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**Study of cadmium-humic interactions and determination of stability constants of cadmium-humate complexes from their diffusion coefficients obtained by scanned stripping voltammetry and dynamic light scattering techniques**

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**Abstract**

Diffusion coefficients of Cd-humate complexes are dependent on pH and [Cd]/[Humic] Acid (HA)] ratio in a Cd-humic system. These two factors mainly control the mass transport and complexation kinetics of Cd that may influence bioavailability and toxicity of Cd species in environmental systems. The combination of stripping voltammetry and dynamic light scattering techniques to determine the diffusion coefficients of Cd-HA system provided a better understanding of the system are very useful for extracting additional speciation parameters of the system. Cd<sup>2+</sup> ion along with small dynamic Cd complexes were predominantly present in the system at pH 5 with high diffusion coefficients. HA molecules were in disaggregated form at pH 6 and concentrations of Cd<sup>2+</sup> ion and small Cd-dynamic complexes decreased at this pH due to formation of Cd-humate complexes. A decrease in diffusion coefficient of Cd complexes was observed. No further decrease in the hydrodynamic radii of HA was observed with the increase of pH. The Cd-humate system partially lost its lability at pH 7. Conditional stability constants were calculated for Cd-HA complexes by combining the diffusion coefficient data obtained by two techniques. The logK values calculated in this study are in good agreement with the data available from the literature.

**Key words:** Diffusion coefficient, Cd-humate system, Cd speciation, Aggregation/disaggregation of humic acids, scanned stripping voltammetry, Dynamic Light Scattering

## 1 Introduction

One of the most important properties in an environmental system is the diffusion coefficient, which determines the mass transport, complexation kinetics, bioavailability and toxicity of a metal species. Unfortunately, determination of diffusion coefficient of a single species is extremely difficult in natural systems where metal complexes are polydisperse and chemically heterogeneous [1]. However, it is possible to determine an average diffusion coefficient of all dynamic metal complexes by using different techniques such as Diffusive Gradients in Thin films technique (DGT) [2], Stripping Voltammetry (SV) [3], gel permeation chromatography [4,5], ultra-filtration [6], diffusion through activated carbon column [7], and dynamic light scattering (DLS) [8,9]. SV techniques have been applied widely to determine average diffusion coefficients ( $\bar{D}$ ) of metal complexes in natural water systems because of their simplicity. Pinheiro et.al [10] applied the SV technique to determine an average diffusion coefficient of nanoparticles and humic substances. Despite a large numbers of attempts [11-13] using different techniques to determine the average diffusion coefficient of metal complexes in natural aqueous systems, this important speciation parameter has not been used to help understand the basic chemistry involved in metal-humics interaction in natural systems.

Metals in natural waters are predominantly found in complexes with ligands including humic substances, polysaccharides, and proteins. Humic substances are physically and chemically heterogeneous [1] and thus the metal-humic complexes are polydisperse and different in thermodynamic stability. Due to the unknown nature of humics, determination of the absolute diffusion coefficient of a single species is impossible. In general, the reported diffusion coefficient of metal complexes in natural systems by different techniques is an average diffusion coefficient of all dynamic metal complexes (including metal aqua complexes) in the system. The average diffusion coefficient ( $\bar{D}$ ) [14, 15] of a metal M is represented by

$$\bar{D} = \frac{D_M c_M + D_{ML} c_{ML}}{c_{M,T}^*} \quad (1)$$

where,  $C_M$ ,  $C_{ML}$  and  $c_{M,T}^*$  are the concentrations of the free metal ion, dynamic metal complexes and total metal,  $D_M$  and  $D_{ML}$  represents the diffusion coefficient of free metal ions and metal-ligand complexes. This equation suggests that the formation of free metal ion or labile complexes from the strong complexes increase either  $C_M$  or  $C_{ML}$  and as a result  $\bar{D}$  of the species increases. Similarly, complexation of free metal ion with strong complexing sites of humics, i.e formation of inert complexes and formation of weak bulky complexes, may decrease  $\bar{D}$ . It is important to mention that the detection of  $C_{ML}$  depends on the analytical detection window of the technique applied.

Thus, the following equation [14] was introduced to express the average diffusion coefficient and explain the above phenomenon:

$$\bar{D} = D_M \frac{(1 + \varepsilon K')}{(1 + K')} \quad (2)$$

where,  $\varepsilon = \frac{D_{ML}}{D_M}$  and  $K' = KC_L$  ( $K$  is the conditional stability constant of ML complexes and  $C_L$  is the uncomplexed ligand concentration).

The above equation can be reduced to

$$K' = \frac{D_M - \bar{D}}{\bar{D} - D_{ML}} \quad (3)$$

Thus, the determination of  $\bar{D}$  and  $D_{ML}$  of a system can be very effective to understand the metal complexation with a heterogeneous ligand in aqueous system. The objective of this work was to develop a better understanding of metal speciation by determining  $\bar{D}$  and  $D_{ML}$  of metal complexes (which includes metal-humate and other complexes) by using two independent techniques. The interaction of cadmium and humic acid was studied in model solutions with a well-characterized humic acid by using scanned stripping voltammetry (SSV) and DLS.

## 2. Theory

The theory of Scanned Stripping Voltammetry (SSV) technique is well established and has been explicitly discussed in the literature [16]. In the present work, SSV current-potential curves of Cd were analyzed to obtain the limiting current, which has been used to calculate the diffusion coefficient of Cd species in the test solution.

