

## SPECIATION AND STABILITY OF METHYLENE BLUE-METAL-THIOCYANATE ION-ASSOCIATION COMPLEXES

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(Received February 17, 2007; revised June 8, 2007)

**ABSTRACT.** With thiocyanate, zinc(II), cobalt(II) and copper(II) ions form stable anion,  $[M(SCN)_4]^{2-}$ . This anion in turn with methylene blue ( $MB^+$ ) forms ion-pair of the type  $MB_2[M(SCN)_4]$  and turns methylene blue to violet. The stoichiometry and stability constants of the ion-association complexes were determined using Benesi-Hildebrand method and the stoichiometry was confirmed by Job's continuous variation method. The kinetics of formation of ternary complexes was studied using stopped-flow technique. The equilibrium studies showed that the stability of ternary complexes increased in the order  $Co(II) > Cu(II) > Zn(II)$  and the formation rate constants increased in the order,  $Cu(II) > Zn(II) > Co(II)$ . The relative stabilities indicate that cobalt is preferred to other two metals in the speciation of ternary complexes comparable with similar complexes in biosystems. This study also provides a method for the spectrophotometric determination of  $Co(II)$  and  $Zn(II)$  ions at nanogram levels at 25 °C and an ionic strength of 0.15 M.

**KEY WORDS:** Ion-association complex, Kinetics, Zinc, Cobalt, Methylene blue, Thiocyanate

### INTRODUCTION

Zinc, copper and cobalt are essential trace metals to biological systems. Zinc is a vital part of many enzymes like carbonic anhydrase and alkaline phosphatase, and in maintaining tertiary and quaternary structures of biological macromolecules like proteins and nucleic acids. Copper is present along with zinc in superoxide dismutase, which is a component of body's defense mechanism [1]. Cobalt is present in coenzymes which are involved in isomerisation and transmethylation reactions.

Methylene blue (3,7-bis(dimethylamino)phenothiazin-5-ium,  $MB^+$ ) belongs to the phenothiazinium dyes. It is a kind of photosensitizer molecule exhibiting promising applications in photodynamic therapy [2] for anticancer treatment [3-7]. It is used as a stain and agent for blood product decontamination [8] and an antidote for cyanide poisoning [9]. Methylene blue is one of the most frequently used counter ions for ion-pair formation [9, 10]. It is used for the determination of hydrogen sulfide [11, 12]. Palladium(II) was determined as ion-associated complex of thiocyanate with methylene blue [13] and malachite green [14]. Traces of zinc were determined by the formation of a ternary complex with thiocyanate and crystal violet [15] or Rhodamine 6G [16]. The thiocyanate ion forms anionic complexes,  $[M(SCN)_x]^{y-}$  [17], which generate an ion-association complex with methylene blue. Interaction of  $MB^+$  with  $Hg(II)$ ,  $Cd(II)$ ,  $Ag(I)$  for their removal from waste water [18] and with gallium chloride [19] was postulated. Hence, the speciation and formation kinetics of ion-association complexes of  $Zn(II)$ ,  $Co(II)$  and  $Cu(II)$  with thiocyanate and  $MB^+$  was studied.

### EXPERIMENTAL

All the chemicals used were of analytical-reagent grade and were used without further purification. The stock solutions were made in A grade glassware using deionized water. The

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concentrations of methylene blue ( $\text{MB}^+\text{Cl}^-$ ) (BDH, England) and metal ion solutions were  $10^{-3}$  M, from which subsequent dilutions were made. The concentration of the stock potassium thiocyanate solution was 0.50 M.

Varian Cary 1E double beam spectrophotometer was used for recording spectra, determining stability constants and for absorbance measurements with an identical pair of quartz cuvettes. Stoichiometry of ion-association complexes was determined by the method of continuous variation (Job's method) and stability constants were calculated by Benesi-Hildebrand method [20]. In a typical experiment for the determination of stability constants, 1.0 mL of  $1.60 \times 10^{-5}$  M  $\text{ZnCl}_2$  solution, 2.0 mL of 0.50 M KCNS and 1.0-7.0 mL of  $1.01 \times 10^{-4}$  M methylene blue were made up to 10.0 mL. The absorbances of the solutions were measured at 661 nm.

The formation kinetics of ion-association complexes were studied using a Hi-Tech SF-61 DX2 double mixing stopped-flow apparatus equipped with photomultiplier/diode array spectrometer. The overall concentrations of the solutions in the reaction mixture were:  $[\text{MB}^+] = 1.01 \times 10^{-5}$  M;  $[\text{KSCN}] = 0.15$  M; metal,  $[\text{M}] = 2.00 \times 10^{-4}$  M. Change in the absorbance of  $\text{MB}^+$  was monitored for 30 seconds at 661 nm wavelength. Factor analysis and global fitting were performed for the calculation of rate constants, using SPECFIT from Spectrum Software Associates. All the rate constants reported are the average of at least five runs.

