

Quantitative study of Co(II) complexation by synchronous fluorescence spectroscopy with Sundarban mangrove habitat humic substances

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Attempt has been made to isolate and characterize humic substances and their relative role for complexation of Co (II) in the mangrove sediment. Conditional stability constant (K_c) for Co (II) complexes with humic and fulvic acids were determined by studying quenching of fluorescence intensity of humic substances with Co (II) using synchronous fluorescence spectroscopy. Fulvic acid forms more stable complex than humic acid with K_c of the order of 4.7×10^{11} and 1.02×10^{11} , respectively at pH 7, ionic strength of 0.1 and temperature of 25°C. Rigid frame work of fulvic acid having more electron donor groups as -OH, -NH₂ and less incorporation of -COOH than humic acid could be the reason for its formation of more stable complex with Co (II) than humic acid.

[**Key words:** Humic substances, spectral characteristics, Co (II) complexation]

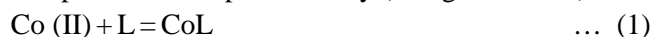
Naturally occurring polyelectrolytes referred to by such names as humic acid (HA) and fulvic acid (FA) are ubiquitous in soil and natural waters, where they form water-soluble and water insoluble complexes with metal ions. These weakly acidic polyelectrolytes play prominent role in the binding of micronutrients in soil. They are involved in the migration and subsequent deposition of mineral substances in sediment. From an environmental standpoint, complexing of essential and toxic heavy metals by humic substances is of importance in determining their fate in the environment.

Cobalt is an essential trace element and also toxic at quite low concentration. Numerous studies have established the important influence of trace element speciation on metal uptake by organisms or toxicity¹. Assimilation efficiencies² of trace metals in bivalve molluscs varied between 22 and 83%. The partition coefficient of cobalt in coastal sediment is two orders in magnitude higher than those in marine phytoplankton³. The mechanism by which geochemistry affects metal bioavailability from sediments are not as well known as are influences of metal speciation in solution⁴. Anthropogenic input of cobalt through the use of fertilizer and deposition (dry and wet) indicates its enrichment in the soil⁵. Humic substances play an im-

portant environmental role both in the inactivation of toxicity and in the mobilization/immobilization processes of cobalt⁶. Systematic studies of the interactions of humic substances and cobalt ion, Co (II)^{7,8} are few and synchronous fluorescence spectroscopy (SyF) has proved to be a valuable tool for the analysis of complexation properties of humic substances⁹. The objective of this work was to obtain a first set information about the relative role of FA and HA occurring in the mangrove sediment to form stable complexes with Co (II). This paper presents the result of a study of the interaction of HA and FA samples isolated from mangrove sediment of Sundarbans with Co (II) at pH 7 and would help to understand the mobility and fate of cobalt in the mangrove environment.

Materials and Methods

The complexation reaction between the ligand (L) and Co (II), considering only the formation of (1:1) complex can be represented by (charges omitted):



The corresponding conditional stability constant¹⁰, (K_c) for the formation of the complex in the ground state is given by:

$$K_c = [\text{CoL}] / [\text{Co}] [\text{L}] \quad \dots (2)$$

where, Co = is the concentration of all cobalt species not bound to ligand, L = is the concentration of ligand not bound to cobalt ion, CoL = is the concentration of cobalt bound to ligand by (1:1) complex.

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For static quenching, the decrease of synchronous fluorescence (SyF) signal is proportional to the concentration of the complex, [CoL] and the following relation is valid¹¹:

$$I_0 - I / I_0 = \alpha (I - I_{FM} / I) \quad \dots (3)$$

where, I_0 is the fluorescence intensity in absence of Co (II), I is the intensities at the intermediate point of titration, I_{FM} corresponds to the intensity at the complete complexation of L with Co (II) and α is the fraction of the total ligand, C_L bound to cobalt ($\alpha = [CoL]/C_L$).

