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Comparison of the Stability Constants of Cd(II)-, Cu(II)-, and Pb(II)-Humate Complexes

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A comparative investigation of the complexations of divalent metal ions (Cd, Cu, and Pb) by a well characterized soil humic acid (HA) from Okchun Metamorphic Belt was carried out in 0.05 M KNO_3 and pH of 4.5 using ion selective electrodes. A continuous distribution model based on the Scatchard Plot was used to determine the stability constants, because the constants obtained by this modeling technique takes the variations in binding energies into consideration without regards to the manner in which M(II) ion is bound on HA. The mean value of log K_i were 4.05 ± 0.60, 4.92 ± 0.36, and 5.63 ± 0.34 l mol^{-1} for Cd(II)-, Pb(II), and Cu(II)-humate complexes respectively. The values of intrinsic constant (log K_{int}; binding at strongest site) were 7.12 ± 0.30, 6.59 ± 0.32, and 5.07 ± 0.56 l mol^{-1} in the order Cu(II)>Pb(II)>Cd(II) ion.

Introduction

The rapidly industrialization nations of Asia are becoming more aware of the consequences of the discharge of heavy metals into freshwater systems, and their subsequent migration to other environmental compartments such as irrigated agricultural lands.[1] The bioavailability of environmentally sensitive metals (*e.g.* Cd, Cu, Pb, Zn, and Ni) is strongly influenced by the chemical speciation of a given metal, defined as the partitioning of total metal into all possible chemcial forms in a given system. In an irrigated agricultural land, one of the major components is the species of metals complexed to humic substances. There are many investigations of the complexation of divalent Cd, Cu and Pb with humic (HA) and fulvic (FA) acids reported in the literature.[2-6] However, conflicting information has been obtained regarding the nature of M(II) binding by humic substances.[5,7,8] Many types of modeling techniques have been used in the calculation of stability constants of the complexes thus formed.[5,9-11] The humic and fulvic acids used in these investigations are different, and their properties depend on their origin and on the source materials.[12] All these factors make it difficult to compare the results of one investigation with those of others carried out with different humic substances or methods.

A recent study of the probe of metal binding sites on humic acids were carried out using ^{113}Cd NMR spectroscopic method, and the preliminary results seem to suggest that there are several types of carboxylate moieties for binding of Cd(II) ions on HA. Therefore, the stability constants of M(II)-humate complexes were evaluated using a continuous distribution model based on the Scatchard Plot. This modeling technique was chosen because this method takes into consideration the variations in the binding energies without regards to manner in which M(II) ions are bound on HA.

The purpose of this work is the determination of the stability constants of the complexes between environmentally sensitive divalent metal ions (Cd, Cu, and Pb) and a representative soil humic acid from the Okchun Metamorphic Belt in Korea. The concentrations of Cd(II), Cu(II), and Pb(II) species were determined using ion selectve electrodes (non-separation method).

Experimental

Materials and reagents. The humic acid used in this investigation was extracted from topsoils (0-5 cm) obtained from Okchun Metamorphic Belt by base digestion (1 M NaOH, 48 h) under N_2 atmosphere. The HA was recovered by acid precipitation (HCl), then was washed repeatedly with acid to remove trace of inorganic impurites, followed by extensive dialysis against double deionized water to remove other impurities such as Cl^- ion, and the final product was freeze-dried in protonated form. It has been characterized by chemical and spectroscopic methods.[13]

The stock HA solution was prepared by dissolving 0.0548 $\pm$ 0.0001 g of accurately weighed HA with a minimum quantity of 0.1 M KOH (~3 ml, 1 h) and further diluting it with 0.05 M KNO_3 to 100 ml, and pH adjusted to 4.5 with HNO_3 and KOH solutions. The stock Cd, Cu, and Pb solutions were prepared by dissolving accurately weighed nitrate salts in

0.05 M KNO_3 to 500 ml and pH adjusted to 4.5. The concentrations of the stock Cd, Cu, and Pb solutions were diluted in 1 M HCl and analyed by AAS (Instrumental Laboratory, Video 12) as 1.18×10^{-2} M, 5.40×10^{-3} M, and 1.08×10^{-2} M respectively. The pH was measured using a glass electrode coupled to a digital pH meter (Metrohm type 632).