### *Diffusion coefficients*

In stripping voltammetry, for a spherical electrode in the presence of labile complexes, the limiting current,  $i_{lim}$ , at a constant potential,  $E$ , is given by [17]

$$i_{lim_{M+L}}^* = nFAD\bar{c}_{M,T}^* (1/\delta + 1/r_o) \quad (4)$$

which in the absence of ligand, reduces to

$$i_{lim_M}^* = nFAD_M c_{M,T}^* (1/\delta_M + 1/r_o) \quad (5)$$

where  $D_M$  and  $\bar{D}$  are the diffusion coefficients of the free metal and its complexes in aqueous solution [18],  $c^*$  is the concentration of the metal complexes (ML) or total metal (M, T) in solution,  $n$  is the number of electrons involved in the process,  $A$  is the electrode surface area exposed to the analyte,  $\delta$  is the diffusion layer thickness in

presence of complexing ligand,  $\delta_M$  is the diffusion layer thickness in absence of complexing ligand and  $r_o$  is the radius of the electrode; all other terms have been defined in the appendix.

The diffusion layer thickness depends on the hydrodynamic condition during the deposition step. It can be evaluated from a power function of  $D^\alpha$ :

$$\delta = \gamma D^\alpha \quad (6)$$

where  $\alpha$  is related to the hydrodynamic nature of mass transport [19] and  $\gamma$  corresponds to the constant part of  $\bar{\delta}$  that does not depend on the diffusion coefficient. The  $\alpha$  value of 1/3 was reported for the similar kind of work in the literature [20].

Combining Eq 4, 5 and 6, we can write,

$$\frac{i_{\lim M+L}^*}{i_{\lim M}^*} = \frac{\bar{D}((1/\gamma\bar{D}^{-1/3}) + (1/r_o))}{D_M((1/\gamma D_M^{-1/3}) + (1/r_o))} \quad (7)$$

For macroelectrodes,  $1/r_o \ll 1/\delta$ , so that the Eq 7 reduces to

$$\frac{i_{\lim M+L}^*}{i_{\lim M}^*} = \left( \frac{\bar{D}}{D_M} \right)^{2/3} \quad (8)$$

Using Equation 8, it is possible to determine the diffusion coefficient of metal complexes in the solution.

In heterogeneous systems (like natural waters containing humics), Eq 8 provides an average diffusion coefficient,  $\bar{D}$ , which is a weighted average of  $D_M$  and  $\bar{D}_{ML}$ .

$$\bar{D} = \frac{D_M c_M + \bar{D}_{ML} c_{ML}}{c_{M,T}^*} \quad (9)$$

The voltammetric behavior of metal complexes (i.e. fully labile, fully inert or intermediate) depends on pH and the [M]/DOC ratio used in the experiment. In this study, diffusion coefficients were calculated by considering the whole system as labile.

## 2.2 Fundamentals of DLS

Dynamic light scattering (DLS) is an effective and a quite straightforward technique for determining the hydrodynamic size of species going from large polymer chains to micelles and to microspheres, among others.

The output of this technique is the scattered intensity autocorrelation function,  $C(\tau)$ , for correlation time, which is given by

$$C(\tau) = A(1 + \beta \int_0^{\alpha} P(\Gamma) \exp(-\Gamma t) d\Gamma) \quad (10)$$

where  $A$  is a baseline value,  $\beta$  is an instrumental constant and  $\alpha$  is the characteristic line width of the distribution function  $P(\Gamma)$ . The value contains information regarding the diffusion coefficient  $D$  of the scattering species, these two values being related by the following expression:

$$\Gamma = Dq^2 \quad 11$$

where  $q$  is the scattering vector, which is constant for a given observation angle and incident light wavelength. In addition, if one assumes that the scattering species can roughly be taken as spheres, then the apparent hydrodynamic diameter,  $d_h$ , of the said species can be calculated through the Stokes- Einstein equation:

$$d_h = k_B T / (3\pi\eta D) \quad (12)$$

where  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature and  $\eta$  is the viscosity. The sizes discussed throughout this work are the Stokes- Einstein diameters, which can be considered as apparent diameters since the aggregates in solution, in most cases, are not hard spheres.

The dynamic light scattering results obtained in the form of Eq. (7) have to be analyzed by a fitting procedure. The most straightforward procedure is to assume that only one type of scattering species is present, and to fit the data to a single exponential expanded as a Taylor series (cumulants method).

The diffusion coefficients determined by the DLS technique of Cd-HA complexes were considered as the diffusion coefficients of the Cd-humate complexes ( $D_{ML}$ ). The mobility of Cd-HA complexes mainly depends on the size of the humic acid molecule and as a result, the mobility of a complex is dependent on the hydrodynamic radius of the humate. Thus,  $\bar{D}$  determined by DLS technique for Cd-HA system was considered as  $D_{ML}$ . It is important to note that this technique is independent of the lability of the system and depends on the size and concentration of the particles. The value of  $\bar{D}$  determined by SSV measurement is combined with the  $\bar{D}$  value determined by DLS technique.

