

Article

Evaluation of the Iron(II)-binding Abilities of Humic Acids by Complexometric Titration using Colorimetry with *ortho*-Phenanthroline

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Abstract

When an *ortho*-phenanthroline (*o*-phen) is added to an aqueous solution containing Fe(II) and humic acid (HA), the Fe(II)-HA resulting complex is stable and does not readily dissociate via a ligand-exchange reaction between HA and *o*-phen. This indicates that the Fe(II)-HA complex is kinetically stable and that free, uncomplexed Fe(II) species, are involved in color formation with *o*-phen in an aqueous mixture of Fe(II) and HA. This finding was applied to a complexometric titration to evaluate the iron(II)-binding abilities (conditional binding constants and binding capacities) of HAs that were extracted from sediments in dam reservoirs.

Keywords: *Ortho*-phenanthroline; Lake sediment; Iron(II); Humic acid; Complexometric titration

Introduction

Humic acids (HAs), which contain acidic functional groups such as phenolic hydroxyl and carboxyl groups that can bind to a variety of metal ions, are widely distributed in the soil and in aquatic environments (Aiken et al., 1985). It is known that, in the presence of metal ions, the resulting HA-metal ion complexes enhance the transport of essential trace metals to plants (Lobartini et al., 1998). From a marine biological point of view, HAs can serve as carriers of Fe(II) from land to coastal areas, and Fe(II)-HA complexes play important roles in the management of ecosystems in coastal areas (Rose and Wait, 2003; Willy et al., 2008). In coastal areas, barren ground, which is a phenomenon associated with seaweed depletion, now poses a serious

environmental problem (Fujita, 2002). The lack of soluble Fe(II) species in coastal areas has been reported to be a major factor in the production of barren ground (Matsunaga et al., 1999). In a previous report, we proposed a technique for fertilizing with a soluble Fe(II)-HA complex using a preparation consisting of a mixture of sediment from a dam reservoir including HA and slag from a steel mill, which contains Fe(II) (Toyoda et al., 2009). The Fe(II)-binding abilities (conditional binding constants and binding capacities) of HAs are crucially important factors in determining the quality of sediments from dam reservoirs.

The binding characteristics of HA to metal ions have been evaluated using a variety of methods, but our knowledge of the binding abilities of HAs to Fe(II) remains limited. While cation- and anion-ex-

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change resins have been used to evaluate the Cu(II)-binding abilities of HAs (Taga et al., 1989, 1990 and 1991), these procedures are complicated and time-consuming. Potentiometric titration methods using ion-selective electrodes (ISEs) have been employed to measure free-metal ion concentrations (Town and Powell, 1993). However, the use of ISEs is limited to selected metal ions (*e.g.*, Ca^{2+} , Cu^{2+} , Pb^{2+} and Cd^{2+}). Anodic stripping voltammetry (ASV), using a mercury electrode, is also a highly sensitive method (Fukushima et al., 1992 and 1996; Florence, 1982). However, ASV is also limited to metal ions that can form amalgams (*e.g.*, Cu^{2+} , Pb^{2+} and Cd^{2+}). Thus, a simple and direct method for evaluating the ability of Fe(II) to bind to HA would be highly desirable.

It is well-known that a colorimetric method using an *o*-phenanthroline (*o*-phen) can be applied to the analysis of Fe(II) species (Tamura et al., 1974). For analyzing iron in natural water, dissolved organic matter such as HAs are first decomposed by an acid hydrolysis treatment, and the total iron is then reduced to Fe(II) by treatment with a reducing agent to form a color in the presence of *o*-phen. However, measurements of Fe(II) using *o*-phen in the presence of dissolved organic matter without the above described pretreatment have not been examined. In preliminary experiments, the absorbance values for color formation with *o*-phen in aqueous mixtures of HA and Fe(II) were much smaller than those in the absence of HA. This suggests that the Fe(II)-HA complex does not contribute to color formation in the presence of *o*-phen, and this may be applicable to the speciation analysis of free-Fe(II) species and Fe(II)-HA complexes in aqueous mixtures of HA and Fe(II). The objective of the present study was to confirm whether *o*-phen produces a color by reacting only with free-Fe(II) species or not. In addition, a speciation analysis based on the above findings was applied to the determination of the Fe(II)-binding abilities of HAs from sediments in dam reservoirs.