Considering the material balances, the total molar concentration of cobalt (C_{Co}) and the ligand (C_L) are given by:

$$C_{Co} = [Co] + [CoL] \quad \dots (4)$$

$$C_L = [L] + [CoL] \quad \dots (5)$$

Combining Eqs. (2), (4) and (5), following relation is obtained:

$$\alpha = K_c [Co] / 1 + K_c [Co] \quad \dots (6)$$

where [Co] is the realistic root of the equation :

$$K_c [Co]^2 + [Co] \{1 + K_c C_A - K_c C_{Co}\} - C_{Co} = 0 \dots (7)$$

Conditional stability constant, K_C , can be estimated by using the following relation obtained from Eqs. 6 and 7:

$$I_0 / (I_0 - I) = (I / I - I_{FM}) - (I / I - I_{FM}) \frac{[2 / (1 + K_C C_A - K_C C_{Co}) \pm \{ (1 + K_C C_A + K_C C_{Co})^2 - 4 K_C^2 C_{Co} C_A \}^{1/2}]}{\dots} \quad \dots (8)$$

Sediment samples were collected from the intertidal zone of Lothian Island (20°50' N and 88°9' E), Sundarbans, covered with deep mangrove forest. Humic acid and fulvic acid were extracted from the sediment by Methyl Isobutyl Ketone (MIBK) method¹². About 1 g of dried sediment sample was added to 100 ml 0.5 N NaOH solution and stirred for 24 h in automatic shaker in the N₂ atmosphere. The mixture was then filtered through GF/C glass fibre filter. The filtrate was transferred to a separatory funnel along with 75 ml MIBK and acidified to a pH 2-3 with 6 N HCl. The mixture was shaken vigorously. HA entered into the MIBK phase as suspension, leaving the FA in the aqueous phase. The aqueous phase was collected and dried in a rotary evaporator under vacuum. The residue left was extracted with absolute alcohol. The extract was dried in a rotary evaporator to get solid FA. A fresh 100 ml aliquot of 0.5 M NaOH was added to the MIBK phase in the separatory funnel and shaken vigorously. The HA was ex-

tracted from the MIBK phase into the aqueous alkaline phase. The alkaline aqueous phase was again acidified to get precipitate of HA. Yields of FA obtained between 474.7 and 662.1 $\mu\text{g g}^{-1}$, and that of HA, between 138 and 277.2 $\mu\text{g g}^{-1}$.

HA and FA of 120 mg l⁻¹, were prepared in 0.1 N KOH. The pH of the solution was adjusted to a constant value of 7 by using 0.1 N HNO₃ and 0.1 N KOH. Concentrated solution of Co (II), 1.5x 10⁻³ (M) was prepared by dissolving Co (NO₃)₂ salt in 0.1 M KNO₃ solution. A series of mixtures were prepared containing (0.1 to 1.8) x 10⁻⁴ M Co (II) along with a constant proportion of HA and FA solution. The mixture was diluted to a constant volume of 10 ml with 0.1 M KNO₃ solution and was maintained at pH 7.0. Synchronous fluorescence spectrum was recorded for each solution against reference solution of 0.1 M KNO₃ solution using Perkin-Elmer LS-50 luminescence spectrometer with the following instrumental settings: 250–550 nm excitation wavelength, 0.5 nm resolution between the points, 10 nm excitation and emission slit width, wavelength difference of 35 nm and scan rate of 200 nm /min.

Absorption spectra were recorded in Perkin- Elmer 5506 uv-vis spectrophotometer. IR spectrum was recorded in a Beckmann IR-20 instrument in KBr pellets. Elemental analyses were carried out using Perkin Elmer 240B CHN analyzer. Silicate- silicon¹³, carbohydrate¹⁴ and protein¹⁵ were determined by spectrophotometric method. Titrable alkalinity was determined by potentiometric titration⁸ up to pH 7.0. Air-dried sediment samples were used for the grain size analysis following pipette method¹⁶.

Results and Discussion

A summary of analytical results of FA characteristics is presented in Table 1. From the elemental composition, humic acid contained 54.25% carbon, 4.94% hydrogen, 4.24% nitrogen and 1.77% silicate-silicon.

Table 1—Characteristics of humic substances

Elemental Characteristics (%)	Humic acid	Fulvic acid
C	54.25	46.23
H	4.94	4.37
N	4.24	3.34
SiO ₄ ⁻⁴ - Si	1.77	1.68
Chemical characteristics		
Titrate acidity (meqv/g)	2.2	1.6
Carbohydrate(%) residue	1.46	2.08
Protein(%) residue	39.4	24.3