Procedure for M(II) titration. Titrations were carried out using a automatic titrator (Metrohm 645 Multidosimat) at 25± 1℃. Some 50 ml of the HA stock solution, diluted to 100 ml with 0.05 M KNO_3, was placed in an enclosed titration vessel. Measured aliquots of 1.18×10^{-2} M $Cd(NO_3)_2$ stock solution (also $Cu(NO_3)_2$ and $Pb(NO_3)_2$ stock solutions) were dispensed into the sample solution under N_2 atmosphere with gentle stirring. Since the pH of the two reagents were both initially adjusted to 4.5, the pH of the sample solution changed very little on mixing, and was corrected by addition of a small quantity of 0.01 M KOH. Free Cd(II) ion was monitored by measuring the steady mV reading on Expandable Ion Analyzer (Orion, EA 920) with a well polished Cd(II)-ion selective electrode (Orion, NO. 94-48) coupled to a double junction reference electrode (Orion, N. 94-02). The Cd(II)-ISE was calibrated using a series of standard solutions prior to use, and the electrode response was found to be linear in the experimental range pCd 3.14-6.01 with Nernstian slope of -24.66 mV. The free Cu(II) and Pb(II) ions were monitored using Cu(II)- and Pb(II)-ion slective electrodes (Orion, No. 94-29, 94-82) in a similar manner. A titration run was conducted over 3.5 h and each titration was repeated for accuracy and reproducibility. Those data obtained near the region where M(II)-humate complexes began to precipitate were not used in the evaluation. All reagents used were reagent grade or better and extra care was taken to reduce the effects of interfering CO_2.

Calculations. The approach used in the determination of the stability constants of M(II)-humate complexes are described in the literature.[5,14] In this graphic approach, HA was regarded as the central group and the M(II) ion became bound on the macromolecular HA(L) to form LM, $LM_2\cdots$, LM_n complexes. The stability constant of k_n at n-th binding site of the M(II) ion can be written as

$$k_n=[LM_n]/[LM_{n-1}][M] \tag{1}$$

The extent of M(II) binding on HA can be expressed by a formation function, v, defined by Eq. (2).

$$v=\frac{(LM)+2(LM_2)\cdots+n(LM_n)}{(L)+(LM)+\cdots+(LM_n)}=\frac{M_b}{L_t} \tag{2}$$

where M_b and L_t are the bound M(II) and total ligand concentrations, and n is the number of metal ions bound per macromolecule. M_b is the difference between the total $[M_t]$ and free $[M]$ metal ion concentrations: $M_b=[M_t]-[M]$.

When the metal binding sites are all considered identical and independent, then the formation constant (v) can be expressed by Adair's equation shown below:

$$v=nK_o[M]/(1+K_o[M]) \tag{3}$$

where K_o is the microscopic binding constant and n is the number of binding sites per macromolecules. The Scatchard equation (4) was obtained from the rearrangement of the Eq. (3):

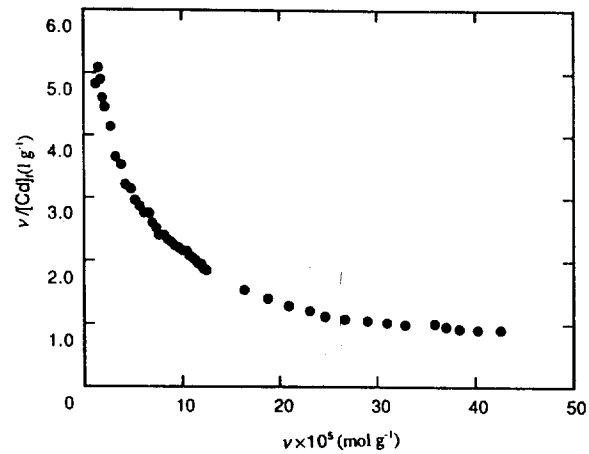

Figure 1. The Scatchard plot for binding of Cd(II) ion on soil humic acid. ([HA]=274 mg l^{-1}; 1=0.05; pH=4.5; T=25± 1 ℃).