### 3 Materials and methods

#### 3.1 Humic Acid

Soil humic acid, HA, supplied by Dr. L. Evans (University of Guelph, CA), had been characterized and purified according to the procedure recommended by the International Humic Substances Society [21]. It was reported [21] that the dissociation reaction that takes place below pH 6.5 in this humic acid is attributed to carboxylic acid groups and above pH 6.5, may be attributable to phenolic groups. As the titration pH range for this humic acid was quite wide, it was assumed that a series of binding sites were available for complexation.

A stock solution of  $\sim 1.00 \text{ g L}^{-1}$  HA was prepared by dissolving approximately 0.100 g of the soil HA in  $100 \text{ cm}^3$  of ultrapure water and electrolytically purified NaOH at pH 10. The pH was then adjusted to 8.0 with ultrapure  $\text{HNO}_3$ . The resulting HA solution was stored in the dark at  $4 \text{ }^\circ\text{C}$ . The molar mass of the HA was estimated by using its bidentate complexing capacity of  $4.88 \text{ mmol g}^{-1}$  [21]. All solutions were prepared in ultrapure water from a Milli-Q-Plus water purification system (resistivity  $18.2 \text{ M}\Omega\text{-cm}$ ).

#### 3.2 Humic Acid solution composition

Four sample solutions were prepared in ultrapure water containing various concentrations of HA (5, 10, 12.5, and  $20 \text{ mg L}^{-1}$ ) and  $7.9 \times 10^{-7} \text{ mol L}^{-1}$  of Cd(II). The heavy-metal concentrations in ground and surface water, soil, and crop plants in farmers' fields near the industrial cities in India were reported to be high. Thus, the concentration used for Cd in this study is environmentally significant. The pH of the solutions were adjusted to  $5.0 \pm 0.1$ ,  $6.0 \pm 0.1$  and  $7.0 \pm 0.1$  using ultrapure NaOH and  $\text{HNO}_3$ , and the ionic strength was maintained at  $10 \text{ mmol L}^{-1}$  with  $\text{KNO}_3$ . The solutions were left to equilibrate for 72 hr in the dark prior to analysis. The pH of the solutions was checked again immediately before analysis and found to be the same.

#### 3.3 Apparatus

Voltammetric measurements were made with an Autolab PGSTAT30 potentiostat/galvanostat, equipped with a Metrohm 663 VA stand. The working electrode was a static mercury drop electrode. The reference electrode was a Ag/AgCl electrode in a glass tube filled with  $3 \text{ mol L}^{-1}$  KCl and fitted with a porous Vycor tip (Bioanalytical Systems, Inc., USA). The counter electrode was made of a platinum rod (Metrohm, Switzerland). Measurements were made in direct current mode at a range of deposition potentials, with a deposition time of 45 s, and an equilibration time of 15 s. For the stripping step the initial and final potentials were  $-0.40 \text{ V}$  and  $-0.80 \text{ V}$ , respectively, and the scan rate was  $0.01 \text{ V s}^{-1}$ . Solutions were initially purged with nitrogen for 10 mins, and then a  $\text{N}_2$  blanket was maintained over the solutions during measurements.

Dynamic light scattering measurements were performed using a Malvern Zetasizer Nano ZEN 1600 (Malvern Instruments Ltd., Malvern, Worcestershire, UK), that uses a 4.0 mW He/ Ne laser with a wavelength of  $\lambda_0 = 633$  nm, and collects the scattered light at a fixed scattering angle  $\theta$  of  $173^\circ$ . Sizes reported are the effective hydrodynamic radii (or diameters) obtained from the major peak of a multimodal fit of the intensity autocorrelation function  $g_2(t)$  as determined by the Malvern Nanosizer, using the built in DTS (Nano) software version 5.0. Depending on the diameter, the diffusion coefficient of the particles are calculated

## 4 Result and discussion

### 4.1 Determination of the diffusion coefficient ( $\bar{D}$ ) of Cd species as a function of Cd /Humic Acid (HA) ratio by SSV technique

The average diffusion coefficients ( $\bar{D}$ ) of Cd-Humate complexes were determined at different [Cd]/ [HA] ratios at three different pH values. It is evident from the Figure 1 that the  $\bar{D}$  of cadmium species decreased with an increase in HA concentration under the experimental conditions. It is necessary to mention that the average diffusion coefficient of metal-Humate complexes in aqueous solution depends on the size, structure and complexing capacity of the HA molecule which forms metal complexes. Thus it is essential to know how the properties (aggregational, and complexing capacity) of humic acid changes with the change in the concentration of humic acid and pH.

It is also reported that HA may form relatively large aggregates (by H-bonding and van der Waal's attraction) and that these aggregates are able to disaggregate/reaggregate, depending on the nature and concentration of HA and pH of the medium [22-24]. It is thought that these HA aggregates may contain regions that have a micelle-like behavior. Micelle-forming amphiphilic compounds are characterized by the presence, on the same molecule, of hydrophobic region (aromatic groups and/ or aliphatic groups) and hydrophilic groups (phenolic, carboxylic, alcoholic) [23-25]. Aggregation of humic acid increases with an increase in HA concentrations. Thus, one could expect a gradual decrease of the  $\bar{D}$  of Cd-humate complexes with an increase in HA concentrations. Figure 1 shows a similar trend where  $\bar{D}$  of Cd-humate complexes gradually decreased with increasing HA concentration.