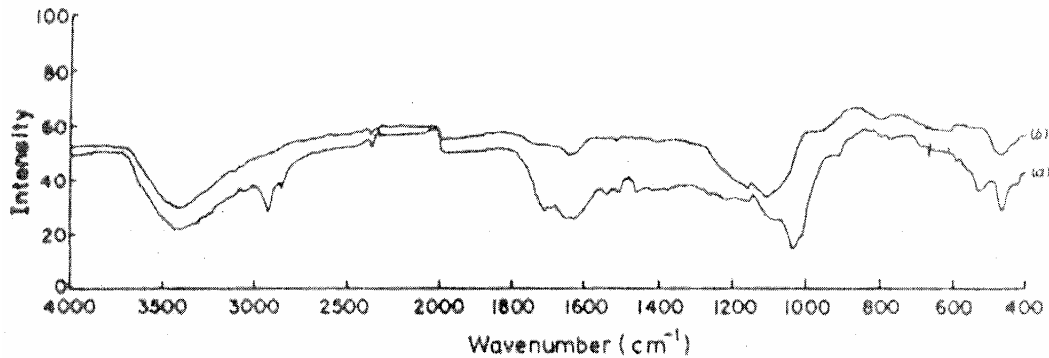


Fig. 1—IR spectrum of (a) Fulvic acid (b) Humic acid

Corresponding fulvic acid was less at 46.23%, 4.37%, 3.34% and 1.68%. The titrable acidity in humic acid was found to be 2.2 meq/g; fulvic acid showing lower acidity, 1.6 meq/g. Percentage of carbohydrate residue in humic acid showed lower value of 1.46 than that of 2.08 in fulvic acid. Protein residue was 39.4% for humic acid and 24.3% for fulvic acid. Results do not differ markedly as compared to other reports¹⁷ and indicate that humification of the organic matter in the sediment increases mineral contents, protein residue and carboxylic acid group.

IR spectrum of HA and FA (Fig. 1) exhibit strong absorption bands at 3400 to 3450 cm^{-1} (weak acid group, -OH), 1710–1640 cm^{-1} (-COOH group). Beside these particular characteristic bands, HA shows stronger absorption band at 2920 and 2850 cm^{-1} compared to FA; which is due to aliphatic C-H stretching. Aromatic C-H stretching band was observed at 3060 (s) and 3040 cm^{-1} (w) for HA and FA, respectively. The strong bands at 1100–1040 cm^{-1} corresponding to C-O stretching in carbohydrate were observed for both FA and HA.

The absorption spectrum of FA and HA; and with Co (II) in 1:1 ratio at pH 7 is given in Fig. 2 A and B. The absorption of HA and FA in the UV region is mainly caused by the excitation of lone-pair electrons of oxygen ($n \rightarrow \pi^*$) and by conjugated C=C double bonds ($\pi \rightarrow \pi^*$). The absorption in the visible range, responsible for the yellow brown color of HA and FA, is due to lone-pair electrons and charge transfer systems. The optical density in both cases decreases as the wavelength increases. However, a slight maximum was observed around 260 – 280 nm. The spectral absorbance from 300 – 250 nm is steeper for FA compared with HA. The specific UV absorption is higher for HA compared to FA. A core with higher aromaticity can explain this in the case of HA. Two

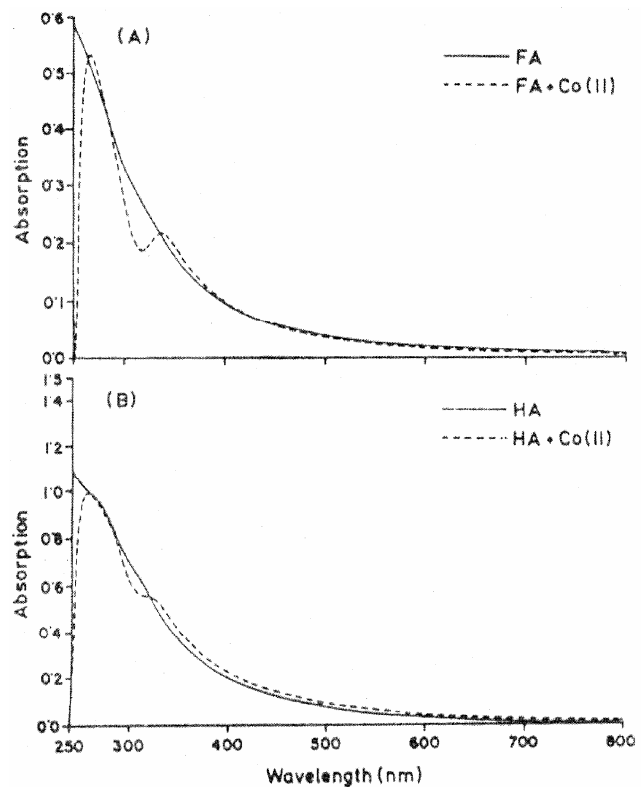


Fig. 2—Absorption spectra of (A) Fulvic acid, Fulvic acid with Co (II) in (1:1) ratio and (B) humic acid, humic acid with Co (II) in (1:1) ratio at pH 7 and I = 0.1

new peaks at 256 nm, and 322 nm for HA and at 256 nm and 327 nm for FA after the addition of Co (II) were observed indicating complexation.