$$v/[M]=nK_o-vK_o \tag{4}$$

The Scatchard plot was obtained by plotting $v/[M]$ versus v with K_o as the slope. From the Scatchard plot thus obtained, a series of incremental conditional stability constants (K_i) were calculated. The individual K_i values were considered to reflect variation in binding energies between various sites. Because the accurate molecular weight of HA was not known, HA concentration was given in mg l^{-1}. Consequently, v was indicative of the fraction of potential sites occupied by the metal ion. Since individual value of K_i was obtained from the data on the slope of a Scatchard plot, it is not affected by the unit of measurement chosen for L_t. The log K_i data thus obtained were further examined using the Gaussian distribution function Eq. (5).

$$\frac{C_i}{C_L}=\frac{1}{\sigma\sqrt{2\pi}}\exp\left[-\frac{1}{2}\left(\frac{\mu-\log K_i}{\sigma}\right)^2\right]d\log K_i \tag{5}$$

where C_i/C_L is the mole fraction of binding sites in the interval $d\log K_i$. Using Eq. (5) the fractional binding site distribution of log K_i was obtained, where μ is the mean value of log K_i and σ is the standard deviation about the mean.

Results and Discussion

The Scatchard plot of $v/[M]$ versus v as applied to Cd(II) binding of HA is shown in Figure 1. The Scatchard plot is shown to be curvilinear in Figure 1. In the earlier study, the two stability constants (log K_1 and log K_2) for binding at two classes of sites were calculated by arbitrarily dividing a similar Scatchard plot into two linear segments.[6] However, more recent evidences suggest that complexation is likely to occur at severl types of binding sites (heterogeneous polyfunctionality) which may have slightly different binding energies for M(II) ion.[2,9,15] The Scatchard plots of Cu(II) and Pb (II) binding on HA are shown in Figure 2 and Figure 3, and these plots are also curvilinear. Numerous experimental difficulties were encountered in an attempt to generate a series of log K_i values at equal increment of v. Instead, the series of log K_i values were generated at equal concentration intervals, and they were subsequently evaluated using the

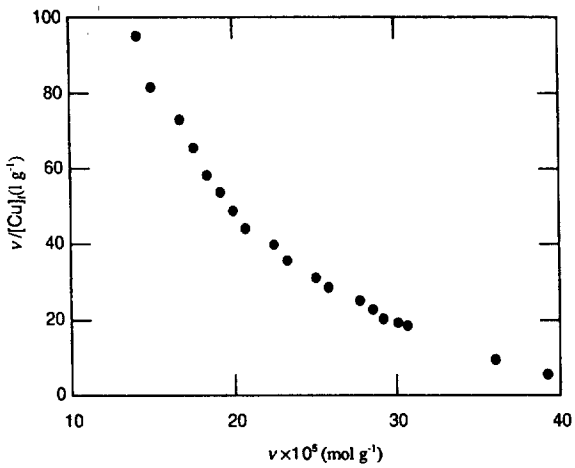

Figure 2. The Scatchard Plot for binding of Cu(II) ion on soil humic acid. ([HA]=274 mg l^{-1}; I=0.05; pH=4.5; T=25± 1 ℃).

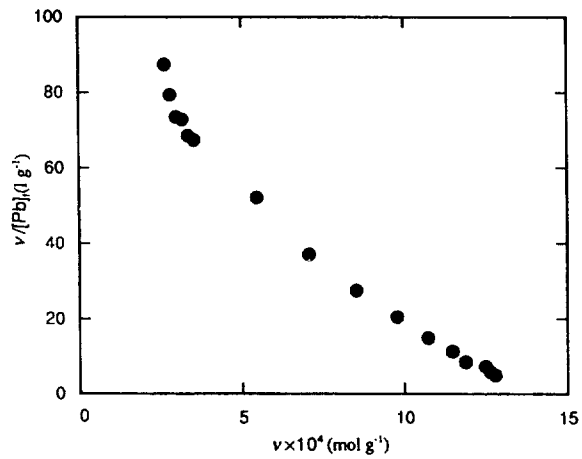

Figure 3. The Scatchard Plot for binding of Pb(II) ion an soil humic acid. ([HA]=274 mg l^{-1}; I=0.05; pH=4.5; T=25± 1 ℃).

Gaussian distribution function (Eq. (5)).

