

19. R. R. Holmes, R. O. Day, V. Chandrasekhar, J. F. Vollano, and R. R. Holmes, *Inorg. Chem.*, **25**, 2490 (1986).
20. R. G. Swisher, J. F. Vollano, V. Chandrasekhar, R. O. Day, and R. R. Holmes, *Inorg. Chem.*, **23**, 3147 (1984).
21. K. C. Molloy, T. G. Purcell, M. F. Mahon, and E. Minshall, *Appl. Organomet. Chem.*, **1** 507 (1987).
22. S. W. Ng, V. G. Kumar Das, F. Van Meurs, J. D. Schagen, and L. H. Straver, *Acta Crystallogr. C.*, **45**, 568 (1989).
23. P. G. Harrison, K. Lambert, T. J. King, and B. Majee, *J. Chem. Soc., Dalton Trans.*, 363 (1983).
24. O. S. Jung and Y. S. Sohn, Unpublished result.

Complexation of Cadmium(II) with Humic Acids: Effects of pH and Humic Acid Origin

Mee Hae Lee, Se Young Choi, Kun Ho Chung, and Hichung Moon*

*Department of Chemistry, Korea Advanced Institute of Science and Technology
373-1 Kusong dong, Yusong ku, Teajun-shi 305-701*

Received July 9, 1993

A comparative study on cadmium (II) complexation with three well characterized humic acids (SHA: soil humic acid from the Okchun Metamorphic Belt; AqHA: aquatic humic acid from Gorleben underground aquifer, Germany; CoHA: commercially available humic acid from the Aldrich Co.) was carried out in 0.1 M NaClO₄ at different solution pH (5.0, 5.5, and 6.0) using the ultrafiltration technique. The maximum binding ability (MBA) of the humic acids for cadmium (II) was observed to vary with their origins and solution pH. The results suggest that 1 : 1 complex predominates within the experimental range, and the conditional stability constants were calculated based on the assumption of cooperative binding, yielding log *K* values that were quite similar (CoHA: 4.17 ± 0.08; AqHA: 4.14 ± 0.07; SHA: 4.06 ± 0.12 l mol⁻¹ at pH 6.0) irrespective of humic acid origins or pH. By contrast a nonlinear Schatchard plot was obtained, using the cadmium (II) ion selective electrode speciation analysis method, which indicated that humic acid may have two or more classes of binding sites, with log *K*₁ and log *K*₂ of 4.73 ± 0.08 and 3.31 ± 0.14 l mol⁻¹ respectively.

Introduction

Cadmium toxicity is of great environmental concern in the rapidly industrializing nations of the East Asian region. Due to increased industrial usage and related mining activities, elevated levels of cadmium are found in terrestrial and aquatic environments¹. During the hot summer season, torrential downpour of monsoon rain causes floodwater to flush away much of soil organic matters and other pollutants into river systems and subsequently into flooded rice fields². Well documented cases of itai-itai disease in the neighbouring Japan are known to be caused by eating cadmium contaminated rice grown on polluted lands³.

In natural aquifer systems the salinity, water hardness and pH are known to affect the cadmium speciation. However, the naturally occurring organic matters in freshwater systems such as humic and fulvic acids (HA and FA) are known to possess high affinity for metal cations ($Z \geq 2+$) and can also affect the cadmium speciation^{4,5}. Two parameters are required for the quantification of the migrational behaviour and availability of trace metals in freshwater systems. They are 1) basic understanding on the nature of humic acids, and 2) determination of the conditional stability constants. The reviews on these topics are reported in the literature^{6,7}. Many factors can influence the results of complexation studies between humic substances and trace metal

ions. One such factor is the variable nature of humic acid; humic acid extracted from different source materials is different and depends on botanic and climatic conditions, age, and depth of burial. Other factors include relative humic acid to metal ion concentrations, ionic strength, pH, method of speciation analysis, and model used to describe the complexation behaviours⁸.

In this work, the results of the studies on the complexation of cadmium (II) with three different humic acids of differing origins conducted at pH of 5.0, 5.5 and 6.0 are described. The investigations were conducted at constant ionic strength (*I* = 0.1) and at humic acid concentrations typically found in summer storm floodwaters (50 and 100 mg l⁻¹) resulting from monsoon rain. Both the separation and nonseparation methods of speciation analysis were used for these investigations, namely ultrafiltration technique (UF, separation) and cadmium (II) ion selective electrode (Cd(II)-ISE, nonseparation). In using the ultrafiltration method, the effects of sorption of labile metal ions on the filter membrane and equilibria shift during the separation stage were minimized by using appropriately designed apparatus and suitable membrane filters^{9,10}. Two models were used to graphically illustrate the experimental data: 1) a positive cooperative binding model based on the Hill plot (1 : 1 complex predominates) and 2) a Schatchard plot which assumes that there are two or more classes of binding sites¹¹.

