

**PAPER IONOPHORETIC TECHNIQUE IN THE STUDY OF BIOLOGICALLY
IMPORTANT BERYLLIUM(II) / COBALT(II)-HOMOSERINE
BINARY COMPLEXES IN SOLUTION**

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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. A method, involving the use of paper electrophoretic technique is described for the study of binary complex system in solution. Present method is based upon the migration of a spot of metal ions on a paper strip at different pH's of background electrolyte. A graph of pH against mobility gives information about the binary complexes and permits calculation of their stability constants. The first and second stability constants of [Be(II)-homoserine] and [Co(II)-homoserine] complexes were found to be $(7.13 \pm 0.02; 6.11 \pm 0.09)$ and $(4.27 \pm 0.07; 3.47 \pm 0.11)$ (logarithm stability constant values) for Be(II) and Co(II) complexes, respectively, at ionic strength of 0.1 mol/L and a temperature of 35 °C.

Key words: paper electrophoresis technique; overall mobility; beryllium(II) complexes; cobalt(II) complexes; homoserine; stability constant

**ЈОНОФОРЕТСКА ТЕХНИКА НА ХАРТИЈА ПРИ ИЗУЧУВАЊЕ НА БИОЛОШКИ ВАЖНИ
ХОМОСЕРИНСКИ БЕРИЛИУМ(II) / КОБАЛТ(II) БИНАРНИ КОМПЛЕКСИ ВО РАСТВОР**

Квантитативната индикација на процесот на образување комплекс потекнува од процената на константата на стабилност која ја карактеризира рамнотежата која соодветствува на последователното додавање на лигандите. Опишан е метод кој вклучува употреба на електрофореза на хартија за проучување на бинарен комплексен систем во раствор. Методот е заснован на миграција на петна од метални јони врз хартиена лента при различни вредности на рН на основниот електролит. График на зависноста на рН од мобилноста дава информација за бинарните комплекси и овозможува пресметка на нивните константи на стабилност. Најдено е дека првата и втората константа на стабилност на комплексите на [Be(II)-хомосерин] и [Co(II)-хомосерин] изнесуваат $7,13 \pm 0,02; 6,11 \pm 0,09$ и $4,27 \pm 0,07; 3,47 \pm 0,11$ (константни вредности на логаритамот на стабилноста) за комплексите на Be(II) и Co(II), соодветно, за јонска сила при 0,1 mol/L и температура од 35 °C.

Клучни зборови: електрофоретска техника на хартија; вкупна мобилност; комплекси на берилиум(II); комплекси на кобалт(II); хомосерин; константа на стабилност

1. 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].

$$K_n = K(ML_{n-1} + L = ML_n)$$

$$\beta_n = K(M + nL = ML_n)$$

Metal complexes play an important role in various biological systems [2] and in different field of chemistry [3]. Cobalt is an integral part of vitamin B₁₂. Dietary cobalt is acquired mostly from meat and milk. Deficiency may be observed in strict vegetarians. Long term vitamin B₁₂ deficiency can result in demyelination of large nerve trunks and the spinal cord, in reduced white blood cells, and in pernicious anemia. Cobalt is highly soluble in lung tissues. There are four types of cobalt disorders. First, asthma, second, anorexia, malaise and dyspnea. The third disorder is intestinal pneumonitis of the usual intestinal pneumonia type. The fourth disorder, gland cell intestinal pneumonitis is slightly more responsive to corticosteroid therapy. Beryllium is used in the space, aircraft, and nuclear industries in a variety of components. The respiratory tract is the major target organ system in humans followings the inhalation of beryllium and disease known as Chronic Beryllium Disease (CBD). CBD is treatable, but not curable. Treatment may involve use of steroids to reduce inflammation, which may slow the progress of CBD by reducing the buildup of scar tissue and delaying permanent lung damage. Beryllium and cobalt are well known for their medical applications and toxicity [4–25].

Homoserine is a α -amino acid and intermediate in the biosynthesis of three essential amino acids: methionine, threonine and isoleucine. It forms by two reductions of aspartic acid via the intermediacy of aspartate semialdehyde. Homoserine has several applications in biological systems [26–34]. Kiso [35] has done comprehensive study on paper electrophoretic migration of metal

complexes. The paper electrophoretic technique usually suffers from a number of defects. Temperature during electrophoresis, capillary flow on paper, electro-osmosis and adsorption affect the mobility of the charged moieties [36]. The technique described here is almost free from these vitiating factors. The technique is very convenient in use. It gives results in fair agreement with the accepted literature values.

Publications [37–40] from our laboratory described a new method for the study of binary complexes. The present work is an extension of the above-mentioned technique and observations on the binary system viz. Be(II)/Co(II)-homoserine are herein reported.