It is reported by Avena and Wilkinson [26] that a small but significant increase in the diffusion coefficients of the HA were observed as a function of increasing pH. The mechanism they proposed appeared to involve the release of monomers from the surface of the aggregates. It was reported that the pH markedly affect the disaggregation rate of humics. They reported that at pH higher than 4.5, the disaggregation rate increased more than 3 orders of magnitude per pH unit increase.

Hosse and Wilkinson [27] employed Capillary electrophoresis (CE) and fluorescence correlation spectroscopy (FCS) to study the electrophoretic mobilities and hydrodynamic radii of humic substances and found that diffusion coefficient of humic substances remain constant or increase slightly as a function of ionic strength [28, 29]. It was also reported that no evidence of aggregation was observed as a function of increasing ionic strength for HS. Thus, the ionic strength of all the solutions were kept constant ( $I = 0.01\text{mM}$ ) in this study and the pH of the test solutions were varied for further experiments.

#### 4.2 Determination of diffusion coefficient ( $\bar{D}$ ) of Cd species as a function of pH by SSV technique

Figure 2 also reveals that the  $\bar{D}$  of Cd-humate complexes was high at pH 5 compared to pH 6 and 7. However, one could expect the lowest  $\bar{D}$  of Cd-humate complexes at pH 5. This is because of the fact that at lower pH (~5) a considerable increase of aggregation of HA is expected due to neutralization of negative charges of polyelectrolyte HA by  $\text{H}^+$  ions. Thus one could expect a decrease in the  $\bar{D}$  of Cd-humate complexes. This contradictory result clearly suggests that the competition by  $\text{H}^+$  with  $\text{Cd}^{2+}$  ion for binding sites in HA is higher at pH 5 than at pH 6 or 7 [30]. Thus, a considerable amount of cadmium metal remained as cadmium ion or small dynamic Cd complexes at pH 5 with fast diffusion coefficient and as a result the average diffusion coefficient of Cd- complexes ( $\bar{D}$ ) was high at pH 5.

However,  $\bar{D}$  of cadmium-humate complexes decreased with the increase of pH from 5 to 6. The increase of pH is expected to initiate disaggregation of aggregated HA (present at pH 5) by increasing the negative charge of HA (by deprotonation of polyelectrolyte groups of HA) and an increase in average diffusion coefficient of Cd-humate complexes is expected. Pinheiro et al [31] reported that with increasing pH, the aggregates of HA start to loose some of their building blocks due to the increasing charge density.

The experimental results showed a decrease in the  $\bar{D}$  value of Cd-humate complexes. The contradictory results (Figure 2 and Table 1) can be rationalized by considering the complexation reactions between  $\text{Cd}^{2+}$  and HA involved in this pH. At pH 6, the disaggregation of humic acid might have occurred with the formation of smaller HA molecules with relatively higher diffusion coefficient (compare to the aggregated HA molecule at pH 5). However at pH 6, the concentration of free  $\text{Cd}^{2+}$  ion or small dynamic Cd complexes decreased due to availability of more binding sites in disaggregated HA molecules. These complexes were smaller in size than the aggregated humic species at pH 5, but much larger than the free  $\text{Cd}^{2+}$  ion and small dynamic complexes (which were predominantly present at pH 5) and as a result the average diffusion coefficient of Cd-humate complexes decreased compared to the average diffusion coefficient at pH 5.



It is well known that the average diffusion coefficient of metal-humics complexes in natural system (under fully labile conditions) is expressed by the following equation

$$\bar{D} = \frac{D_{ML}C_{ML} + D_M C_M}{C_{M,T}}$$

When,  $D_{ML}C_{ML} \gg D_M C_M$  i.e., all metal ions are complexed (i.e.,  $C_{M,T} \approx C_{ML}$ ), then  $\bar{D} \approx D_{ML}$ .

At the other extreme case,

when  $D_M C_M \gg D_{ML}C_{ML}$  i.e., very little complexation of metal ions with humics (i.e.,  $C_{M,T} \approx C_M$ ) has occurred, then  $\bar{D} \approx D_M$ .

At pH 5, it is found that  $\bar{D}$  calculated from SSV data for Cd complexes are very close to the diffusion coefficient of free  $Cd^{2+}$  ion ( $7 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ). This also supports our explanation that at pH 5,  $Cd^{2+}$  ion interacted weakly with humics due to competition of  $H^+$  ion and predominantly remained as  $Cd^{2+}$  ion or small weak complexes having similar diffusion coefficients to the  $Cd^{2+}$  ion in the solution.

At pH 6,  $Cd^{2+}$  ion interacted strongly with disaggregated humics and  $\bar{D}$  decreased compared with  $\bar{D}$  at pH 5. It was reported by Pinheiro et al [31] that Cd-LFA and Cd-PPHA complexes were labile at pH 6 (even though capacity of those humics were more than the humic acid used in this case and as a result, a fully labile system was assumed at pH 6).