Experimental

Materials and reagents. Three humic acids used in this investigation are as follows: The soil humic acid (SHA) was extracted by base digestion of air dried soil obtained from the Okchun Metamorphic Belt region under N_2 atmosphere (Kuye San, Korea); the aquatic humic acid (AqHA), provided by G. Buckau of the Technical University of Munich, was isolated from an underground aquifer (Gorleben, Germany); the third humic acid was the commercially available humic acid (CoHA) from the Aldrich Co. These humic acids were purified according to the same procedure and the details of the characteristics of these humic acids are described elsewhere.^{12,13}

Each humic acid stock solution was prepared by first dissolving the purified solid material in 0.1 M NaOH (carbonate free, Baker Co.), and further diluting it with 0.1 M NaClO₄ containing 10^{-3} M of either MES (2-morpholineethane sulfonic acid) or PES (4-pyridineethane sulfonic acid) buffers. Three replicate HA stock solutions (HA concentration of 200 mg l^{-1}) were pH adjusted to 5.0, 5.5, and 6.0 by adding appropriate amounts of 0.1 M HClO₄. Three replicate cadmium (II) stock solutions were prepared by dissolving CdO (Aldrich Co., 99.9%) with 1.0 M HClO₄ and diluting them with 0.1 M NaClO₄ (10^{-3} M MES or PES buffer), and pH adjusted (5.0, 5.5, and 6.0) with 0.1 M NaOH. The cadmium (II) concentration in the stock solutions, analyzed by atomic absorption spectrophotometer (AAS, Instrumental laboratory, Video 12) was 2.0×10^{-2} M. Other cadmium solutions were subsequently prepared by further dilution of these stock solutions. All solutions were prepared using reagent grade chemicals and double deionized water free of interfering carbon dioxide in an N_2 inert gas box. Glasswares were first soaked in an acid bath and then repeatedly washed with double deionized water prior to use. Glass electrode (Metrohm Co.) coupled to a digital pH meter (Metrohm type 632) are used to measure pH.

Ultrafiltration procedure. Series of the sample solutions (50 ml) were prepared by mixing equal volumes of HA stock solutions and cadmium (II) solutions: while maintaining the HA concentration constant (100 or 50 mg l^{-1}), the cadmium (II) concentration was varied from 1.0×10^{-5} M to 5.0×10^{-3} M. In parallel, series of the reference solutions are made by mixing the cadmium (II) solutions with the blank solution (0.1 M NaClO₄). The sample and reference solutions were allowed to stand for three days in darkness to equilibrate.

The sample solutions were filtered using a ultrafiltration cell, (Amicon Co., model 8050) modified to take 25 mm YM 1 membrane filter (molecular weight cut-off 1000 daltons) under 40 psi N_2 pressure. Filters were soaked in water and washed thoroughly to remove the trace of coating material. The solution was gently stirred during filtration to prevent clogging on the membrane surface. The proper functioning of the membrane was assured when the flowrate was maintained constant at 0.1 ml/min. Only 10% of the total volume was filtered: first 1.0 ml of the filtrate was discarded (dead volume of the cell), and subsequent 4.0 ml was collected for analysis by AAS. HA precipitates formed in high cadmium to humate concentration ratio samples were centrifuged for 30 min. at 4000 rpm using a benchtop centrifuge.

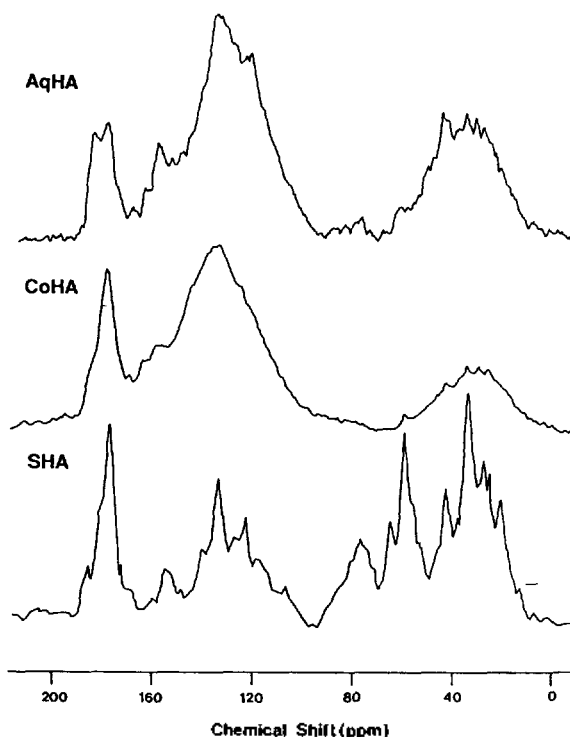


Figure 1. Normal ^{13}C -NMR spectra of soil (SHA) and water (AqHA) derived humic acids, and Aldrich humic acid (CoHA): 200 mg m^{-1} HA in 1.0 M NaOD.

