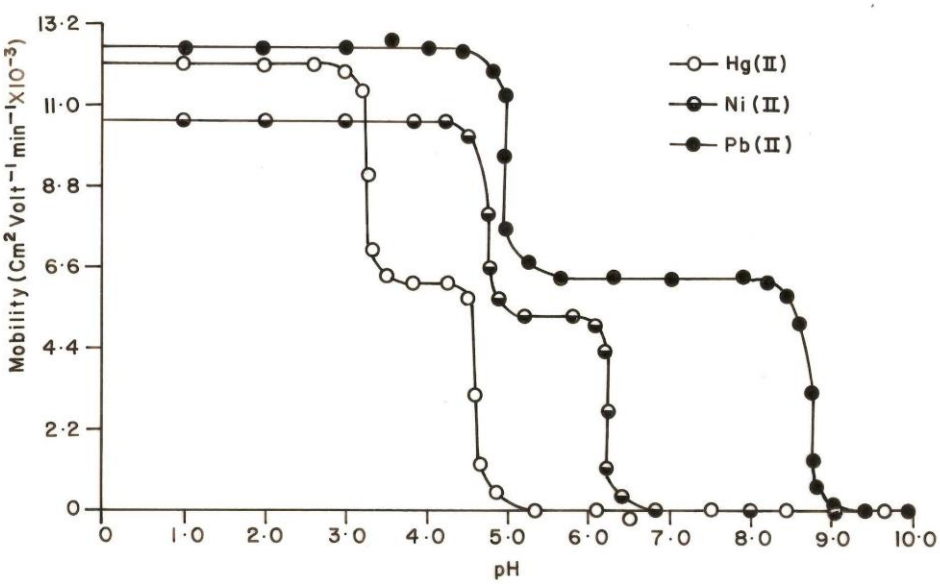


Fig. 4 : Mobility curves for the metal(II) – proline systems. —○— = Hg(II) – proline = —◐— nickel(II) – proline —●— = Pb(II) – proline. Background electrolyte = 0.1 M perchloric acid and 0.01 M proline. pH was maintained by addition of sodium hydroxide. Concentration of Hg^{2+}, Ni^{2+} and Pb^{2+} = 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).