For a typical determination of metal ion, a suitable aliquot of the metal ion solution containing not more than 3.0  $\mu\text{g}$  was transferred to a 10.0 mL volumetric flask. 2.0 mL of 0.50 M potassium thiocyanate solution followed by 2.0 mL of  $10^{-4}$  M methylene blue solution were added. The solution was diluted to the mark with water. After 10 min the absorbance was measured in 10 mm cell at 661 nm against a reagent blank prepared with all the solutions mentioned above except metal ion solution. A calibration curve was constructed for 0.2-3.0  $\mu\text{g}$  of zinc and 0.1-1.5  $\mu\text{g}$  of cobalt. Beer's law was obeyed over the specified concentration range.

## RESULTS AND DISCUSSION

It was observed that Zn(II), Co(II) and Cu(II) ions reacted with  $\text{MB}^+$  in presence of thiocyanate ions. Due to the formation of ion-association complexes between  $\text{MB}^+$  and the anionic metal(II)-thiocyanate complex, methylene blue turned violet. There are evidences for the formation of  $[\text{M}(\text{SCN})_4]^{2-}$  anions for Co(II) [21], Cu(II) [22] and Zn(II) [23]. Based on IR spectral data, both Co-NCS and Co-SCN bonding modes were established [24].

In the case of Fe(III), thiocyanate is bonded through nitrogen [25]. Sultan and Bishop reported that even at 1:5000 ratio of Fe(III) to thiocyanate only 1:1 complex is formed in an acidic medium [26]. Hence, a large excess of thiocyanate was maintained to ensure the complete conversion of metal ion to  $[\text{M}(\text{SCN})_4]^{2-}$  complex. It was also reported that the stability of the complex decreased with increasing acid concentration. In acid medium, the terminal sulfur separates from the thiocyanate and precipitates. The residual cyano group then released and protonated, evolves as hydrogen cyanide. Hence, to avoid the formation of HCN, no acid is used in the present studies.

Figure 1 shows typical absorption spectra of methylene blue in presence of varying amounts of Zn(II) and fixed amount of thiocyanate. The absorbance had decreased with the metal ion concentration (hypochromic effect) and it was stable in between certain wavelength regions. This hypochromic phenomenon suggests the strong interaction between the electronic states [15] of the chromophore of MB and the anionic complex. The marked hypochromism and bathochromic shift (Figure 1) of the chromophore indicate close proximity of the  $\text{MB}^+$  to the complex anion.

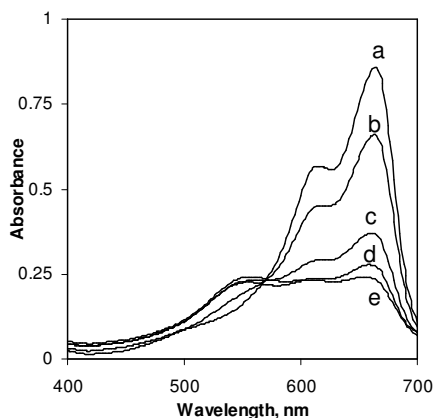


Figure 1. Absorption spectra of methylene blue-zinc(II)-thiocyanate complexes with varying amounts of zinc.  $[KSCN] = 0.15\text{ M}$ ;  $[MB^+] = 2.02 \times 10^{-5}\text{ M}$ ;  $[Zn(II)], 10^{-6}\text{ M}$ : a) 1.00, b) 2.00, c) 3.00, d) 4.00, and e) 5.00.

The rates of formation of ion-association complexes of Cu(II), Co(II) and Zn(II) were studied using stopped-flow apparatus. The traces of typical kinetic runs (Figure 2) indicate that complexation reactions are not instantaneous. The observed *pseudo* first-order rate constants given in Table 1 indicate that under other-wise identical conditions, the formation of the complex containing Cu(II) is faster than those of cobalt(II) and zinc(II), but the rate of formation of zinc complex is higher than that of cobalt. The values of the observed  $k'$  values agree well with the reported order of stability of thiocyanate complexes [27] that is  $Cu(II) > Co(II) > Zn(II)$ . Possibly, the instability of zinc complex forces it to readily form ion-association complex.

