Interaction of Hydroxyproline with Bivalent Metal Ions in Chemical and Biological Systems

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ABSTRACT

A recent technique involving the use of paper electrophoresis is described for the study of equilibria in binary complex systems in solution. The stability constants of the ML and ML$_2$ complex species of some metal ions, namely beryllium(II) and cobalt(II), with hydroxyproline were determined in 0.1 mol L^{-1} perchloric acid and a temperature of 35 °C. The logarithm stability constants of the ML and ML$_2$ complexes of hydroxyproline were found to be 7.39 ± 0.07 and 5.90 ± 0.03, and 4.73 ± 0.08 and 3.71 ± 0.11 for the beryllium(II) and cobalt(II) complexes, respectively.

KEYWORDS

Hydroxyproline, paper electrophoresis, stability constants.

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]

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

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

A knowledge of the stabilities of binary complexes plays an important role in many metabolic and toxicological functions. Several attempts have been made to correlate stabilities of metal ligand complexes with their biocidal activities.[2] In this study the complexes of beryllium and cobalt were studied since these metal ions have several significant applications in biological systems.[3–20] Beryllium is one of the most toxic elements and is a class A carcinogen. Skin exposure to beryllium can cause beryllium sensitization, while inhalation can lead to a beryllium lung burden and eventually chronic beryllium disease (CBD), a currently incurable and often fatal immunological lung disease. The current treatment for CBD is an anti-inflammatory medication that lowers the immune response to beryllium. However, the treatment specifically fails to remove beryllium from the lung and exhibits side effects including susceptibility to infections, water retention, and loss of bone density. Cobalt is a mineral required by the body for blood formation. It is an integral part of vitamin B$_{12}$ (cobalamin), a vitamin essential for producing red blood cells and maintaining the nervous system. Cobalt activates several metabolic enzymes and is necessary for myelin formation and thyroid hormone production. It supports red blood cell production and is essential for the metabolism of fats and carbohydrates, the synthesis of proteins and the production of DNA and RNA. Cobalt is used to treat anaemia in pregnant woman, because it stimulates the production of red blood cells. It is also used by athletes to increase the oxygen-carrying ability of the blood. The complexes formed by beryllium(II) and cobalt(II) with the ligand hydroxyproline were investigated since this ligand is important in biological systems.[21–27]

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Hydroxyproline is a non-essential amino acid, which means that it is manufactured from other amino acids in the liver and it does not have to be obtained directly through the diet. Hydroxyproline is necessary for the construction of the body's major structural protein, collagen. Defects in collagen synthesis lead to easy bruising, internal bleeding, breakdown of connective tissue of the ligaments and tendons, and increased risk to blood vessel damage. The complexes were studied by the paper electrophoretic technique which usually suffers from a number of defects, namely, temperature during electrophoresis, capillary flow on paper, electro-osmosis and adsorption all affect the mobility of charged moieties.[28] The present technique described here is almost free from these negative factors and is very convenient to use. It gives results in fair agreement with the accepted literature values. Communications[29–31] from our laboratory have described a new method for the study of metal complexes. A search of the literature indicated few reports on Co(II)-hydroxyproline binary complexes and no reports on Be(II)-hydroxyproline binary complexes. In view of this, attempts were made to establish the optimum conditions for metal(II)-hydroxyproline complex formation. In addition, the present paper describes a paper electrophoretic method for the determination of the stability constants of Be(II)/Co(II)-hydroxyproline binary complexes.