After carefully discarding the supernatant, they were centrifuged for further 30 min, and left standing at an angle for 1 hr. To collect remaining supernatant which was then discarded. A small volume (3.0 ml) of deionized water was added repeatedly and the inner walls of the vessel carefully washed without disturbing the precipitate surface. The HA precipitate was digested by a standard nitric acid attack and its cadmium content was analyzed by AAS.

Ion-selective electrode method. The HA stock solution (CoHA) and cadmium solutions in 0.1 M KNO₃ media (10^{-3} M MES, pH=6.0) were prepared according to the method described earlier. A series of sample and reference solutions were prepared: HA concentration is kept constant (100 mg l^{-1}) and cadmium (II) concentration was varied from 3.12×10^{-5} M to 5.93×10^{-3} M. The speciation analysis was carried out with a cadmium (II) ion-selective electrode (model No. 94-48, Orion Co.) and a double junction reference electrode (model No. 90-02) coupled to an EA-920 expandable ion analyzer (Orion Co.). The Cd(II) ISE was polished with a polishing strip prior to each use. With standard solution, the electrode response was found to be linear in the range pM from 2.0 to 5.1 with Nernstian slope of -26.5 mV.

Results and Discussion

The normal ^{13}C -NMR spectra of the three humic acids, shown in Figure 1, illustrate the variable nature of these humic acids under investigation. The spectra of the AqHA and CoHA are somewhat similar, displaying broad resonances in the aliphatic (0-105 ppm) and aromatic regions

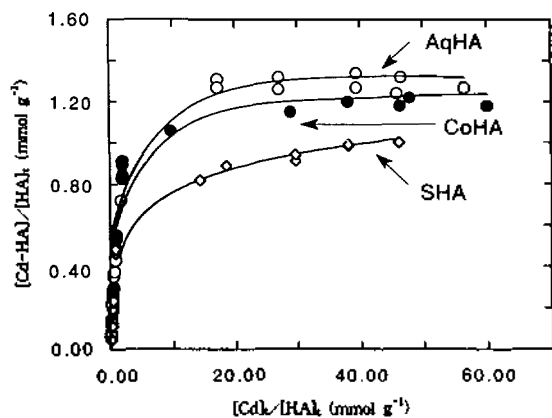


Figure 2. The graphic illustration of cadmium (II) binding on humic acids of differing origins.

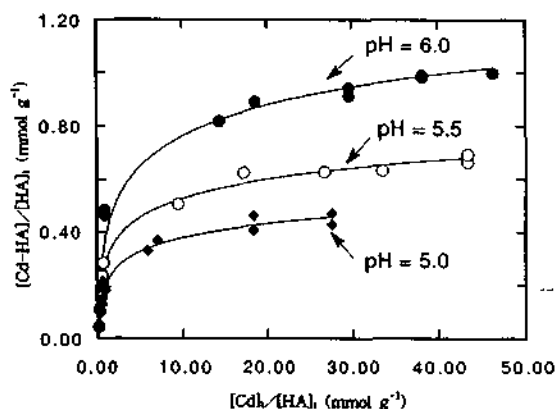


Figure 3. The graphic illustration demonstrating the effects of pH on cadmium binding ability of the soil derived humic acid.

(105-165 ppm). A relatively high proportion of intensity in the aromatic region suggests that these humic acids are made up predominately of aromatic constituents and contain acidic functional groups such as COOH and phenolic OH. The total acidities of AqHA and CoHA, measured by a direct pH titration, are 5.38 ± 0.20 and 5.03 ± 0.13 meq g^{-1} respectively. By contrast, the spectrum of SHA shows a number of distinct peaks in the aliphatic and aromatic regions, suggesting that the aromatic constituents of this humic acid are more extensively bridged by methylene and branched hydrocarbons, and polysaccharides. The total acidity of the SHA is 4.59 ± 0.10 meq g^{-1} , reflecting somewhat lower acidic functional groups content. The cadmium (II) binding ability of the three humic acids under investigation at pH of 6.0 is shown in Figure 2. At low cadmium to humate concentration ratios, the experimental points from all three humic acid data lie close to a linear plot, showing a linear relationship between the quantity of the cadmium bound to the humic acids and the total cadmium concentration. At higher total cadmium concentrations HA began to flocculate, leading to the formation of HA precipitates. The experimental data began to deviate from the linear plot. The analysis of the HA precipitate shows that the quantity of cadmium bound to the humic acids remain constant regardless of further inc-