The  $\bar{D}$  of Cd-Humate complexes decreased with increasing pH from 6 to 7 (Table 1 and Figure 2). However, one could expect a further major increase in  $\bar{D}$  when the pH increased from 6 to 7. This is because HA is supposed to undergo further disaggregation. This further decrease of  $\bar{D}$  of Cd-humate complexes at pH 7 was not expected. At this pH, the concentration of binding sites probably increased within the humic acid for complexation with  $Cd^{2+}$  in the solutions [31] and the concentration of  $Cd^{2+}$  ion further decreased at this pH. Secondly, there may be a chance of decreased lability and formation of inert Cd-humate complexes. These factors might have played an important role in reducing the signal at pH 7. It is important to mention that  $Cd^{2+}$  ion was used in this work as a probe to determine the  $\bar{D}$  of Cd-humate complexes. Calculation of diffusion coefficients of Cd-humate complexes by SSV technique was done under the assumption that the system is fully labile. However, this assumption can not be taken as an absolute truth since very little is known for complex systems like humic acid and as a result, it is questionable to determine the  $\bar{D}$  of metal-humate complexes at higher pH by this method.

Thus, it was necessary to determine the diffusion coefficients of Cd-humate complexes by another independent technique, where loss of lability would not affect the determination of diffusion coefficient of Cd-humate complexes. Dynamic light scattering (DLS) technique was used as an independent technique for the determination of  $\bar{D}$  of Cd-humate complexes at different pH.

#### 4.3 Determination of Diffusion coefficient ( $\bar{D}$ ) of Cd species by DLS

The  $\bar{D}$  of Cd-humate complexes determined by DLS technique (Table 2) was consistent with the literature values. The value of  $\bar{D}$  of Cd-humate was lowest at pH 5 and the value was found to increase with the increase of pH. This observation suggests that at pH 5, aggregation of humic acid predominated but disaggregation increased with the increase of pH. This supports the reaggregation/ disaggregation properties of humic acid and the basis of our explanation for SSV results (i.e., at pH 5, Cd<sup>2+</sup> ion and small dynamic Cd-complexes predominates due to competition from H<sup>+</sup> ion).

The DLS technique indicated that the value of  $\bar{D}$  of Cd-humate complexes increased with the increase of pH from 5 to 6. This suggests that at pH 6, HA started to disaggregate and as a result  $\bar{D}$  increased. However, a decrease in  $\bar{D}$  value was observed in the SSV technique (where Cd<sup>2+</sup> acts as a probe to find out the diffusion coefficient of humics). This data suggest that at pH 6, Cd<sup>2+</sup> ion was complexed with humate ion (less competition with H<sup>+</sup> ion and opening up of new binding sites in HA due to disaggregation at pH 6) and as a result the average  $\bar{D}$  decreased compared to the  $\bar{D}$  value at pH 5.

However, the  $\bar{D}$  value determined by DLS of Cd-humate complexes remained almost unchanged (small change within the uncertainty of the measurement technique) when the pH raised from 6 to 7 (Figure 3). This observation suggests that there was no further change in the hydrodynamic radius of humic acid at pH 7 compared to pH 6.

However, the  $\bar{D}$  value determined by SSV was found to undergo further small decrease. This is because of the fact that at pH 7, the Cd-humate system is losing lability and thus SSV technique may not be a suitable technique to measure  $\bar{D}$  of Cd-humate system at this pH.

The value for  $\bar{D}$  obtained by DLS is consistent with the  $\bar{D}$  value reported by Pinheiro et al [9] using the same technique. However, the  $\bar{D}$  values of Cd-humate complexes measured by DLS technique are much lower than the values obtained by SSV. This is because of the fact that the DLS measurements are more sensitive to large aggregates even when their contribution to the total mass is small, hence are not so affected by the presence of

small aggregates. However, a clear decrease in the size of the Cd-humate complex was observed by DLS when the pH was increased from 5 to 6.

Secondly, in case of SSV,  $\text{Cd}^{2+}$  ion acted as a probe to determine  $\bar{D}$ . At different pH, the complexation of Cd and humic acid and formation of free  $\text{Cd}^{2+}$  ion and weak complexes were also varied and  $\text{Cd}^{2+}$  ion has much faster diffusion coefficient than HA molecules and thus the average  $\bar{D}$  value was found to be higher by SSV technique than the  $\bar{D}$  measured by DLS.

These observations by SSV and DLS may help us to visualize the phenomenon as shown in Figure 4.

Combining the observations by these two techniques the following conclusions can be drawn.

1. HA molecules were present in aggregated form at pH 5.  $\text{Cd}^{2+}$  interacted with HA very weakly due to competition between  $\text{H}^+$  and  $\text{Cd}^{2+}$  ion.  $\text{Cd}^{2+}$  ion along with small dynamic complexes predominated in the solution at pH 5.
2. Humic acid molecules disaggregated at pH 6 and Cd interacted with HA and concentrations of  $\text{Cd}^{2+}$  and small dynamic complexes decreased at this pH due to formation of Cd-humate complexes.
3. At pH 7, no further decrease in the hydrodynamic radii of HA was observed by DLS technique but a decrease in signal by SSV was observed. This suggests that Cd-humate system lost its lability at pH 7.

This finding can be very important to reveal the speciation of bioavailable Cd species under different environmental conditions.

#### **4.4 Determination of logK of Cd-HA complexes from the diffusion coefficients determined by SSV and DLS techniques**

The results of this investigation demonstrate that combination of two analytical techniques having complementary analytical capabilities can provide a better physicochemical picture of metal speciation than either one of the analytical technique can do alone. The combined use of these techniques to find out the stability constant of Cd-HA complexes is new. Equation 3 was used to calculate  $K'$ .

$$K' = \frac{D_M - \bar{D}}{\bar{D} - D_{ML}} \quad (3)$$

The value of  $D_M$  has been used from the literature ( $7 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ ). The diffusion coefficient determined by SSV technique has been used as  $\overline{D}$  and the diffusion coefficient determined from DLS technique has been considered as  $D_{ML}$ . This measurement is independent of lability of the system. In the model solution, the concentration of ligand is known and thus the K value of Cd-humate complexes at different pH was calculated. The data are presented in Table 3.