2. Experimental

2.1. Apparatus

A Systronics (Naroda, India) Model 604 electrophoresis system was used. The apparatus consisted of a polyvinyl chloride (PVC) moulded double tank vessel. In our laboratory significant changes to the instrument have been made. Two hollow rectangular iron plates, each weighing 1 kg, and covered with thin polythene sheets, have been used through which thermostatted water circulated for controlling the temperature. The tanks were closed with a transparent PVC moulded lid. The whole assembly was made tight to prevent moisture changes, which might upset the equilibria in the paper strip. This assembly design thus keeps to a minimum the disturbing effect of evaporation from the unwanted liquid flow in the paper strips. Each electrolyte tank contains a separate electrode chamber in which a Pt-wire anode
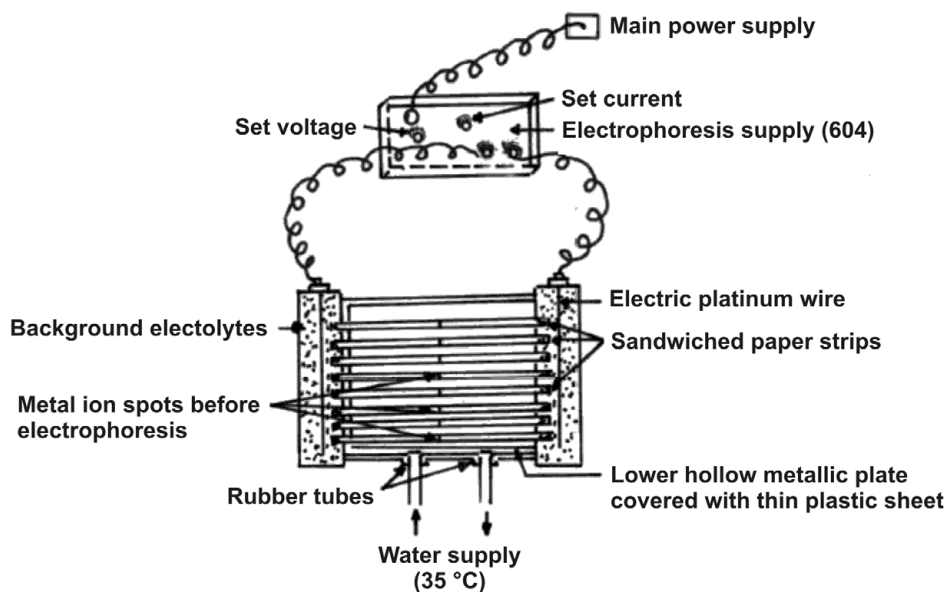
Figure 1 Electrophoresis cell showing sandwiched paper strips.

and a cathode are placed. The applied voltage was from a stabilized source. The electrophoresis cell showing sandwiched paper strips and water supply are shown in Fig. 1. Whatman No. 1 filter paper for chromatography was used for the purpose of electrophoresis. An Elico (Hyderabad, India) model L_{1-10} pH meter using a glass and calomel electrode assembly working on 220 V/50 Hz established a.c. mains, was used for the pH measurements. The pH meter was calibrated with buffer solution of pH 7.0.

2.2. Chemicals

2.2.1. *Metal Solutions*

Beryllium(II) and cobalt(II) metal perchlorate solutions were prepared by the preliminary precipitation of metal carbonates from a 0.1 mol L^{-1} solution of sodium carbonate (Analytical Reagent 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 was determined and the final concentration was kept at 0.005 mol L^{-1}.[32,33]

2.2.2. *Standardization of Metal Perchlorate Solutions*

Beryllium(II) perchlorate solution was standardized by a gravimetric method. A volume of 25 mL of beryllium perchlorate solution was taken in a beaker and to it ammonia solution was added dropwise until the hydroxide started to precipitate. The precipitate was redissolved by adding a few drops of 0.1 mol L^{-1} hydrochloric acid. A mass of 1 g of ammonium chloride and a sufficient amount of EDTA solution was added to it to complex all other trace metal ions (if present). Again a small amount of ammonia solution was added to it and the contents filtered through a gravimetric filter paper. The paper was placed in a silica crucible, dried and then heated by an open flame. The crucible was cooled in a covered desiccator over magnesium perchlorate and weighed immediately when cool. The cobalt(II) perchlorate solution was standardized by a volumetric method. The cobalt(II) solution was taken in a conical vessel to which standard EDTA solution was added in known excess and to it were then added 5 mL of pH 5 buffer and 3–5 drops of 1-(2-pyridylazo)-2-naphthol (PAN) indicator. The solution was

diluted to 60 mL. The uncomplexed EDTA was titrated against standard copper(II) perchlorate solution until the colour changed first to violet. A few drops of EDTA solution were then added to restore the yellow colour. The amount of Co(II) present could be determined from the known amount of EDTA consumed by it.

2.2.3. *Detecting Reagent for Metal Ions and Glucose*

A 0.1 % solution of 1-(2-pyridylazo)-2-naphthol (PAN) (Merck, Darmstadt, Germany) in ethanol was used for detecting the metal ions. A 0.005 mol L^{-1} glucose (Analytical Reagent grade, BDH) solution 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 solution and then with a 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 Fig. 2.

2.2.4. *Background Electrolyte (BGE)*

The BGE used in the study of binary complexes was 0.1 mol L^{-1} perchloric acid and 0.01 mol L^{-1} hydroxyproline. The binary system was maintained at various pHs by the addition of sodium hydroxide. Stock solutions of 5.0 mol L^{-1} perchloric acid were prepared by dilution of the 70 % solution (Analytical Reagent grade, SDS). Stock solutions of 0.5 mol L^{-1} hydroxyproline (BDH) and 2.0 mol L^{-1} sodium hydroxide (Analytical Reagent grade) solutions were also prepared.

