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Original Scientific Article

Electrophoretic Studies of Biologically Important Mixed Metal – Ascorbic Acid – Nitrilotriacetate Complexes

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Abstract. Quantitative indication of a complex formation comes from the estimation of the stability or formation constants characterizing the equilibria corresponding to the successive addition of ligands. The binary equilibria of metal (II)/(III) – ascorbic acid and also mixed equilibria metal (II)/(III) – ascorbic acid – NTA have been studied using ionophoretic technique. The stability constants of metal – ascorbic acid binary complexes are found to be $10^{3.77}$, $10^{2.47}$,$10^{2.27}$ and that of metal – ascorbic acid – NTA mixed complexes have been found to be $10^{6.05}$, $10^{5.93}$, $10^{5.75}$, for Fe(III), Cu(II) and Co(II) complexes, respectively at 25 °C and ionic strength $I_c = 0.1$ mol dm^{-3} ($HClO_4$). (doi: 10.5562/cca1778)

Keywords: binary complex, mixed complex, overall mobility, stability constants, ionophoretic technique

INTRODUCTION

The stabilities of binary and mixed complexes are known to play an important role in a number of metabolic and toxicological functions. Vitamin C (ascorbic acid, AA) is a water soluble organic compound involved in many biological processes. It plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism.[1] Most likely it is also involved in maintaining the reduced state of metal cofactors at monooxygenase and dioxygenase. The interaction of Vitamin C with metal ions has been studied by Khan and Martell[2,3] and the transition metal complexes of L-ascorbic acid were reviewed by Davies.[4] In the present communication an attempt has been made to study the optimal condition to metal – ascorbic acid binary and metal – ascorbic acid – Nitrilotriacetate (NTA) mixed complex formation. From these studies it has been inferred that the combining species of the ascorbic acid with the metal ion is its ascorbate monoanion. A simple ionophoretic tube has been designed which after standardization yields remarkable results. Ionophoresis set-up is shown in Figure 1.

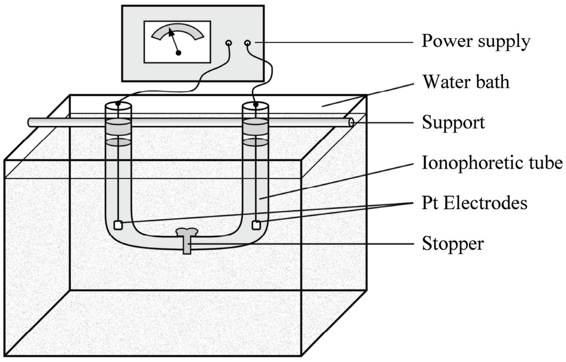

Figure 1. Ionophoresis set-up.

EXPERIMENTAL

Instruments

Ionophoresis equipment from Systronic (Naroda, India) model 604 was used. It has a built in power supply (AC/DC) that is fed directly to an ionophoretic tube (18 cm long and 0.5 cm bore) with a stopper in the middle and fused perpendicularly at the ends with short wider tubes of 1.2 cm bore. This tube is kept in a thermostatic water bath at 25 °C. pH measurements were made with century CP 901 digital pH meter using a glass electrode. Absorbance was measured with SPECTROCHEM MK II (PEI) spectrophotometer. A SICO made constant temperature water bath has been used to ensure uniform temperature.

Chemicals

Fe(III), Cu(II) and Co(II) perchlorate solutions were prepared by precipitating the corresponding carbonates

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from solution of chlorides (AnalaR grade) with the solution of sodium carbonate, washing the precipitates thoroughly with boiling water and dissolving in a suitable amount of perchloric acid. The resulting solutions were heated to boiling on a water bath and then filtered. The solutions were standardized and diluted with distilled water as required. AnalaR grade ascorbic acid, NaOH, $HClO_4$ and specific colour developing reagents were used for different metal ions of binary system sets: for Fe(III) ammonium thiocyanate solution ($w = 20\ \%$), for Cu(II) saturated solution of potassium persulphate ($w = 50\ \%$), solution of ammonium thiocyanate, acetone, concentrated HCl AnalaR grade and for Co(II) stannous chloride ($w = 20\ \%$), ammonium thiocyanate solution ($w = 50\ \%$).