Table 1. Maximum Binding Ability (MBA) of Humic Acids of Differing Origin Measured by Ultrafiltration Technique

Materials	pH	Humic Acid concentration (mg l^{-1})	MBA (mmol g^{-1})	Total acidity (meq g^{-1})
CoHA	5.0	101	0.729	5.03 ± 0.13
	5.5	101	0.949	
	6.0	101	1.255	
AqHA	5.0	104	0.814	5.38 ± 0.20
	5.5	101	1.070	
	6.0	101	1.299	
SHA	5.0	102	0.455	4.59 ± 0.10
	5.5	101	0.655	
	6.0	101	0.966	

rease in the total cadmium concentration. Therefore these saturation values were taken as the maximum binding ability (MBA). The results in Figure 2 show that MBA is different among humic acids of different origins.

The MBA of a humic acid is expected to be dependent on the solution pH. This is because metal cations bind predominately to anionic sites, formed from ionization of weak acidic functional groups of humic acids. This is illustrated by the experimental data shown in Figure 3 (SHA at pH of 5.0, 5.5, and 6.0). At low cadmium to humate concentration ratios, the experimental points lie cluster close to a linear plot as before. The analyses showed that more cadmium are bound to HA precipitates at higher pH. The MBA data of the three humic acids, obtained in such a manner are summarized in Table 1. The results show the cation binding ability of humic acid is dependent on both the acidic functional group content (variability in the total acidity related to humic acid origin) and solution pH. The two humic acids (AqHA and CoHA) with higher total acidity contents gave MBA values significantly larger than that of the soil derived humic acid (SHA) with low total acidity. In the case of SHA, the MBA increased more than twofold when pH is raised from 5.0 to 6.0.

Many modeling techniques have been used to calculate complexation reactions between humic acids and trace metal cations in natural system¹¹. This is because there are difficulties associated with heterogeneous nature of humic acid: 1) a multisite system possessing polyfunctionality, 2) polyelectrolyte character due to a large number of functional group per unit entity, and 3) may undergo conformational changes linked specifically to those properties related to geometrical factors¹⁴. In addition there are many experimental difficulties, especially when a separation method is used. For the ultrafiltration technique, these difficulties may include 1) the adsorption of labile species on membranes, 2) the possibility of shifting equilibria during the filtration stage, and 3) separation into species may not be complete since small amounts of low-molecular weight HA fraction may pass through the filter materials. Furthermore, data obtained at very high metal concentrations may not have high reliability, since the bound metal concentration is calculated as the difference between two large numbers.

The incremental stability constant K_i at the i -th site of

Table 2. Experimental data from the Complexation Studies of Cadmium (II) with SHA using Ultrafiltration Technique at pH=5.0, 5.5, 6.0 in 0.1 M NaClO₄

[Cd] _t (μ mol l ⁻¹)	[Cd] _f (μ mol l ⁻¹)	[Cd] _{2f} (μ mol l ⁻¹)	[CdHA] ₁ (μ mol l ⁻¹)	[CdHA] ₂ (μ mol l ⁻¹)	[HA] _f (μ mol l ⁻¹)	[HA] _{2f} (μ mol l ⁻¹)	log K (l mol ⁻¹)	log K (l mol ⁻¹)
18.9	13.7	13.5	5.20	5.41	41.2	41.0	3.96	3.99
38.1	28.3	27.5	9.85	10.6	36.6	35.8	3.98	4.03
57.4	42.2	44.4	15.2	13.0	31.2	33.4	4.06	3.94
71.5	49.8	52.5	21.7	19.0	24.7	27.4	4.25	4.12
91.4	71.9	73.2	19.5	18.1	26.9	28.3	4.00	3.94
pH=5.0			HA content=102 mg l ⁻¹			average value=4.03±0.10		
38.4	20.7	23.0	17.7	15.5	48.5	50.7	4.25	4.12
57.6	33.7	33.9	23.8	23.7	42.3	42.5	4.22	4.22
76.6	48.5	47.8	28.1	28.8	38.1	37.4	4.18	4.21
98.6	64.0	59.7	34.6	38.9	31.5	27.2	4.24	4.38
pH=5.5			HA content=101 mg l ⁻¹			average value=4.23±0.07		
21.3	10.5	10.2	10.8	11.1	86.8	97.6	4.07	4.05
44.2	26.0	24.7	18.2	19.5	79.4	86.5	3.95	3.96
58.9	35.2	34.6	23.7	24.3	73.9	78.1	3.96	3.95
92.3	45.3	43.3	47.0	49.0	50.6	73.3	4.31	4.19
14.5	79.2	91.7	65.8	53.3	44.3	48.6	4.06	4.08
pH=6.0			HA content=101 mg l ⁻¹			average value=4.06±0.12		