2.3. Procedure

For recording observations of particular metal ions, two paper strips were spotted with the metal ion solution along with two additional strips spotted with glucose using a 0.1 μL pipette and then mounted on the insulated plate. The hollow base plate in the instrument was made horizontal using a spirit level and a 150 mL volume of BGE containing 0.1 mol L^{-1} perchloric acid and 0.01 mol L^{-1} hydroxyproline was placed in each of the two tanks of the electrophoretic apparatus. The paper became moistened with the BGE solution due to diffusion. The second insulated plate was placed on paper strips and then thermostatted water (35 °C) was circulated in the plates to keep the temperature
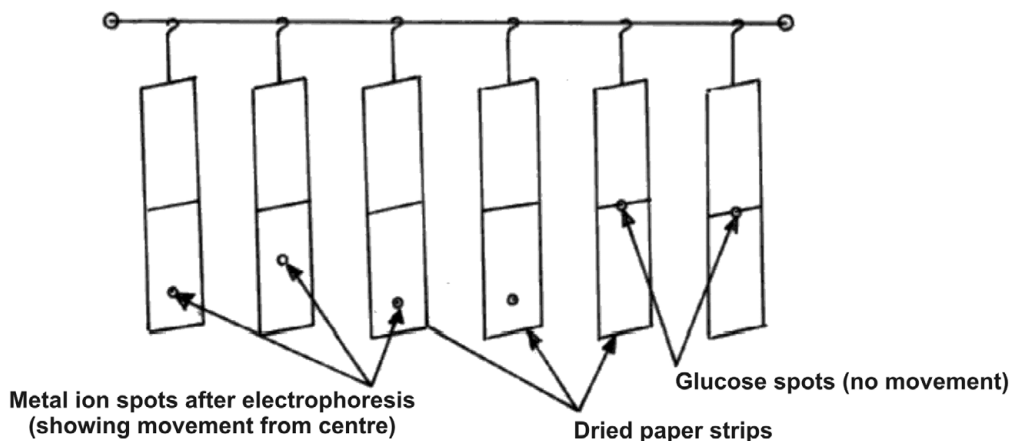
Figure 2 Paper strips showing position of metal ion spots after electrophoresis.

constant. The lid was then placed on the instrument to make it airtight. It was left for 15 min to ensure wetting of the strips. Subsequently, a 200 V potential difference was applied between the tank solutions to initiate electrophoresis. The electrophoresis was carried out for 60 min, after which the paper strips were taken out by means of a glass rod, dried on a horizontal platform and the spots detected. 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 recorded in the duplicates were within ±5 % and the average distances in the duplicates were noted for the calculation. The distance travelled toward the anode was taken to be negative and that toward the cathode positive. The actual distance of the sample spot was measured after taking into account the distance travelled by the reference glucose spot. Ionophoretic observations of metal ions were recorded at various pH values of the BGE obtained by adding sodium hydroxide solution, the ionic strength being maintained at 0.1 mol L^{-1}. The observed mobility of migrant was calculated by using the equation:

$$U = \frac{d}{xt}$$

After applying the correction factor the observed mobility is given as:

$$U = \frac{d \pm d_G}{xt}$$

where U is the mobility of metal ion/complex ion, d is the mean of duplicate distances travelled by metal ion/complex ion, d_G is the mean of duplicate distances travelled by the glucose spot, x is the field strength and t is the time for electrophoresis. The mobilities of metal/complex ion spots on the paper strips were thus calculated and are reported at different pH values (Fig. 3). The mobilities of beryllium(II) and cobalt(II) with hydroxyproline at various pHs of background electrolyte are given in Tables 1 and 2, respectively. The dissociation constants of pure hydroxyproline were determined by using the same paper electrophoresis technique. For recording the mobility of hydroxyproline two paper strips were spotted with hydroxyproline along with two with glucose using 0.1 mol L^{-1} perchloric acid only as the BGE. The electrophoresis was carried out for 60 min as for the metal ions. The electrophoretic speed was calculated (figure not provided).
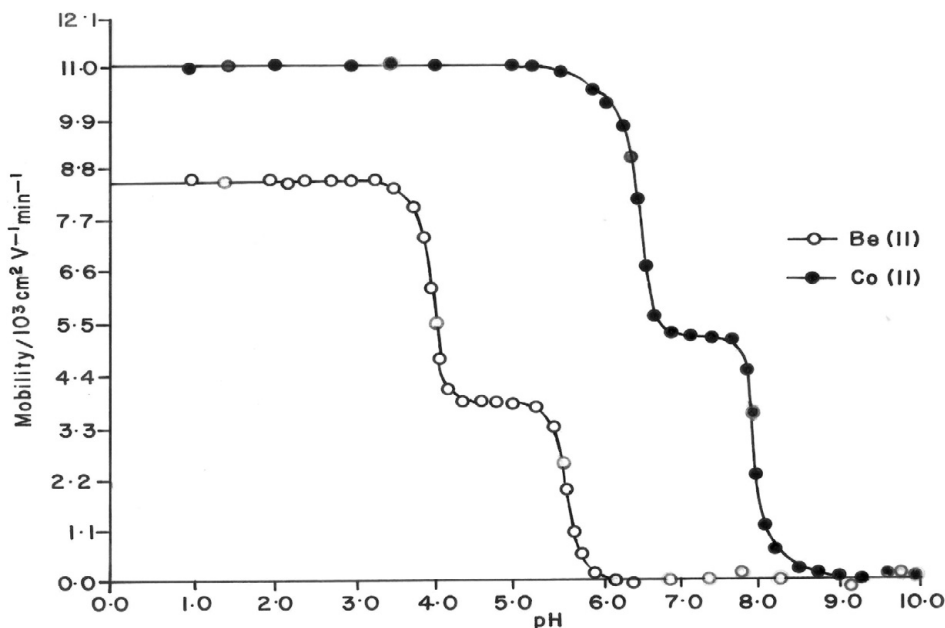

Figure 3 Mobility curves for the metal(II)-hydroxyproline systems. O Be(II)-hydroxyproline; ● Co(II)-hydroxyproline. Background electrolytes: 0.1 mol L^{-1} perchloric acid and 0.01 mol L^{-1} hydroxyproline. pH was maintained by addition of sodium hydroxide. Concentration of Be^{2+} and Co^{2+}: 0.005 mol L^{-1}. Ionic strength = 0.1 mol L^{-1}, temperature = 35 °C. The paper strips were spotted with 0.1 μL of sample solutions and glucose (for making osmotic corrections).
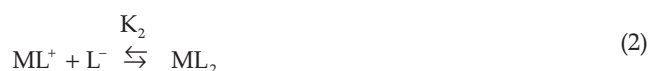
Table 1 Mobility of metal ion spot at various pH values of background electrolyte in the beryllium(II)-hydroxyproline system.

pH	Mobility/ 10^3 cm^2 V^{-1} min^{-1}	pH	Mobility/ 10^3 cm^2 V^{-1} min^{-1}
0.90	8.51	4.80	3.63
1.30	8.47	5.20	3.52
1.80	8.47	5.40	3.41
2.00	8.43	5.50	2.56
2.25	8.47	5.60	1.76
2.50	8.47	5.70	1.32
2.75	8.47	5.80	0.77
3.00	8.47	5.90	0.18
3.25	8.36	6.10	0.00
3.60	8.25	6.20	–0.02
3.75	7.37	6.90	0.00
3.85	6.27	7.45	0.00
3.90	5.42	7.80	0.02
3.95	4.62	8.30	0.00
4.10	3.85	9.20	–0.02
4.25	3.96	9.75	0.02
4.40	3.70	10.00	0.00
4.60	3.67		

Concentration of hydroxyproline in background electrolyte = 1.0×10^{-2} mol L^{-1}; concentration of Be(II) in spot = 5.0×10^{-3} mol L^{-1}; potential = 200 V; potential gradient = 7.5 V cm^{-1}; time = 60 min; temperature = 35 °C; average distance travelled by glucose spot = 0.00 cm.