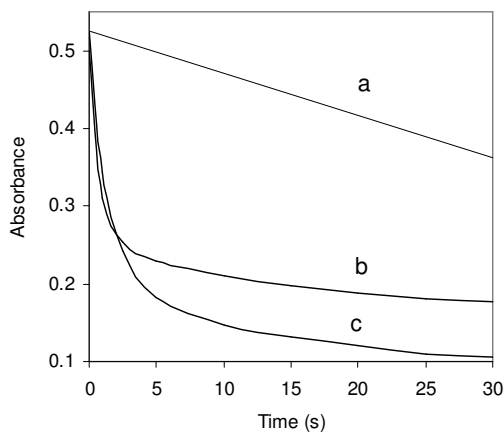


Figure 2. Kinetic traces for formation of ion-association complexes of metals:  $[MB^+] = 1.01 \times 10^{-5}\text{ M}$ ;  $[KSCN] = 0.15\text{ M}$ ;  $[M(II)] = 2.00 \times 10^{-4}\text{ M}$ . Curves: a) Co(II), b) Cu(II), and c) Zn(II).

Table 1. Pseudo first-order rate constants for the formation of  $MB_2[M(SCN)_4]$  [ $MB^+$ ] =  $1.01 \times 10^{-5}$  M; [KSCN] = 0.15 M; [M(II)] =  $2.00 \times 10^{-4}$  M.

Metal	$k'$ ( $s^{-1}$ )
Co(II)	$0.013 \pm 0.001$
Zn(II)	$0.556 \pm 0.005$
Cu(II)	$0.657 \pm 0.001$

*Determination of stoichiometry and stability constants of ion-association complexes*

In the Benesi-Hildebrand method, the formation of an ion-association complex can be represented as Eq. (1)



where D and M are the dye and metal-thiocyanate anionic complexes, respectively, and  $\beta_{D_nM}$  is overall formation constant of the ion-association complex,  $D_nM$ . If the absorbances of various amounts of D are measured at a fixed concentration of metal ion, a relationship can be expressed as

$$\log \{(A-A_0)/(A_c-A)\} = n \log CD + \log \beta_{D_nM} \quad (2)$$

where A is the absorbance at a pre-selected wavelength of a solution having known molarities of dye (CD) and anion complex (CM).  $A_0$  is the absorbance measured at the same wavelength of a solution when  $CD = 0$ , and  $A_c$  is the absorbance of a solution for which  $CD \gg CM$  so that the absorbance is constant with increasing CM. Thus, a plot of  $\log \{(A-A_0)/(A_c-A)\}$  versus  $\log CD$  should be linear, whose slope is equal to the stoichiometric coefficient, n of the dye and intercept corresponds to  $\log \beta_{D_nM}$ .

Since very high concentration of thiocyanate was used, all the metal ions were assumed to be in the form of anionic complex, and so the concentration of metal ion solution was equal to CM. The plots of  $\log \{(A-A_0)/(A_c-A)\}$  versus  $\log CD$  are illustrated in Figure 3. The stoichiometric coefficients and stability constants of the ion-association complexes obtained from these plots are summarised in Table 2. The stoichiometric ratio of  $MB^+$  to  $[M(SCN)_4]^{2-}$  was found to be 2:1 for Co(II), Cu(II) and Zn(II) and so the molecular formula of the complexes is  $MB_2[M(SCN)_4]$ . This was also confirmed by Job's method. The stability constants indicate that the order of stability of ion-association complexes is  $Co(II) > Cu(II) > Zn(II)$ .

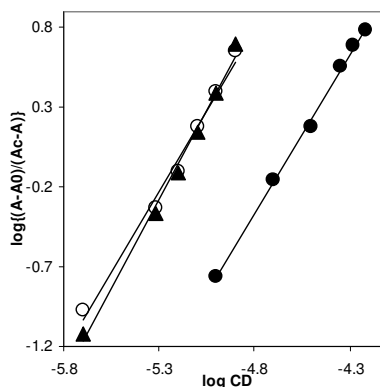


Figure 3. Linear plots for calculation of stability constants by Benesi-Hildebrand method.

●Zn(II), ○Cu(II), ▲Co(II).

Table 2. Stoichiometric coefficients and stability constants of ternary complexes obtained by Benesi-Hildebrand method.