(suffix t: total; s: soluble; 1 and 2 correspond to replicate experiment)

a multisite polyelectrolyte system can be written as

$$K_i = [\text{Cd-HA}]_i / [\text{HA}]_i [\text{Cd}^{2+}] \quad (1)$$

where $[\text{Cd-HA}]_i$ is the concentration of cadmium bound with the i -th chelating site, $[\text{HA}]_i$ is the concentration of the i -th site and $[\text{Cd}^{2+}]$ is the free ion concentration. Since the UF method is inadequate for measuring the complexation reaction at each of the incremental sites, the conditional stability constant K was calculated from the experimental data using the expression shown in Eq. (2) or Eq. (3), where K stands for the weighted average of the stability constants for each increment of binding sites,

$$K = [\text{Cd-HA}] / [\text{HA}]_f [\text{Cd}^{2+}]_f \quad (2)$$

or
$$\log[\text{Cd-HA}] / [\text{Cd}^{2+}]_f = \log[\text{HA}]_f + \log K \quad (3)$$

where $[\text{Cd-HA}]$ is the concentration of the bound cadmium, $[\text{HA}]_f$ is the unbound HA binding site concentration, and $[\text{Cd}^{2+}]_f$ is the free ion concentration. The overall concentrations of cadmium (II) and humic acid are indicated as $[\text{Cd}]_t$ and $[\text{HA}]_t$. $[\text{Cd}^{2+}]_f$ is the measured quantity represented by the concentration of cadmium in the filtrate. $[\text{Cd-HA}]$ was calculated using Eq. (4):

$$[\text{Cd-HA}] = [\text{Cd}]_t - [\text{Cd}^{2+}]_f \quad (4)$$

In the absence of the known molecular weight, $[\text{HA}]_f$ is expressed in terms of binding site concentration: the product of MBA ($\mu\text{mol mg}^{-1}$) and HA content (mg l^{-1}). Since $[\text{HA}]_f$ is calculated using Eq. (5) below

$$[\text{HA}]_f = [\text{HA}]_t - [\text{Cd-HA}] \quad (5)$$

The expression shown in Eq. (3) is quite similar to the Hill plot based on cooperative binding. When binding of a metal cation at one site greatly enhance binding at subsequent sites, then binding of all n metal cations can be considered to occur in one single step as shown below:



The single step reaction can then be expressed in terms of θ

$$\log[\theta/(1-\theta)] = \log K + n \log[M] \quad (7)$$

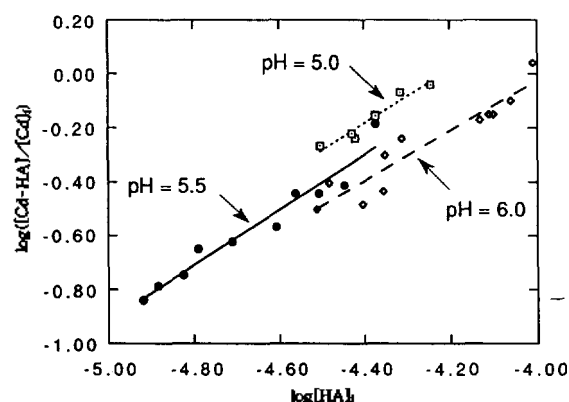
where θ = site bound/total number of active sites¹⁵. The experimental data from the complexation studies of cadmium (II) with SHA at different pH using the ultrafiltration technique are summarized in Table 2. A graphical plots of $\log[\text{Cd-HA}]/[\text{Cd}^{2+}]_f$ versus $\log[\text{HA}]_f$, using the analytical data of Table 2, is shown in Figure 4. All three data set (pH 5.0, 5.5, and 6.0) yielded linear plots with slopes of close to unity (i.e. 1.03, 1.00, and 0.93). Therefore, the expression in Eq. (3) represents the Hill plot for the situation where $n=1$. This means that 1:1 complex predominates and cadmium binding occurs at nearly identical and independent sites^{16,17}.

The conditional stability constant ($\log K$) was calculated for each experimental data using Eq. (3), and this is shown in Table 2. The values fall in the range 3.95-4.38 l mol⁻¹ in a random manner. Therefore, the average value of $\log K$ was taken as the conditional stability constant of SHA. $\log K$ values for SHA, AqHA, and CoHA measured at different solution pH are summarized in Table 3. All nine $\log K$ values are found to be quite similar with one another,

Table 3. The Summary of Conditional Stability Constants of Cadmium Complexes of Humic Acids of Differing Origin