Electrolytic Solutions

For binary systems, 15 mL solutions, each containing 1×10^{-3} M Cu(II)/Co(II), 0.1 M $HClO_4$ and 1×10^{-2} M ascorbic acid, were prepared at different pH values (by adding NaOH solution). In case of Fe(III), 1×10^{-4} M Fe(III), 0.1 M $HClO_4$ and 1×10^{-3} M ascorbic acid was used. For mixed systems the study was carried out by preparing sets of 15 mL solutions by progressive addition of secondary ligand NTA from 1×10^{-6} M to 1×10^{-2} M at a fixed value of pH = 7.0 to a mixture containing metal ion (having same concentration as in binary systems), 1×10^{-2} M ascorbic acid and 0.1 M perchloric acid.

Procedure

Metal–Ascorbic Acid Binary System

10 mL of electrolytic solution as mentioned for binary system is taken in an ionophoretic tube and then thermostated at 25 °C. The position of the tube was adjusted in such a way that the level of the solution in one wide end arm reached a circular mark on it. This adjustment fixed the volume of the solution on both sides of the middle stopper. Two platinum electrodes were dipped in each arm cup and a 50 V potential difference was applied between them. Ionophoresis of the solution was allowed for 45 minutes, after which the middle stopper of the tube is closed. The solution of the anodic compartment was taken out in a 15 mL flask. The Cu(II) content of the solution was converted into copper thiocyanate.[5] The volume was raised to a mark in the flask and the absorbance at $\lambda = 408$ nm was measured with spectrophotometer. The Co(II) content of the anodic compartment was converted into cobalt thiocyanate[5] complex and absorbance at $\lambda = 625$ nm was measured after making up the volume to 15 mL. Similarly the Fe(III) content of the anodic compartment was converted to iron thiocyanate[6] complex and absorbance was measured at $\lambda = 480$ nm against a reagent blank. The observations were repeated for different pH values of different background electrolyte (variations in pH were made by the addition of NaOH solution). The plot of the absorbance difference *vs.* pH is shown in Figure 2.

Metal–Nitrilotriacetate Binary System

The experiments for binary complexes of the Fe(III)/Cu(II)/Co(II)–NTA are prerequisite for the study of metal–ascorbic acid–NTA mixed complexes. These studies have been carried out by Aziz *et al.*[7] and Singh *et al.*[8,17]. Their observations have been taken for comparison with our observations found in case of mixed complexes.

Metal–Ascorbic Acid–Nitrilotriacetate Mixed Systems

The studies of mixed complexes were carried out by progressive addition of secondary ligand NTA from 1×10^{-6} M to 1×10^{-2} M at a fixed value of pH = 7.0 to a mixture of metal ion (having same concentration as in binary systems), 1×10^{-2} M ascorbic acid and 0.1 M perchloric acid. The reason behind keeping the reaction mixture at pH = 7.0 is that much ahead of this pH the simple binary complexes of metal ascorbic acid and M–NTA are formed and remain intact even beyond this pH. The amount of secondary ligand NTA was increased each time in the ionophoretic tube and the ionophoresis of the solution and its observations were recorded each time as it was done in case of binary complexes. These observations are graphically represented in Figure 3. A number of factors[9] (*e.g.* diffusion, ionic

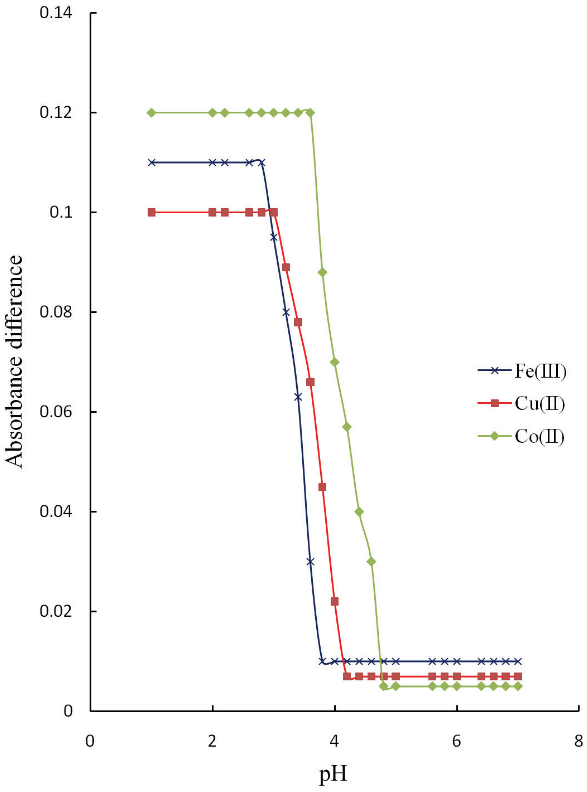

Figure 2. Mobility curve M–ascorbic acid system.

strength and temperature) obviously vitiate the ionophoretic mobility of a particular ion. The technique described here is almost free of these vitiating factors and the reliability may vary to ± 2 %.