2. EXPERIMENTAL SECTION

2.1. Instruments

Electrophoresis equipment from Systronics (Naroda, India) model 604 was used. The apparatus consists of a poly(vinyl chloride) (PVC) moulded double tank vessel. In order to avoid errors due to the heat generated during electrophoresis, two hollow rectangular plates each weighing 1 kg, and covered with thin plastic paper were used through which thermostated water circulated. The tanks were closed with a transparent PVC molded lid to prevent moisture changes that might upset the equilibrium in the paper strip. Each electrolyte tank contained a separate Pt-wire electrode. Applied voltage was from a stabilized source.

Measurements of pH were made with an Elico model L₁₋₁₀ pH meter using a glass electrode. Electrophoresis cell showing sandwiched paper strips and water supply is shown in Figure 1.

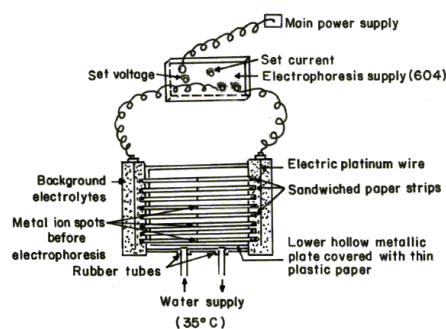


Fig. 1. Electrophoresis cell showing sandwiched paper strips

2.2. Chemicals

Beryllium(II) and cobalt(II) metal perchlorate solutions were prepared by the precipitation of metal carbonate from a 0.1 mol/L solution of Be(II) and Co(II) chlorides with solution of sodium carbonate (chemically pure grade, BDH, Poole, UK). The precipitates were washed with boiling water and treated with calculated amounts of 1 % perchloric acid. They were heated and filtered. The metal contents of the filtrates were determined and final concentration was kept at 0.005 mol/L.

A 0.1 % solution of 1-(2-pyridylazo)-2-naphthol (PAN) (Merck, Darmstadt, Germany), in ethanol was used for detecting the metal ions. 0.005 mol/L 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 (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 sodium hydroxide, when a black spot was formed. Paper strips showing position of metal ion spots after electrophoresis is shown in Figure 2.

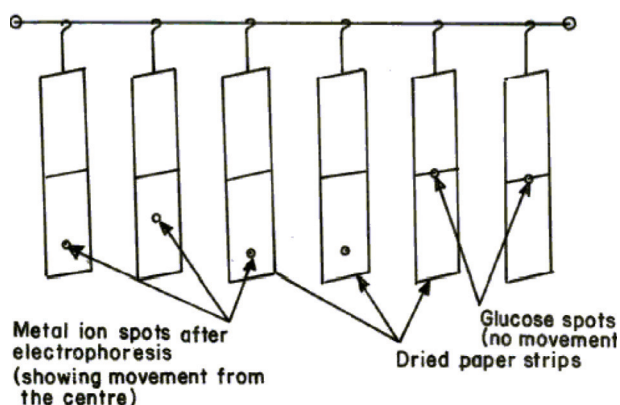


Fig. 2. Paper strips showing position of metal ion spots after electrophoresis

2.3. Background electrolyte

Stock solution of 5.0 mol/L perchloric acid was prepared from its 70 % solution (SDS, Analytical Reagent grade). 2.0 mol/L sodium hydroxide (Analytical Reagent grade) and 0.5

mol/L homoserine (BDH, Poole, UK) solutions were prepared. Each solution was standardized using the appropriate method.

The background electrolytes used in the study of binary complexes were 0.1 mol/L perchloric acid and 0.01 mol/L homoserine. The binary system was maintained at various pH values 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 strips were spotted with the metal ion solution along with additional two spotted with glucose using 1.0 μ L pipette and then mounted on the insulated plate. Each of the two electrolyte vessel was filled with 150 mL of background electrolyte containing 0.1 mol/L perchloric acid and 0.01 mol/L homoserine. 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 of strips. Subsequently a direct 200 V potential was applied between the electrodes. Electrophoresis was carried out 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 mol/L. 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.