Materials	Method	pH	HA concentration (mg l ⁻¹)	Cd concentration (μ mol l ⁻¹)	log <i>K</i> (l mol ⁻¹)
CoHA	UF	5.0	101	9.96-190	3.95 ± 0.08
	UF	5.5	101	9.48-168	3.87 ± 0.09
	UF	6.0	101	10.0-198	4.17 ± 0.08
AqHA	UF	5.0	104	18.6-210	3.97 ± 0.08
	UF	5.5	101	20.1-189	3.88 ± 0.11
	UF	6.0	101	9.94-175	4.14 ± 0.07
SHA	UF	5.0	102	10.1-194	4.03 ± 0.10
	UF	5.0	50	18.2-169	4.09 ± 0.02
	UF	5.5	101	19.3-199	4.23 ± 0.07
	UF	6.0	101	12.9-145	4.06 ± 0.12
	UF	6.0	51	18.6-160	4.09 ± 0.18

(UF: Ultrafiltration)

**Figure 4.** A plot of $\log [CdHA]/[Cd^{2+}]_f$ versus $\log [HA]_f$, demonstrating the cooperative binding ability of humic acids.

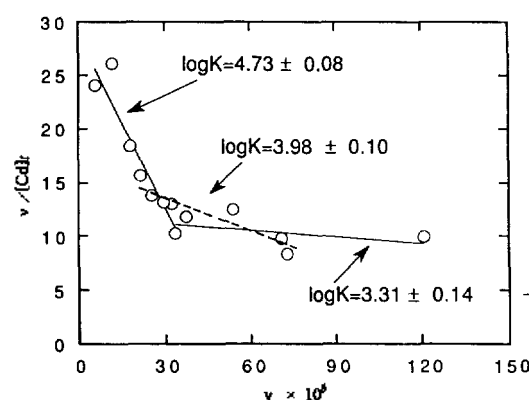
showing little dependency on humic acid type or pH. Another set of experiment was carried out using half the HA concentration (SHA, 50 mg l⁻¹), and comparable log *K* values of 4.09 ± 0.02 and 4.06 ± 0.18 l mol⁻¹ were obtained at pH of 5.0 and 6.0 respectively.

The method of speciation analyses can have pronounced influence on the results, since each method measures different aspects of the system. Due to experimental difficulties state earlier (UF experiments), only the weighted average of the stability constants for each increment of binding sites (log *K*) was obtainable using the ultrafiltration technique. However, naturally occurring heterogeneous compounds are known to have more than one class of binding sites¹⁴. A complementary experiment was carried using Cd(II) ISE method of speciation analysis. The Scatchard plot approach, using the expression in Eq. (8), was used for the analysis of experimental binding data¹¹.

$$v/[M] = nK_o - vK_o \quad (8)$$

where the extent of binding is expressed in terms of a formation function, *v* defined as

$$v = \text{site bound/polyelectrolyte concentration} = [M]_b/[L]_t$$

**Figure 5.** The Scatchard plot demonstrating the complex nature of the binding sites of humic acids: Based on CoHA and Cd²⁺ experimental data in 0.1 M KNO₃ at pH of 6.0.

A plot of *v*/[*M*] (*Y* axis) versus *v* (*X* axis) yielded a nonlinear Scatchard plot and this is shown in Figure 5. The plot seems to be composed of two straight line segments, from which stability constants for binding at two classes of sites (log *K*₁ and log *K*₂) were calculated to be 4.73 ± 0.08 (log *K*₁) and 3.31 ± 0.14 l mol⁻¹ (log *K*₂) respectively. Although the division of Scatchard plot into two linear segments is somewhat arbitrary, the result seems to indicate that there are at least two classes of sites in humic acids. Those sample solutions with very low or very high cadmium concentrations may not be accurately measurable using the ultrafiltration technique (experimental difficulties stated earlier). When those data points on either ends of the Scatchard plot (Figure 5) are neglected, then the rest of the data is represented by a single straight line yielding a log *K* value of 3.98 ± 0.10 l mol⁻¹ which is compatible with those results obtained earlier in Table 3. This points out the limitation of the ultrafiltration technique in obtaining accurate data over a wide range of Cd(II) to humic acid concentration ratios.

A range of values of conditional stability constant of cadmium humate and fulvate are reported in the literature, and some are listed in Table 4. The range of values given in

Table 4. Literary Values of Conditional Stability Constant of Cadmium (II) Humate and Fulvate Complexes

Materials	pH	<i>I</i> (M)	Method	log <i>K</i>	log <i>K</i> ₂	comment	reference
HA, Water	5.0		UF	5.32 ± 0.24			
	6.0		UF	5.66 ± 0.20		1 : 1 complexes	[16]
	7.0		UF	5.94 ± 0.06			
HA, Soil	4-5	0.01	PT	6.38		1 : 2 complexes	[18]
		0.1	PT	5.14			
HA, Soil	6.5-7.2	0.01-0.1	PT		6.9-5.4(<i>I</i>) ^{1/2}	1 : 2 complexes	[19]
HA, Coal	5.5	0.1	ISE	4.9		1 : 1 complexes	[20]
FA, water	5.0	0.1	ISE	3.47			
	6.0	0.1	ISE	3.68			
						1 : 1 complexes	[17]
FA, soil	5.0	0.1	ISE	3.80			
	6.0	0.1	ISE	4.08			