However, it is well known that DLS is not able to detect the presence of small particles in presence of bigger particles. The intensity of the scattered light is a function of both the particle number and size. The intensity of scattered light is proportional to the sixth power of size for a constant number of particles. Therefore, dynamic light scattering measurements in polydisperse systems underestimate the mean diffusion coefficient, and consequently the average molecular weight is overestimated [32]. Thus, the use of the diffusion coefficient obtained from DLS technique as  $D_{ML}$  may underestimate the logK value of a metal-humate complex.

Table 3 shows a small change in the logK values of Cd(II)-HA complexes at pH 5 even after the increase of HA concentration in the model solutions, which suggest that molecular size of humic fractions had smaller influence on metal-complex stability at pH 5. Similar observation was reported by Evangelou et al [33].

The logK values were found to increase when the pH increased to 6. This observation suggest that the aggregated form of HA are in disaggregated form and different binding sites were available for complexation with Cd(II). With the increase of pH, the aggregates of HA started to loose some of their building blocks due to the increasing charge density and these opened up new binding sites in the HA molecules, which is reflected as an increase in the logK value of Cd-HA complexes at pH 6.

At pH 7, there was much less competition from proton and more binding sites were available for Cd(II) binding and logK values were found to increase with the increase of HA concentration at this pH. The variation of logK values as a function of pH is presented in Figure 5.

There is a body of data reported for logK of Cd complexes with humic substances. It is well known that an appropriate comparison of the results with literature data requires a similar [Cd]/[HA] mole ratio, as well as similar pH and ionic strength. The log K values of Cd (II)-LFA (Laurentian Fulvic Acid) complexes reported by Murimboh [34] was 5.26 and 4.92 at two different Cd (II)/LFA mole ratio at pH 5. Chakraborty et al [35] also reported the logK values for Cd (II)-SRFA complexes, which were 5.26, 5.41, and 5.47 at three different [Cd]/[SRFA] mole ratios at pH 5. logK for Cd-illite-humic fraction complexes was studied at different pH by Evangelou et al [33]. They reported that logK values of Cd-HA complexes varied in between 4.56 to 5.18. The logK data calculated in this study are in very good agreement with the literature values.

The finding of this research is that the combination of scanned stripping voltammetry and dynamic light scattering techniques to determine the diffusion coefficients of Cd-HA system can provide a better understanding of the system and can be very useful to calculate conditional stability constants for Cd-HA complexes. This finding is new and novel; however, further development is required to look into the dependence of the diffusion coefficient of humic entities on pH and whether such dependence can be utilized to improve our understanding on metal-humics interactions.

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**Table 1** Average diffusion coefficient of Cd-humate complexes under different [Cd]/[HA] ratio and pH conditions determined by SSV technique.

Samples	Average Diffusion coefficient ( $\bar{D}_{ML}$ ) of Cd-Humate complexes Determined by SSV technique ( $m^2.s^{-1}$ )			
	[Cd]/[HA]	pH 5	pH 6	pH 7
0.16		$(2.45 \pm 0.12) \times 10^{-10}$	$(1.45 \pm 0.07) \times 10^{-10}$	$(1.00 \pm 0.05) \times 10^{-10}$
0.08		$(1.75 \pm 0.08) \times 10^{-10}$	$(6.61 \pm 0.03) \times 10^{-11}$	$(5.10 \pm 0.02) \times 10^{-11}$
0.06		$(1.31 \pm 0.06) \times 10^{-10}$	$(5.22 \pm 0.02) \times 10^{-11}$	$(3.46 \pm 0.02) \times 10^{-11}$
0.04		$(1.02 \pm 0.05) \times 10^{-10}$	$(4.42 \pm 0.02) \times 10^{-11}$	$(1.75 \pm 0.01) \times 10^{-11}$



**Table 2** Average diffusion coefficient of Cd-species complexes in Cd-humic acid system at different pH by DLS technique.

<b>Samples</b>	<b>pH</b>	<b>Average Diffusion coefficient (<math>\overline{D}_{ML}</math>) of Cd-Humate complexes Determined by DLS technique (<math>\text{m}^2.\text{s}^{-1}</math>)</b>
Cd-Humate system	5	$(1.93 \pm 0.09) \times 10^{-12}$
	6	$(2.52 \pm 0.01) \times 10^{-12}$
	7	$(2.56 \pm 0.01) \times 10^{-12}$
	9	$(2.58 \pm 0.01) \times 10^{-12}$
	11	$(2.59 \pm 0.01) \times 10^{-12}$

**Table 3** Conditional stability constant of Cd-humate complexes calculated by the combination of SSV and DLS techniques under different experimental conditions

pH	[Cd]/[HA]	logK	Average
5	0.16	5.19	5.14 ± 0.08
	0.08	5.10	
	0.06	5.15	
	0.04	5.10	
6	0.16	5.50	5.58 ± 0.10
	0.08	5.60	
	0.06	5.63	
	0.04	5.56	
7	0.16	5.70	5.80 ± 0.25
	0.08	5.73	
	0.06	5.83	
	0.04	5.97	

## Figures Captions and Legends

**Figure 1** Plots of diffusion coefficients of Cd complexes as a function of Cd/HA mole ratios. Model solutions were composed of 0.01M KNO<sub>3</sub>, [Cd] =  $7.9 \times 10^{-7}$  mol. L<sup>-1</sup>, and HA of 5, 10, 12.5, and 20 mg L<sup>-1</sup> at pH 5 (◆), 6 (■), and 7 (▲).