As for the possibility of hydroxy compound formation, we had considered this aspect in our earlier studies. For this we performed the study of binary complex formation at two different concentration of ligand giving complexing species at two different pH values. In that study we find two different plateaus but almost same stability constant. If it would have been a hydroxyl complex only one plateau at fixed pH value must have been formed irrespective of different concentration of ligand (ascorbic acid here) used. This clearly indicates that at very low concentration of metal (*i.e.* c(Fe(III)) = 10^{-4} mol dm^{-3}) no hydroxy compound are formed. Hence the possibility of hydroxy compound formation at very low concentrations of metal ions is ignored in these studies. Structurally, ascorbic acid (H_2A) is a sugar acid, a γ-lactone and an ene-diol. As a weak dibasic acid ($pk_1 = 4.25$ and $pk_2 = 11.79$), the monoanion (HA) forms at pH values between 4 and 5 with deprotonation of O(3)–H and the dianion (A) forms at pH between 11 and 12 with deprotonation[10] of the O(2)–H. The monoanionic form is more stable due to the delocalization of the negative charge between the oxygen atoms at the 1- and 3-positions[11] as shown in Figure 4 and is main coordinating species in our studies.

RESULTS AND DISCUSSION

Metal–Ascorbic Acid Binary System

Figure 2 illustrates the relationship between absorbance differences and the pH's and thus gives an idea of the change of overall mobility of the metal ion species with change in hydrogen ion status of the system containing Fe(III)/Cu(II)/Co(II) and ascorbic acid. A curve with a number of plateaus indicates the formation of certain complex species. A plateau is obviously an indication of a pH range where mobility is practically constant. The first plateau (at low pH) in each case corresponds to a region of pH where metal ions are uncomplexed. In this low pH region, the protonated form of ascorbic acid is maximum. Further increase in pH from this region onward which naturally leads to increase in ligating ascorbic acid anion concentration [L$^-$] and brings about a progressive decrease in overall ionic mobility of the metal ion species. This decrease indicates formation of complex of the metal ion with the ligand. A point is reached beyond which mobility of the metal ion species remain constant regardless of the increase of pH of reaction mixture which indicates no further interaction of metal ions and ligand. This is the second plateau which corresponds to a pH region in which 1:1 complex is predominantly formed. One ascorbic acid anion combines with each metal ion to form [Fe{$C_6H_7O_6$}]$^{++}$, [Cu{$C_6H_7O_6$}]$^+$ and [Co{$C_6H_7O_6$}]$^+$, cationic complexes with Fe(III), Cu(II) and Co(II) respectively. Beyond this plateau increase of pH has no effect and mobility remain constant.

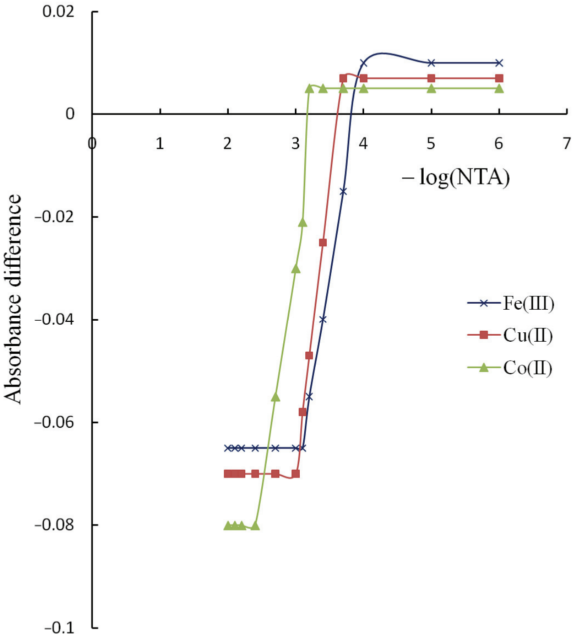

Figure 3. Mobility curve M–ascorbic acid–NTA system.

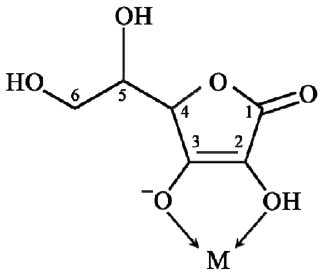

Figure 4. Chelate-type coordination of ascorbate monoanion.