Table 2 Mobility of metal ion spot at various pH values of background electrolyte in the cobalt(II)-hydroxyproline system.

pH	Mobility/ 10^3 cm^2 V^{-1} min^{-1}	pH	Mobility/ 10^3 cm^2 V^{-1} min^{-1}
0.80	10.97	6.85	5.39
1.40	11.00	7.20	5.214
2.00	11.00	7.40	5.06
2.90	11.00	7.60	5.06
3.30	11.00	7.80	4.68
3.90	11.00	7.84	3.62
5.00	11.00	7.90	2.57
5.30	10.93	8.10	1.47
5.60	10.78	8.20	0.92
5.80	10.38	8.50	0.44
5.90	10.23	8.75	0.17
6.25	9.90	9.00	0.00
6.33	9.05	9.25	0.00
6.40	8.14	9.60	0.02
6.60	6.82	10.00	0.00
6.75	5.72		

Concentration of hydroxyproline in background electrolyte = 1.0×10^{-2} mol L^{-1}; concentration of Co(II) in spot = 5.0×10^{-3} mol L^{-1}; potential = 200 V; potential gradient = 7.5 V cm^{-1}; time = 60 min; temperature = 35 °C; average distance travelled by glucose spot = 0.00 cm.

3. Results

The plot of overall electrophoretic mobility of the metal spots against pH is shown in Fig. 3. The first plateau at low pH corresponds to a region in which metal ions are uncomplexed. It is obvious that protonated ionic species of hydroxyproline, which exist in low pH ranges are non-complexing [CH$_2$CH(OH)CH$_2$CH(NH$_2^+$)COOH]. Figure 3 shows that beryllium(II) and cobalt(II) ions form their first complex movements towards the negative electrode. Hence, one hydroxyproline anionic species [CH$_2$CH(OH)CH$_2$CH(NH)COO$^-$] must have combined with beryllium(II) and cobalt(II) to give 1:1 [Be{CH$_2$CH(OH)CH$_2$CH(NH)COO}]$^+$ and [Co{CH$_2$CH(OH)CH$_2$CH(NH)COO}]$^+$ complex cations, respectively. The third plateau in each case is in the zero region of the mobility curve showing the neutral nature of the metal ligand complex. Hence, two anionic species of hydroxyproline [CH$_2$CH(OH)CH$_2$CH(NH)COO$^-$] must have combined with metal ions to give 1:2 [Be{CH$_2$CH(OH)CH$_2$CH(NH)COO}$_2$] and [Co{CH$_2$CH(OH)CH$_2$CH(NH)COO}$_2$] complexes, respectively. Further increase of pH had no effect on the mobility of the metal ions, which indicates no further interaction between metal ions and ligands. The unprotonated anionic species, but not the zwitterions of hydroxyproline, are known to have ligating properties.[34] In general the complexation of metal ions with hydroxyproline anion may be represented as:

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

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

here M^{2+} are Be^{2+} and Co^{2+} metal ions, L$^-$ is the hydroxyproline anion and K$_1$ and K$_2$ are the first and second stability constants, respectively. The metal spot on the paper is thus due to a combination of uncomplexed metal ions, 1:1 and 1:2 metal complexes. The spot moves under the influence of an electric field and the overall mobility U is given by Equation (3).[35]
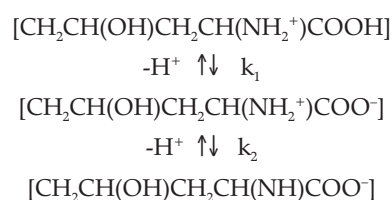
$$U = \frac{u_0 + u_1 K_1 [L^-] + u_2 K_1 K_2 [L^-]^2}{1 + K_1 [L^-] + K_1 K_2 [L^-]^2} \tag{3}$$

where u_0, u_1 and u_2 are the mobilities of the uncomplexed metal ions, 1:1 metal complex and 1:2 metal complex, respectively. The dissociation constants of pure hydroxyproline [$k_1 = 10^{1.80}$; $k_2 = 10^{9.46}$] were determined by the same electrophoretic technique. The mode of dissociation of hydroxyproline can be represented as:

[CH$_2$CH(OH)CH$_2$CH(NH$_2^+$)COOH]

-H$^+$ ⇅ k_1

[CH$_2$CH(OH)CH$_2$CH(NH$_2^+$)COO$^-$]

-H$^+$ ⇅ k_2

[CH$_2$CH(OH)CH$_2$CH(NH)COO$^-$]