The synchronous fluorescence spectra (SyF) of FA and HA along with their quenching due to successive addition of Co (II) at pH 7 are shown in Fig. 3 The shape of the two spectra are different, i.e. bands described as strong (s), medium (m) and weak (w) are observed for FA at 345 (s), 386 (s), 423 (m), and 445 (w) nm and those for HA at 348 (s), 394 (m), 425 (m)

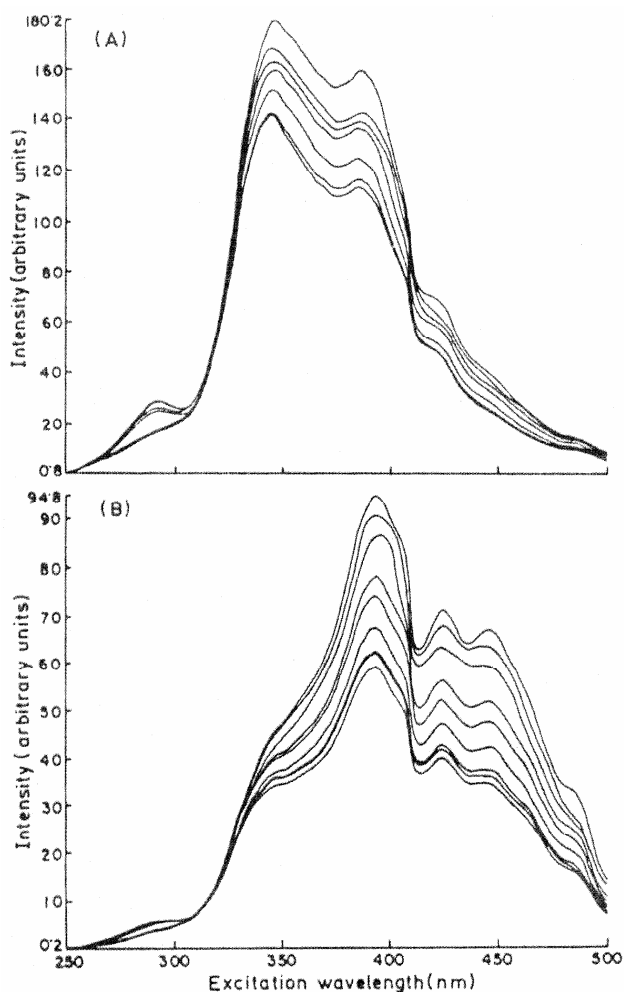


Fig. 3—Stoichiometric quenching of (A) fulvic acid and (B) humic acid with Co (II).

and 445 (w) nm. FA shows higher fluorescence intensity than that observed for HA. This indicates that FA has more rigid framework having more electron donor groups as $-OH$, $-NH_2$, etc. Humification results in more flexibility of the structure with more incorporation of electron withdrawing substituents such as $-COOH$ group resulting lowering fluorescence¹⁸. All four bands in SyF spectrum for FA and HA show the quenching effect for the presence of Co (II). Values of I at different concentrations of metal in between I_0 and I_{FM} were used to calculate K_C using Eq. (8).

In Table 2 average values of conditional stability constants for HA and FA are compared with those obtained for organic acids. It can be seen that both natural polyelectrolyte formed stronger complexes with Co (II) than the simple organic acids. Conditional stability constant (K_C) for FA complex is about five times higher than HA complex indicating that FA

Table 2—Conditional stability constants (K_C) for (1:1) complex between Co (II) and humic and fulvic acids, and some simple organic acids at 25°C (ref. 19).