**Figure 2** Plots of diffusion coefficients of Cd complexes as a function of pH at different mole ratios. Model solutions were composed of 0.01M KNO<sub>3</sub>, [Cd] =  $7.9 \times 10^{-7}$  mol. L<sup>-1</sup>, and HA of 5 (◆), 10 (■), 12.5 (▲), and 20 mg L<sup>-1</sup> (●).

**Figure 3** Plot of diffusion coefficients of Cd complexes as a function of pH by DLS technique.

**Figure 4.** Schematic diagram of humic acid at different pH. A) At pH 5 humic acid molecules are aggregated due to neutralization of negative charges by H<sup>+</sup>. Cd remains mainly as free in the solution that facilitated higher mobility and hence an increase the average diffusion coefficient was observed. B) At pH 6, humic acid disaggregates to smaller molecules. With the availability of complexing sites for Cd<sup>2+</sup> ions to undergo complexation and resulting in the lowered average diffusion coefficient. C) At pH 7, humic acid do not disaggregate. However, Cd started to form inert complexes at this pH.

**Figure 5.** Plot of logK of Cd-humate complexes as a function of pH.

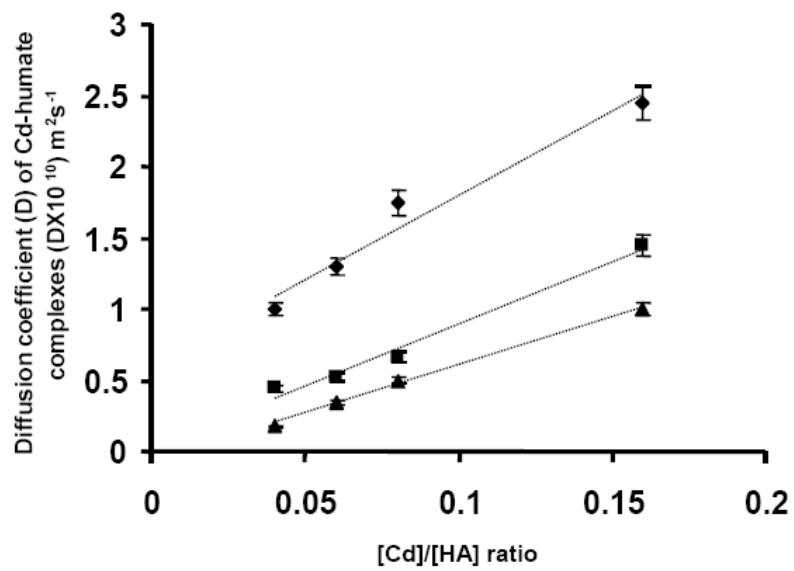


Figure 1

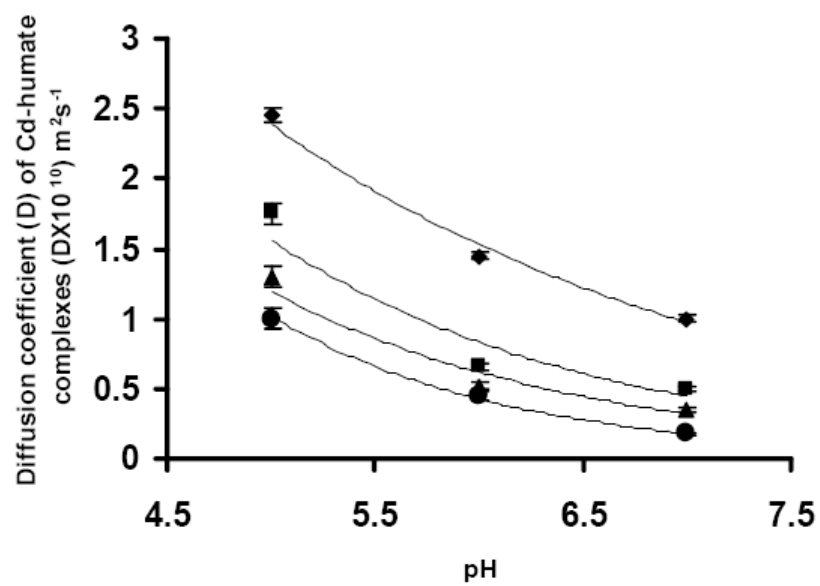
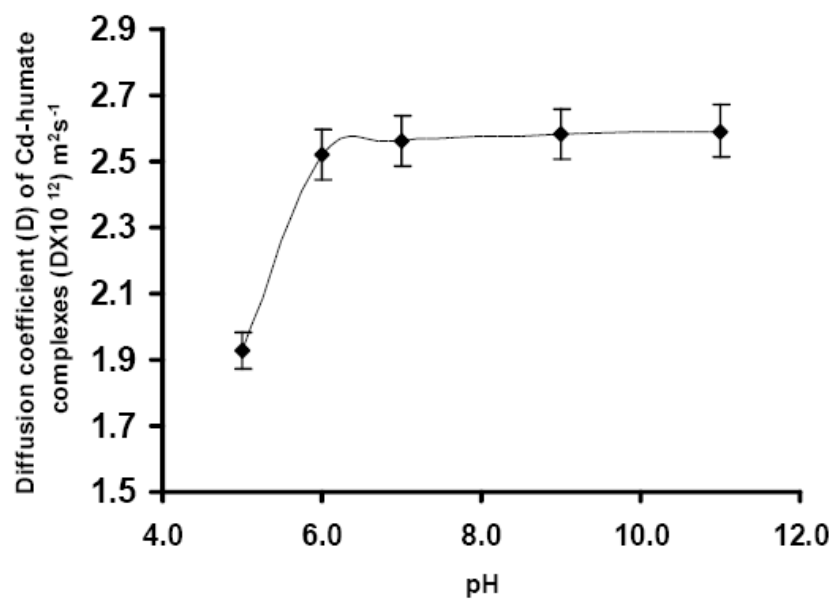