For calculating the first stability constant, K_1, the region between the first and second plateaux is relevant. 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(OH)CH$_2$CH(NH)COO$^-$]. Using the dissociation constants of pure hydroxyproline, the concentration of hydroxyproline anion, [L$^-$], is determined for the pH, from which K_1 can be calculated. The concentration of chelating hydroxyproline anion, [L$^-$], is calculated with the help of equation (4)

$$[L^-] = \frac{[L_T]}{1 + [H]/k_2 + [H]^2/k_1 k_2} \tag{4}$$

where [L$_T$] is the total concentration of ligand hydroxyproline (0.01 mol L^{-1}) and k_1 and k_2 are the first and second dissociation constants of pure hydroxyproline, respectively. The second stability constant, K_2, of the second complex can be calculated by taking into consideration the region between the second and third plateaux of the mobility curve. The calculated values of K_1 and K_2 are given in Table 3.
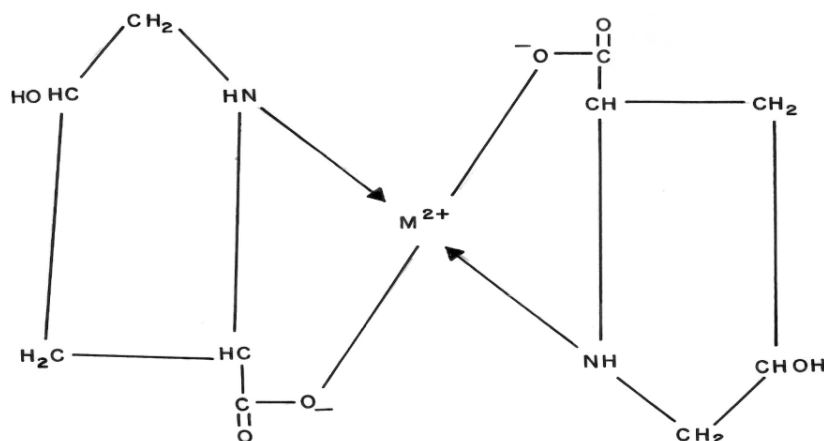
Scheme 1

4. Discussion

It is clear from Table 3 that the first and second stability constants follow the order

beryllium(II) > cobalt(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.[36] It is also clear from Table 3 that the calculated stability constant values are similar to literature values. The slight deviation in the values obtained from different sources is mainly due to the difference in temperature and ionic strength used by different workers. The high stability constant values of the beryllium(II)-hydroxyproline complexes indicate strong bonding between the beryllium(II) cation and the hydroxyproline anion, while the low stability constant values of the cobalt(II)-hydroxyproline complexes indicate weak bonding between the cobalt(II) cation and the hydroxyproline anion. The higher stability of beryllium(II) complexes may be ascribed to the greater affinity of beryllium for the oxygen donor ligands. According to the standard deviation statistics the precision of the method is limited to that of paper electrophoresis, and the uncertainty in the result is ±5 %. Hence, it cannot immediately replace the most reliable methods, even though it is a new approach deserving further development. To examine the possibility of hydrolysis of beryllium(II) at higher pH, experiments have been performed at two concentrations of the ligand, 0.01 mol L^{-1} and 0.001 mol L^{-1}. The mobility curves show that the plateaux at lower ligand concentration are shifted towards the higher pH range, but the calculated stability constants are found to be the same in both cases. Thus the stability constant obtained is independent of the pH, indicating that hydrolysis of beryllium(II) can be ignored here. The probable structure for the ML$_2$ complexes are shown in Scheme 1.

5. Conclusions

The following conclusions can be drawn from the present study. Beryllium(II) and cobalt(II) are significant but since they are toxic, hydroxyproline may be used to reduce the level of these metal ions in biological systems. Beryllium(II) and cobalt(II) are toxic and essential metal ions, respectively. The present 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. Beryllium(II)-hydroxyproline complexes are found to have higher stability constant values in comparison with cobalt(II)-hydroxyproline complexes. Biologically important beryllium(II) and cobalt(II) complexes with hydroxyproline can be prepared on a large scale at a particular pH of background electrolyte. The ML$_2$ complexes are found to have low stability constant values and are less stable in comparison with the ML complexes.

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Table 3 Stability constants of binary complexes of beryllium(II) and cobalt(II) with hydroxyproline.

Metal ion	Complex	Stability constant	Logarithm stability constant values *
Beryllium(II)	ML$^+$	K$_1$	7.39 ± 0.07
	ML$_2$	K$_2$	5.90 ± 0.03
Cobalt(II)	ML$^+$	K$_1$	4.73 ± 0.08 [4.82 (37), 4.81 (38)]
	ML$_2$	K$_2$	3.71 ± 0.11 [4.06 (37), 3.81 (38)]

Ionic strength = 0.1 mol L^{-1}; temperature = 35 °C; M = metal cations (Be^{2+}, Co^{2+}); ligand = hydroxyproline; hydroxyproline anion = [CH$_2$CH(OH)CH$_2$CH(NH) COO$^-$].

* Literature values are given in parentheses.

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