Sample	K_C
Fulvic acid	4.7×10^{11}
Humic acid	1.02×10^{11}
Salicylic acid ⁽¹⁹⁾	3.16×10^7
Phthalic acid ⁽¹⁹⁾	6.3×10^2
Citric acid ⁽¹⁹⁾	0.20×10^{11}
Glycine ⁽¹⁹⁾	1.3×10^5
Acetic acid ⁽¹⁹⁾	31.6

forms a more stable complex than HA. These results show that quenching of fluorescence intensity of humic substances due to the complexation with metal can be used as a valuable tool for measuring stability constant by using synchronous fluorescence spectroscopy and Co (II) associates strongly with soil FA and HA forming soluble complexes which have important influence on Co (II) uptake by benthic fauna and toxicity. Rigid framework of FA having more electron donors groups as $-OH$, $-NH_2$ and less incorporation of $-COOH$ than HA could be the reason for its formation of more stable complex with Co (II).

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References

- 1 Sunda W G, Trace metal interactions with marine phytoplankton, *Biol. Oceanog.*, 6 (1990) 411- 421.
- 2 Fisher N S & Reinfelder J R, The trophic transport of metals in marine system : in *Metal speciation and bioavailability in aquatic system*, Vol. 3, edited by A Tessier & D R Turner, (IUPAC, John Wiley & Sons, New York) 1995, pp. 363-406.
- 3 Fisher N S & Wente M, The release of trace elements by dying marine phytoplankton, *Deep-Sea Res.*, 40 (1993) 671-677.
- 4 Luoma S N, Can we determine the biological availability of sediment-bound trace elements?, *Hydrobiologia*, 176 (1989) 379-390.
- 5 Young R S, *Cobalt in biology and biochemistry*, (Academic Press, London) 1979, pp. 340.
- 6 Mac Carthy J F, Bioavailability and toxicity of metals and hydrophobic organic contaminants, in: *Humic substances - Influence on fate and treatment of pollutants*, edited by I H Suffet & Mc. Carthy, (American Chemical Society, Washington D.C) 1989, pp.263-277.
- 7 Brown G K, Mac Carthy P & Leenheer J A, Simultaneous determination of Ca, Cu, Ni, Zn and Cd binding strengths with fulvic acid fractions by Schubert's method, *Anal. Chim. Acta*, 402 (1999) 169-181.

- 8 Stevenson F J, Stability constants of Cu^{+2} , Pb^{+2} , and Cd^{+2} complexes with humic acids, *Soil Sci*, 40 (1976) 665 – 672.
- 9 Silva C S P C O, Esteves da Silva J C G & Machado A A S C, Evolving factor analysis of synchronous fluorescence spectra of fulvic acids in the presence of aluminium, *App Spectro*, 48 (1994) 363-372.
- 10 Turner D R, Problems in trace metal speciation modeling, in: *Metal speciation and bioavailability in aquatic systems*, edited by A Tessier & D R Turner, (John Wiley & Sons, New York) 1995, pp. 149-203.
- 11 Sarkar B, Das U, Bhattacharya S & Bose S, The quenching of lumichrome fluorescence by β -cyclodextrin: Evidence for inclusion complex, *Bull Chem Soc Japan*, 68 (1994) 1807-1809.
- 12 Rice A J & Mac Carthy P, Characterization of a stream sediment Humin, in *Aquatic humic substances*, edited by H Suffet & P Mac Carthy, (American Chemical Society, Washington) 1989, pp. 41-54.
- 13 Korolef F, Determination of silicon, in: *Methods of seawater analysis*, edited by K Grasshoff, M Ehrhardt & K Kremling, (Verlag Chemie, Germany) 1983, pp. 174-183.
- 14 Dowson R, Liebezeit G & Josefsson B, Determination of amino acids and carbohydrates, in: *Methods of seawater analysis*, edited by K Grasshoff, M Ehrhardt & K Kremling, (Verlag Chemie, Germany) 1983, pp. 314-342.
- 15 Lowry G H, Roxbrough N J, Farr A L & Randal R J I, Protein measurement with folin phenol reagent, *J Biol Chem*, 193 (1951) 265-275.
- 16 Piper C S, *Soil and plant analysis*, (Inter Science Publication, New York) 1950, pp. 167.
- 17 Sardesai S, Humic and fulvic acids in sediments of the Hooghly estuary and some coastal areas in the northern Bay of Bengal, *Indian J Mar Sci*, 18 (1989) 16-20.
- 18 Rohatgi-Mukherjee K K, *Fundamentals of photochemistry*, (New Age international Publisher, New Delhi) 1997, pp. 371.
- 19 Morel M M F & Hering G J, *Principles and application of aquatic chemistry*, (John Wiley & Sons, New York) 1993, pp. 336.