(UF: Ultrafiltration; PT: Potentiometric titration; ISE: Ion selective electrode)

the literature seem to reflect the effects of such factors as pH, ionic strength, humic acid type, and modeling used in the treatment of the data. Due to these factors, comparing the data of one laboratory with that of another is a challenging task. In many cases, the differences are the results of the application of different modeling techniques for calculating the conditional stability constants. For example, the stability constants obtained by Stevenson using the modified potentiometric titration procedure^{18,19} are in fact log *K*₂, based on the formation of 1 : 2 complexes (Cd-HA₂). Whitworth and Pagenkoft, from their cadmium complexation study with coal humic acid²⁰, obtained constant on the formation of 1 : 1 (Cd-HA), 2 : 1 (Cd₂-HA), 3 : 1 (Cd₃-HA) complexes. A single log *K* of 4.9 was quoted of pH 5.5 (1 : 1 complexes), but this is obtained using an average gram formula weight of 6761 ± 842 daltons in the calculation. Other results are calculated on the basis of 1 : 1 complex formation (low metal to humate concentration ratios), using experimental data obtained from UF or Cd(II) ISE methods. John and coworkers obtained the conditional stability constants of cadmium-humate complex (UF) that depend on pH and humic concentration¹⁶. An arbitrary value of 1000 daltons was used as the average molecular weight of humic substances in their calculations. Their rather high log *K* values (5.31-6.02) compared to those of Mantoura *et al.* (4.5-5.0 at pH ≈ 5.0)⁵ were explained as due from the differences in the origin of the samples. Saar and Weber obtained pH dependent conditional stability constants of cadmium-fulvate complexes (log *K* range 3.47-4.08 l mol⁻¹)¹⁷, using the molecular weights of 644 daltons for soil fulvic acid and 626 daltons for water extracted fulvic acid.

Conclusion

The cadmium binding properties of naturally occurring humic acids of differing origins were investigated using the separation and nonseparation methods of speciation analyses. The results show the cadmium binding ability of humic acid is different from one another and depends on the relative concentrations of the binding sites and solution pH. Many

investigators have used the average molecular weight of humic acids in the estimation of the ligand concentration. However, in the absence of known average molecular weight, which is the case with these humic acids, the formation function can be alternatively expressed in terms of binding site concentration. The binding site concentration can be obtained from MBA data or in other ways as discussed in the literature^{21,22}. By this approach, the conditional stability constant for Cd(II)-humate was obtained, which seems to show little dependency for the humic acid type and pH. A more advanced modeling method, such as a continuous distribution model based on the Scatchard plot, is reported in the literature²³. In order to obtain a more accurate estimate of the stability constants between humic substances and trace metal ions in the environment, an improved method of speciation analyses is required enabling a better identification on the nature of the binding sites of these naturally occurring heterogeneous compounds. Such work is being carried out using ¹¹³Cd-NMR and other methods.

Acknowledgements. The authors wish to thank Prof. J. I. Kim of the Technical University of Munich for his generous support, and to Dr. G. Buckau of providing humic acid sample. This work was partially supported by Korea Science and Engineering Foundation.

References

1. C. H. Moon and I. Thornton. The environmental impact of the rapid development of the Republic of Korea, IC-CET Series E No. 1 (1988).
2. C. H. Moon, Y. S. Lee, and T. H. Yoon. *Environ. Technol.* **12**, 413 (1991).
3. K. Kitagashi and I. Yamane. Heavy metal pollution in Japan. Japan Scientific Societies Press, Tokyo (1980).
4. J. Gardiner. *Water Res.* **8**, 23 (1974).
5. R. F. C. Mantoura, A. Dickson, and J. P. Riley. *Est. Coast. Mar. Sci.* **6**, 387 (1978).
6. B. T. Hart. *Environ. Technol. Lett.* **2**, 95 (1981).
7. T. A. Neubecker and H. E. Allen. *Water Res.* **17**, 1(1983).
8. R. A. Saar and J. H. Weber. *Environ. Sci. Technol.* **16**,