The Gaussian distribution curves for log K_i obtained from the Cd(II)-, Cu(II)-, and Pb(II)-humate complexation data are shown in Figure 4. The distribution curves obtained in this manner (see Figure 4) are found to be normally distributed about the mean values. The Gaussian distribution curves representing the Cu(II)- and, Pb(II)-humate complexes are located at higher log K_i regions and have sharper with narrower distribution pattern compared to that of the Cd(II)-humate complexes. The stability constants of Cu(II)-, Pb(II)-, and Cd (II)-humate complexes shown in Figure 4 are summarized in Table 1. The conditional stability constants of Cu(II)- and Pb(II)-fulvate complexes determined by Sterritt and Lester[3] were found to be quite similar (ISE; Cu(II)-fulvate: log K_1= 6.5; Pb(II)- Fulvate: 6.3), suggesting that both Cu(II) and Pb(II) ions are strongly bound by fulvic acid almost equally. However, the results of this investigation show that the soil humic acid from the Okchun Metamorphic Belt region has the affinity for divalent metal ions in an increasing order Cu(II)>Pb(II)>Cd(II): The mean value of log K_i (μ) and the

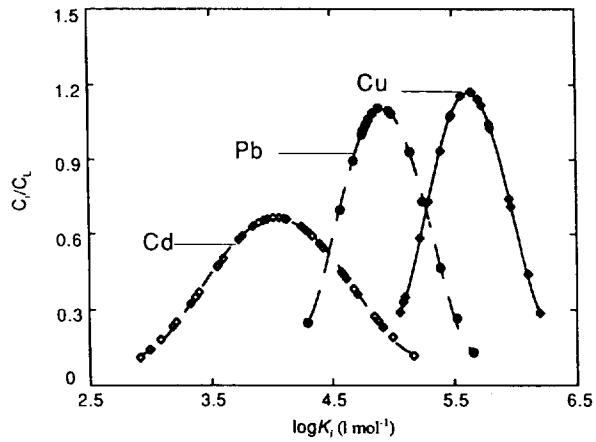

Figure 4. The Gaussian distribution curves for log K_i of Cd(II)- and Pb(II)-humate complexes, evaluated using a continuous distribution model based on Scatchard plot.

Table 1. The summary of the stability constants of divalent metal humate complexes obtained using a continuous distribution model based on scatchard plot (T=25± 1℃)

Metal ion	Method	[HA] (mg l^{-1})	pH	I	[M(II)] (μmol l^{-1})	μ± σ (l mol^{-1})
Cd(II)	ISE	274	4.5	0.05	5.90−551	4.05± 0.60
Pb(II)	ISE	274	4.5	0.05	75.6−614	4.92± 0.36
Cu(II)	ISE	274	4.5	0.05	37.5−178	5.63± 0.34

(ISE : Ion Selective Electrode; μ : mean value of log K_i; σ : standard deviation of log K_i).

standard deviation (σ) for Cu(II)-, Pb(II)-, and Cd(II)- humate complexes are 5.63± 0.34 l mol^{-1} 4.92± 0.36 l mol^{-1} and 4.05 ± 0.60 l mol^{-1} respectively. Similar values of conditional stability constants were obtained for Cd(II) complexed to fulvic and humic acids, showing a similar complexation behaviour of two natural organic ligands.[6,16]

In the earlier study, the stability constants of the complex formed between Cd(II) and the same soil humic acid was evaluated using both the Hill plot (conditional stability log K; 1 : 1 complex) and Scatchard plot (two classes of sites, one weak and one strong; log K_1 and log K_2). When the results in Figure 4 are compared with the those results of the earlier study, the following observations can be made: (1) The mean value of log K_i (μ=4.05± 0.60 l mol^{-1}) obtained by a continuous distribution model based on Scatchard plot is quite similar to value of the conditional stability constant (log K=3.98± 0.10 l mol^{-1}) evaluated using the Hill plot based on cooperative binding (1 : 1 complex). The conditional stability constant (log K) obtained by the Hill plot seems to be a reflection of the incremental stability constant (log K_i) at the highest C_i/C_L (the mole fraction of binding sites in Eq. (5)).[6] (2) The values of stability constants (strong site binding: log K_1=4.73± 0.08 l mol^{-1}; weak site binding: log K_2=3.31± 0.14 l mol^{-1}) obtained by Scatchard plot,[6] on the other hand, are typical of the values of log K_i in the two tail ends of the Gaussian distribution curve for log K_i (see Figure 4). It seemed that the stability constants evaluated