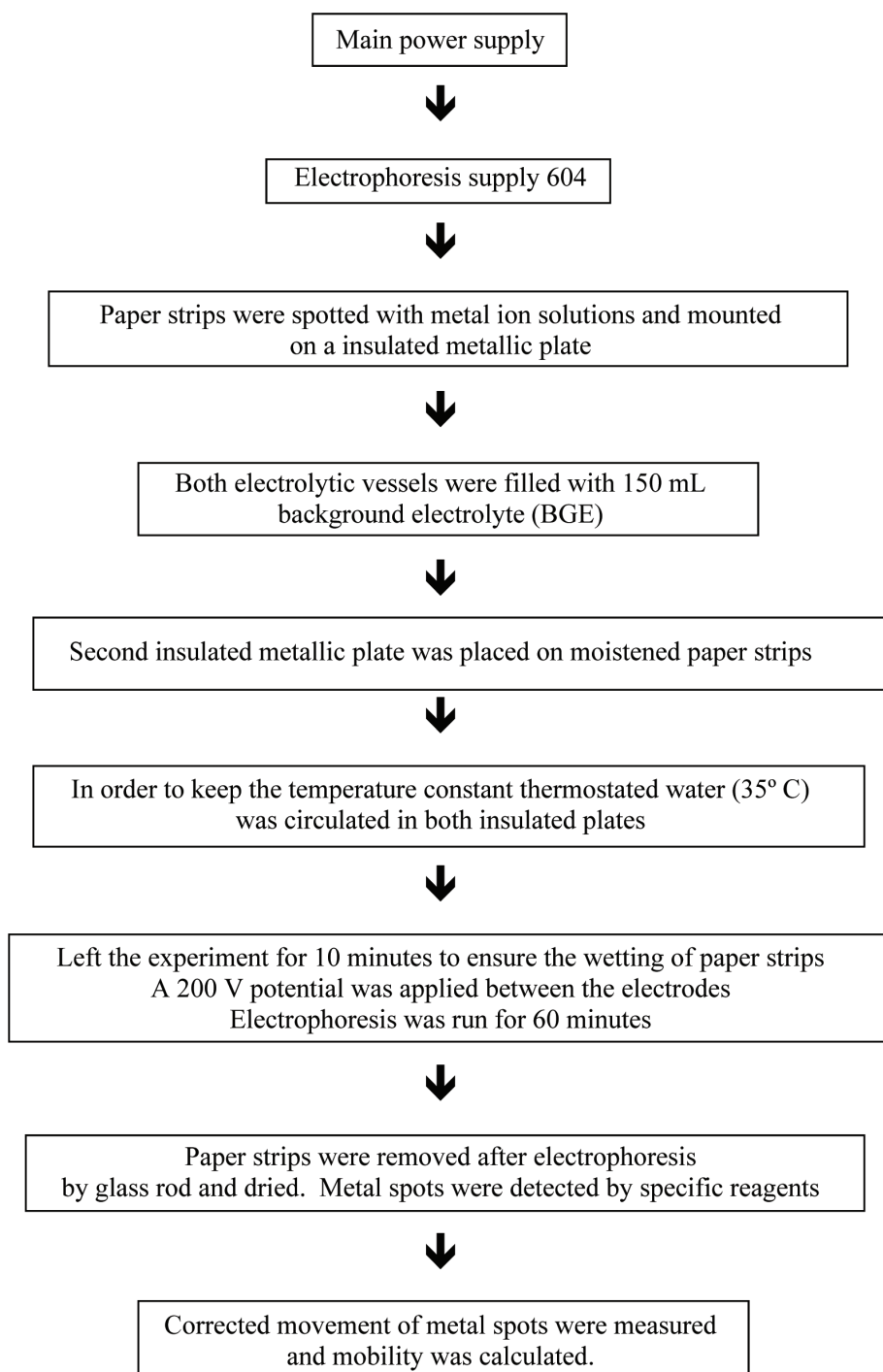


Fig. 3. The scheme for paper electrophoresis set-up

The protonation constants of pure homoserine were determined by same paper electrophoresis technique. The two paper strips were spotted with pure homoserine along with two with glucose using 0.1 mol/L 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/homoserine spots are reported with pH values. The individual speeds of the duplicate spots were found to be fairly equal. The scheme for paper electrophoresis set-up is shown in Figure 3.

3. RESULTS

Metal complexes play a significant role in several biological systems and in different field of chemistry. The plot of electrophoretic mobility of a metal spot vs. pH gives a curve with a number of plateau is shown in Figure 4. A plateau is obviously an indication of a pH range, where the speed is virtually constant and indicates the formation of a single complex species. There are three plateaus in the curves of both beryllium(II) and cobalt(II) cations. The first plateau in each case with positive mobility in the lower pH range is due to non-complexed cations, in this pH range homoserine is present as a non-complexing species $[\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_3^+)\text{COOH}]$. Figure 4 disclose that beryllium(II) and cobalt(II) ions form their first complex movements towards negative electrode. Hence, one homoserine anionic species $[\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COO}^-]$ must have combined with beryllium(II) and cobalt(II) to give, 1:1, $[\text{Be}\{\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COO}\}]^+$ and $[\text{Co}\{\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COO}\}]^+$ 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 homoserine $[\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COO}^-]$ must have combined with metal ions to give 1:2, $[\text{Be}\{\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COO}\}_2]$ and $[\text{Co}\{\text{CH}_2(\text{OH})\text{CH}_2\text{CH}(\text{NH}_2)\text{COO}\}_2]$ complexes, respectively.