- 510A (1982).
9. J. Buffle and C. Staub. *Anal. Chem.* **56**, 2837 (1984).
 10. C. Staub, J. Buffle, and W. Haerdi. *Anal. Chem.* **56**, 2843 (1984).
 11. A. Fitch and F. J. Stevenson. *Soil Sci. Soc. Am. J.* **48**, 1044-1050 (1984).
 12. H. Moon, M. H. Lee, and T. H. Yoon. *Bull. Korean Chem Soc.* **12**, 153 (1991).
 13. J. I. Kim, G. Buckau, G. H. Li, H. Duschner, and N. Psaros. *Z. Anal. Chem.* **338**, 245 (1990).
 14. J. Buffle, R. S. Altmann, M. Filella, and A. Tessier. *Geochim et Cosmochim. Acta.* **54**, 1535-1553 (1990).
 15. A. G. Marshall. *Biophysical chemistry: Principles, techniques and applications.* John Wiley and Sons, New York (1978).
 16. J. John, B. Salbu, and E. T. Gjessing. *Water Res.* **22** 1381 (1988).
 17. R. A. Saar and J. H. Weber. *Can. J. Chem.* **57**, 1263 (1979).
 18. F. J. Stevenson. *Soil Sci.* **123**, 10 (1976).
 19. F. J. Stevenson. *Soil Sci. Soc. Am. J.* **40**, 665 (1976).
 20. C. Whitworth and G. K. Pagenkopf. *J. Inorg. nucl. Chem.* **41**, 317 (1979).
 21. A. Fitch and F. J. Stevenson. Stability constants of metal organic matter complexes: Theoretical aspects and mathematical models. In: *The significance of trace elements in solving petrogenetic problems and controversies* (S. S. Augustithis eds.). Theoprastus Publications S. A., Athen, Greece (1983).
 22. H. Zunino and J. P. Martin. *Soil Sci.* **123**, 188 (1976).
 23. F. J. Stevenson, A. Fitch, and M. S. Brar. *Soil Sci.* **155**, 77 (1993).

Facile Syntheses and Multi-orthofunctionalizations of Tertiary Benzamides

Kyungsoo Paek*, Kyungmo Kim, and Youseung Kim†

Department of Chemistry, Soongsil University, Seoul 156-743

Center for Biofunctional Molecules, P. O. Box 125, Pohang 790-600

†*Division of Applied Science, Korea Institute of Science and Technology, Seoul 136-791*

Received July 9, 1993

Good yields were usually obtained in Pd(O)-catalyzed Suzuki aryl-aryl coupling reaction, even when both coupling partners had an ortho tertiary benzamide functional group. The direct ortho functionalization of oligomeric tertiary benzamides at Snieckus condition is dependent on the chain length. Tertiary benzamide **1** can be *o,o'*-dilithiated only by metal-halogen exchange of the 2,6-dihalo-compound. Bis-tertiary benzamide **9** can be *o,o'*-dilithiated with excess (4.1 equivalents) *s*-butyllithium/TMEDA as the lithiating agent. Tris-tertiary benzamide **21** is hard to *o,o''*-difunctionalize due to steric interactions among the tertiary benzamide functional groups, and due to steric interactions between these functional groups and others (if present) on the termini of the terphenyl unit.

Introduction

The carbonyl group is one of the most important functional groups in biological systems. The base pairing within nucleic acids is based on hydrogen bonding to carbonyl oxygens¹ and many ionophores use carbonyl oxygens as ligands.² Valinomycin and enniatin B are antibiotics capable of inducing alkali metal permeability in various artificial and biological membranes.³ These bind potassium ion in preference to sodium ion, and their antibiotic activities are due to their interference with the ion balances of bacterial cells. In the potassium ion complex of valinomycin, all the six ester carbonyls are coordinated to potassium, forming a distorted octahedral ligand system. The structural framework of this ionophore is stabilized by hydrogen bonds. In the conformation of the potassium complex of enniatin B, six carbonyl oxygens from an equal number of amide and ester are coordinated to cation.⁴

Even though the carbonyl group has such important biologi-

cal functions, only a few hosts have been designed and synthesized whose only binding sites are carbonyl oxygens. The cyclic urea system⁵ and the calixarene derivatives⁶⁻⁸ have recently been reported. The use of amide groups is especially attractive because the highly polarized carbonyl of this functional group should be a superior binding site.

The observed stability order of the 1 : 1 complexes of potassium cation and solvents (K⁺L) in the gas phase is as follows: dimethyl sulfoxide (25), dimethyl acetamide (24), dimethyl formamide (23), 1,2-dimethoxyethane (23), 1,2-diaminoethane (19), acetone (19), acetonitrile (18), aniline (16), diethyl ether (15), pyridine (15), dimethyl ether (13), trimethyl amine (13), methyl amine (13), benzene (12), ammonia (12), water (11), where the numbers in parenthesis are the values of $-\Delta G^{\circ}_{300}$ in kcal/mol.⁹ The strong bonding of dimethyl sulfoxide, dimethyl acetamide, and dimethyl formamide to potassium ion is due to the high dipole moment of these compounds. These bond even more strongly than the bidentate ligands, 1,2-dimethoxyethane and 1,2-diaminoethane.