Metal	Slope (n)	Intercept ( $\log \beta_{DnM}$ )	Correlation coefficient
Co(II)	2.23	11.56	0.993
Zn(II)	2.01	9.26	0.998
Cu(II)	1.98	10.51	0.992

The magnitude of hypochromism can be correlated with the strength of the binding interaction and stability of the ion-association complex. Two modes of  $\pi$ - $\pi$  stacking interactions, viz., parallel and anti-parallel stacking were proposed in ion-association complexes by Raj *et al.* [28]. They determined the crystal structures of  $\text{MB}^+$ - $\text{HgCl}_4^{2-}$  ion-association complexes. The asymmetric units of these crystals consisted of two  $\text{MB}^+$  cations and a  $\text{HgCl}_4^{2-}$  anion. Pair-wise hydrogen bonding motifs were formed between two  $\text{MB}^+$  units via C-H...N interactions. Hence, it is expected that in the present study the ion-association complexes shall have structures similar to those mentioned above. The results of this study clearly show that there is competition between metal ion complexes for association with  $\text{MB}^+$ . The dominance of the species is dictated by the stability, which in turn is established from the stability constants. These results are of significance and can be extrapolated to biosystems, where the metals ions in the present study form binary or ternary complexes [1, 29].

Further, there is scope for devising an analytical method for the determination of Co(II) or Zn(II) based on this study. Although, it is limited to the single cation and in absence of other cations only, it is targeted at the speciation of metal. The following experiments support that possibility. For fixed concentrations of  $\text{MB}^+$  and  $\text{SCN}^-$ , absorbance values were measured for different concentrations of Zn(II) or Co(II) in replicate experiments. Figure 4 shows the plots of absorbance *versus* concentration of metal ions. Under the present experimental conditions, Beer's law was obeyed over the range 0.2 to 3.0 micrograms of zinc and 0.5-1.5 micrograms of cobalt. The precision of the method was evaluated for ten measurements of 1.0  $\mu\text{g}$  of zinc and the results are given in Table 3.

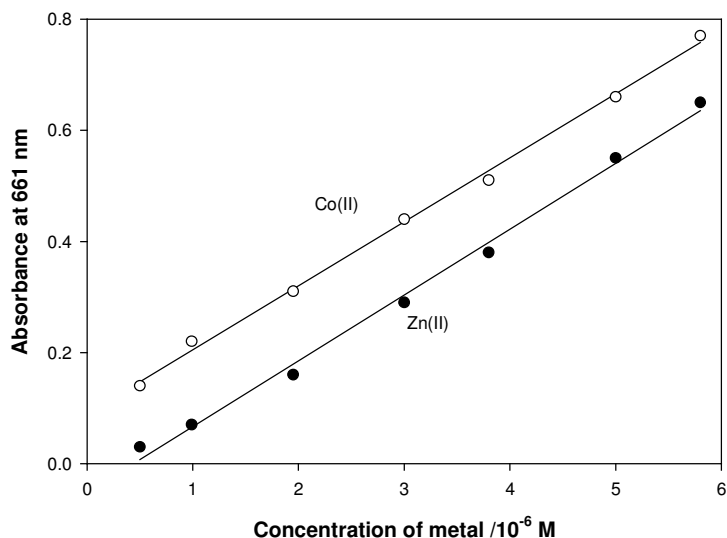


Figure 4. Calibration curves for determination of Zn(II)● and Co(II)○.

Table 3. Precision of the method for measurement of 1.0 µg of zinc.

Sample number	Absorbance
1	0.448
2	0.451
3	0.450
4	0.446
5	0.448
6	0.449
7	0.445
8	0.451
9	0.447
10	0.451
Standard deviation	0.002

In the Kitson method for Co(II) [29], blue color is developed by adding KCNS to the cobalt(II) solution in 50 % acetone solution. The amount of acetone used is limited by the low solubility of inorganic salts. The color intensity changes with the change in acetone concentration. But in our method color intensity is very high and there is no need to add an organic solvent like acetone. The range of the determination of cobalt(II) by the Kitson method is 0-60 ppm, whereas, the present method can be used even at the ppb level.

At high concentrations of metal ions the complexes slowly precipitated. Although precipitation could be avoided by using a stabilizing/dispersing agent like polyvinyl alcohol, gum Arabic or gelatin, at high concentrations of metal ion, the absorbance reduces to very small values and impacting on accuracy of measurements.

As the various reviews imply, a number of kinetic based analytical methods for photometric determination of metal ion concentrations through formation of coloured complexes have been reported [30]. In the indirect kinetic spectrophotometric determination of cobalt based on the redox reaction with iron(III) in the presence of 1,10-phenanthroline, the detection limit for Co(II) is reported as 0.2 µM L<sup>-1</sup> [31]. As described earlier, the proposed method achieves better detection limits for cobalt ion determination.

#### ACKNOWLEDGEMENTS

Authors thank the National Research Foundation, Pretoria for the financial support to the work and the Department of Inorganic and Analytical Chemistry, Andhra University, Visakhapatnam, India for giving permission to NRG to spend his sabbatical at UKZN.

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