The overall mobility U is a composite parameter contributed by different ionic species of the metal ion and is given by following equation:

$$U = \frac{u_0 + u_1K_1[\mathrm{L}^-] + u_2K_1K_2[\mathrm{L}^-]^2 + \dots}{1 + K_1[\mathrm{L}^-] + K_1K_2[\mathrm{L}^-]^2 + \dots} \qquad (1)$$

where K's (K_1, K_2) are the stability constant of complexes and [L$^-$] is the concentration of ascorbic acid ion. u's (u_0, u_1, u_2) are the ionic mobilities of different species of the metal ions which can be assessed from the plateaus of the Figure 2. In the region between first and second plateau the system contains overwhelmingly a mixture of free metal ion and 1:1 complex. The formation constants[12] of the 1:1 complexes are in the range from 10 to $10^{3.6}$. The values are quite small possibly because of the low negative charge on the ligand anion.

The existence of 1:2 complexes can be excluded and hence the third term in the numerator and the denominator of the above equation can be justifiably neglected. U would be equal to $(u_0 + u_1)/2$ provided $K_1[\mathrm{L}^-] = 1$.

Accordingly the pH corresponding to the average value of u_0 and u_1 is found from the Figure 2 and with the knowledge of the dissociation constant of ascorbic acid ($\mathrm{p}k_1 = 4.25$ and $\mathrm{p}k_2 = 11.79$),[13] the concentration of ascorbic acid ion at this pH is calculated. Its reciprocal gives the stability constant K_1 of the 1:1 complex. The concentration of chelating ascorbic acid anion $[\mathrm{L}^-]$ is determined as:

$$[\mathrm{L}^-] = \frac{[\mathrm{L}]_{\mathrm{tot}}}{1 + [\mathrm{H}]/k_2 + [\mathrm{H}]^2/k_1k_2} \tag{2}$$

where $[\mathrm{L}]_{\mathrm{tot}}$ is the total concentration of the ligand ascorbic acid (for Fe(III) 10^{-3} mol dm^{-3} and for Cu(II), Co(II) 10^{-2} mol dm^{-3}) and k_1 and k_2 are the first and second dissociation constants of the ascorbic acid, respectively.

Metal–Ascorbic Acid–Nitrilotriacetate Mixed Systems

It is observed from the mobility curves of the metal–ascorbic acid system that 1:1 binary complex are formed at pH < 7.0. It was therefore considered necessary to study the transformation of binary complex into 1:1:1 metal–ascorbic acid–NTA mixed complexes at pH = 7.0 in order to avoid any side interaction.

Figure 3 depicts the observation of studies carried out in metal–ascorbic acid–NTA mixed complexes. The first plateau is in agreement with the mobility of the binary metal–ascorbic acid complexes. And the mobility of last plateau is more negative than that of the first plateau and indicates the formation of new complex. Further, since the mobility in the last plateau does not coincide with the mobility of the 1:1 metal–ascorbic acid complexes, it is inferred that the coordination of the nitrilotriacetate anion to 1:1 metal–ascorbic acid moiety resulting in the formation of 1:1:1 metal–ascorbic acid–NTA mixed complexes as.

$$\text{M-L} + \text{NTA} \xrightarrow{K'} \text{M-L-NTA} \tag{3}$$

For M-L-NTA complexes the stability constant K' is calculated by using modified equation.

$$U = \frac{u_0 + u_1K'[\mathrm{NTA}]}{1 + K'[\mathrm{NTA}]} \tag{4}$$

where u_0 and u_1 are the mobilities of M-L and M-L-NTA complexes, respectively.

From the Figure 3 concentration of NTA at which overall mobility is the mean of the mobilities of the two plateaus is determined. The concentration of NTA anion at pH = 7.0 for this NTA concentration is calculated. K' is obviously equal to $1/[\mathrm{NTA}]$. The stability constant values for binary (M-L) and mixed (M-L-NTA) complexes are $\mathrm{Fe^{III}}(3.77, 6.05) > \mathrm{Cu^{II}}(2.47, 5.93) > \mathrm{Co^{II}}(2.27, 5.75)$ which follows the Irving William's[14] order for the stability constants of transition metals of the first transition series.

In literature[13] $\log K$ values for binary complexes of $\mathrm{Cu^{II}}$ (at I_c = 0.1 M and temp. 0 °C) is 1.57 and for $\mathrm{Co^{II}}$ (at I_c = 0.0 M and temp. 25 °C) is 1.4. The difference in calculated and literature values can be attributed to different experimental conditions. $\log K'$ values for mixed complexes are not available in literature hence no comparison can be made.

CONCLUSION

It thus follows from these studies that Fe(III)/Cu(II)/Co(II) are essential for biological systems, but since they are toxic if present in excess, ascorbic acid and nitrilotriacetate may be used to reduce the levels of these metal ions in living cells.[15,16]

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