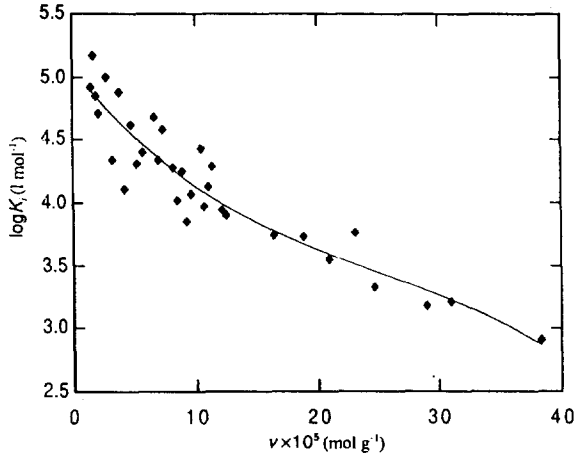

Figure 5. Experimetal plot relating log K_i of Cd(II)-humate to the formation constant v. ([HA]=274 mg l^{-1}; 1=0.05; pH=4.5; T=25± 1℃).

Table 2. The intrinsic constant (log K_{int}) for binding of Cd(II). Pb(II), and Cu(II) ions on the strongest binding site on soil humic acid (T=25± 1℃)

Metal ion	[HA] (mg l^{-1})	I	pH	log K_{int} (l mol^{-1})	ref.
Cd(II)	274	0.05	4.5	5.07± 0.56	this work
Pb(II)	274	0.05	4.5	6.59± 0.32	this work
Cu(II)	274	0.05	4.5	7.12± 0.30	this work
Cu(II)	250	0.005	4.5	7.80	
	250	0.005	4.0	7.62	[5]
	250	0.05	4.0	7.00	
	150	0.05	4.0	7.30	

with Hill and Scatchard plots are both obtainable from a single gaussian distribution curve (see Figure 4) obtained from a continuous distribution model based on Scatchard Plot.

From an environmental standpoint, the stability constants for binding at the strongest sites are said to be of greatest interest.[17] The intrinsic constant (log K_{int}), which is defined as the constant for binding at the strongest site, was obtainable from the plot of log K_i values against the formation constant, v, shown in Figure 5 for Cd(II)-humate complex. The log K_{int} was obtained as the intercept value on the y-axis (log K_i) when the value of v was extrapolated to zero. The log K_{int} values for Cu(II)-, Pb(II), and Cd(II)-humate complexes thus obtained are given in Table 2 and are found in the increasing order Cu>Pb>Cd. The log K_{int} value of 7.12± 0.30 for Cu(II)-humate complex of this investigation (I=0.05; pH=4.5) is in good agreement with the log K_{int} value of 7.00 given for Cu(II)-humate compledx (I=0.05; pH=4.0) and lies within the range of values obtained at differing pH, ionic strength, and HA concentrations.[5]

Conclusion

In this study, the data obtained from the complexation reactions between divalent metal ions (Cd, Cu, and Pb) and local soil humic acid were evaluated by a continuous distribution model based on Scatchard plot. This method of data evaluation is found to have certain advantages over the methods previously used in earlier studies such as Hill and Scatchard plots. They are: (1) Gaussian distribution curves of log K_i obtained by this method seem to take into consideration the variation in the binding strength between sites without regards to the manner in which how M(II) ion is bound on humic acid, and (2) the mean value of log K_i (μ), as well as the log K_i values for strong and weak site bindings can be readily estimated from a single Gaussian distribution curve. In addition the intrinsic constant (log K_{int}) for binding at the strongest site can be obtained from the same series of log K_i values. This method of modeling the data provides little information concerning the nature of binding sites by divalent metal ion on humic materials. A ^{113}Cd NMR spectroscopic probe on the nature of binding sites of Cd(II) ion on humic materials and synthetic polymer, polymethacrylic acid (PMAA), is in progress.

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