Figure 2



**Figure 3**

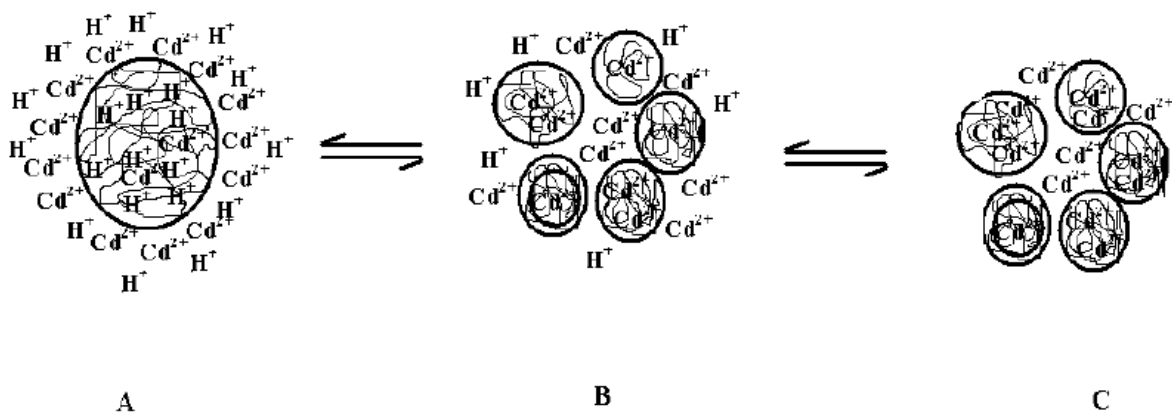


Figure 4

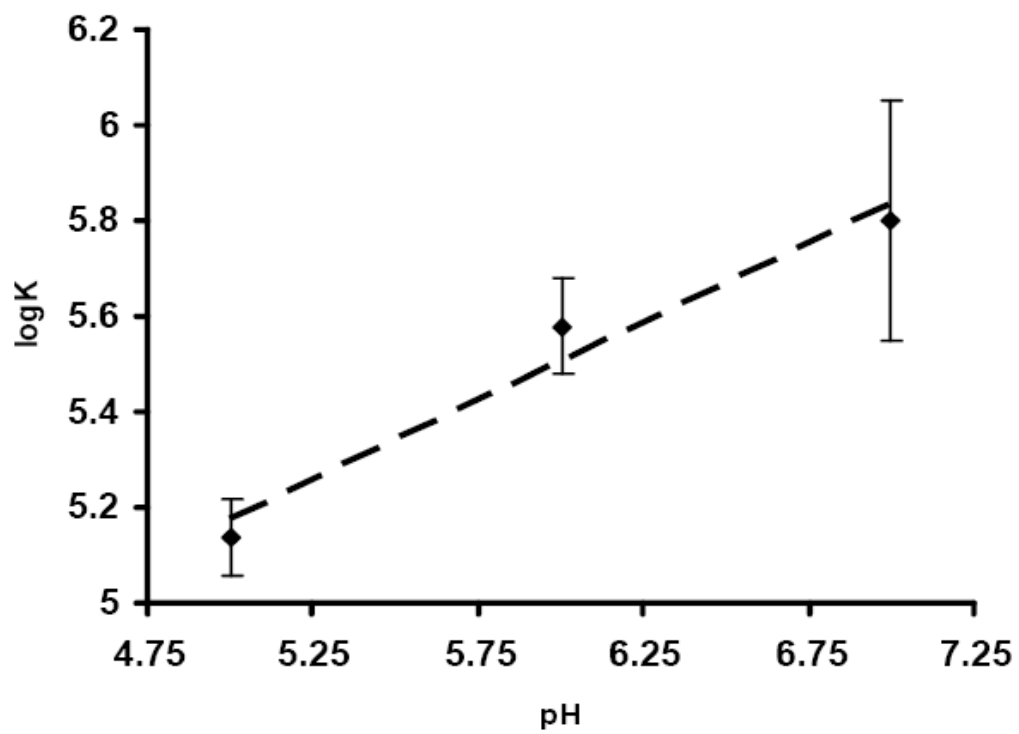


Figure 5