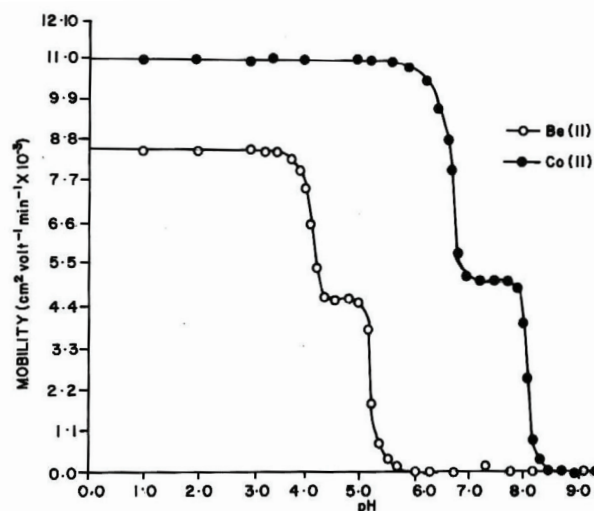


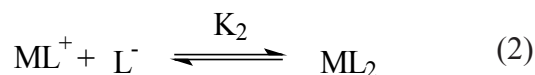
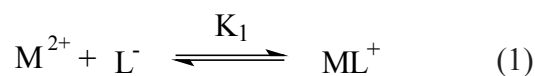
Fig. 4. Mobility curve for the metal(II)-homoserine systems.

○ = Be(II)-homoserine

● = Co(II)-homoserine.

pHs were maintained by addition of sodium hydroxide. 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).

Further increase of pH has no effect on the mobility of metal ions, which indicates no further interaction between metal ions and ligand. The predominant ligating properties of unprotonated anionic species of homoserine have also been reported to ruling out any such property of zwitterions [41]. In general the complexation of metal ions with homoserine anion may be represented as:



where M^{2+} is Be^{2+} and Co^{2+} metal ions; $[\text{L}^-]$ is the homoserine 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):

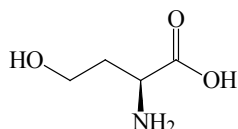
4. DISCUSSION

It is clear from Table 1 that values of first and second stability constants of ML and ML_2 complexes follow the order

beryllium(II) > cobalt(II).

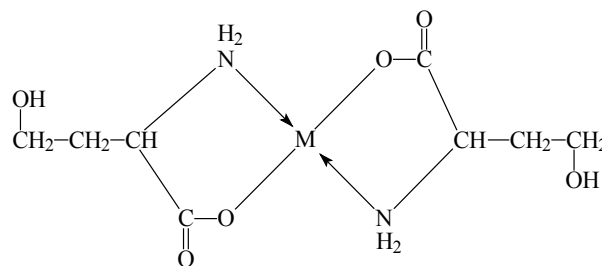
The values of second stability constants are found to be lower in comparison to first stability constant in each case, this may be due to the decrease in coordinating tendency of ligand with higher state of aggregation. High stability constant values of beryllium(II)–homoserine complexes indicate strong bonding between beryllium(II) cation and homoserine anion. Whilst low stability constant value of cobalt(II)–homoserine complexes indicate weak bonding between cobalt(II) cation and homoserine anion.

According to standard deviation (statistics) the precision of the method is limited to that of paper electrophoresis, and uncertainty in the result is $\pm 5\%$. Hence, it cannot immediately replace the most reliable methods, even though it is new approach deserving further development. The molecular structure of homoserine is given below:



To examine the possibility of hydrolysis of beryllium(II) at higher pH range, experiments have been performed at two concentrations of the ligand: 0.01 mol/L and 0.001 mol/L. The mobility curves show that the plateaus at lower ligand concentration are shifted towards higher pH range, but the calculated stability constants are found to be the same in the two cases. Thus the constant obtained is independent of the pH indicating that hydrolysis of beryllium(II) can be ignored here.

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



5. CONCLUDING REMARKS

Several conclusions can be drawn from the present study. Beryllium(II) and cobalt(II) are significant for biological systems as such, because they are essential and at the same time are toxic. The homoserine may be used to reduce the level of these metal ions in the biological systems. Beryllium(II)–homoserine complexes are found to have higher stability constant values in comparison to cobalt(II)–homoserine complexes. Biologically important beryllium(II) and cobalt(II) complexes with homoserine can be prepared on large scale at a particular pH of the background electrolyte solution. The ML_2 complexes are found to have lower stability constant values in comparison to ML complexes. 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.

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