Materials and Methods

Reagents and Materials

The stock solution of HA was prepared by dissolving powdered HA in a 0.01M aqueous solution of NaOH. A stock solution of *o*-phen (0.12%) was prepared by dissolving powdered *o*-phenanthroline chloride (Nacalai Tesque) in pure water. A stock solution of Fe(II) (0.01 M) was prepared by dissolving powdered $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$ (Nacalai Tesque) in an aqueous 0.01 M HCl solution. A stock solution of acetate buffer (3.60±0.05) was prepared by mixing 2 M aqueous solutions of acetic acid and sodium acetate (9:1, v/v). This was diluted ten-fold with pure water for use. An Fe(II)-lactate complex (Fe(II)-La), in the form of $\text{Fe}[\text{CH}_3\text{CH}(\text{OH})\text{COO}]_2 \cdot \text{H}_2\text{O}$, was purchased from Nacalai Tesque.

Pretreatment of sediments sample and extraction of HA

Six sediment samples were obtained from the following sites in Japan: the Niiho Dam (Sado-ga-shima Island, Niigata, October 2008); the Ebihara and Yoneoka catchments (Okushiri Island, Hokkaido, December 2008). The surfaces of the sediments were obtained using a glove sampler. The sediment samples were frozen, freeze-dried and then passed through a stainless-steel sieve (2 mm). Particles less than 2 mm in size were used in the tests. HAs were extracted from sediment samples and purified, based on the method recommended by the International Humic Substances Society (Swift, 1996).

Analyses of HAs

C, H, N, S, and ash contents of the HA samples were determined at the Center for Instrumental Analysis at Hokkaido University, Sapporo, Japan. The percent oxygen was calculated by subtracting the sum of percent of C, H, N, S and ash from 100%. The powdered HA samples were dehydrated under reduced pressure for at least 12 h prior to the anal-

Table 1 Elemental composition of the HA samples.

HA samples	%C	%H	%N	%O	%S	%ash	O/C	N/C
Niiho 1	49.66	5.39	4.10	39.52	0.54	0.79	0.60	0.071
Niiho 2	48.38	5.71	5.09	37.23	1.24	2.35	0.58	0.090
Ebihara 1	50.18	5.16	4.78	36.55	1.50	1.83	0.55	0.082
Ebihara 2	49.97	5.57	4.58	36.07	1.46	2.35	0.54	0.079
Yoneoka 1	46.26	4.81	3.55	41.68	1.23	2.47	0.68	0.066
Yoneoka 2	50.80	5.43	3.95	35.61	1.27	2.94	0.53	0.067

yses. The results of HA elemental analyses are summarized in Table 1. Total acidity and carboxylic group content were determined using the $\text{Ba}(\text{OH})_2$ and $\text{Ca}(\text{CH}_3\text{COO})_2$ methods, respectively, as described in a previous report (Yabuta et al., 2008). Phenolic hydroxyl group content was calculated by subtracting the carboxylic group content from the total acidity.

Colorimetric titration

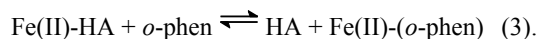
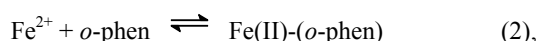
A 10 mL aliquot of 0.2 M acetate/sodium acetate buffer including HA (0 or 10 mg L^{-1}) and Fe(II) (0 – 50 μM) was placed to a test tube. A 0.5 mL aliquot of *o*-phen aqueous was then added, and the solution was allowed to stand for 0.5 hours. Unbound Fe(II) species were determined by measuring the absorbance at 510 nm using a V630-type UV-vis spectrophotometer (Japan Spectroscopic Co. Ltd.).

To evaluate the dissociation rates of the Fe(II)-HA complex, sample solutions including HA (10 mg L^{-1}), Fe(II) (0 and 50 μM) and *o*-phen were allowed to stand for 1, 3 and 5 days, and the absorbance values at 510 nm were monitored. For comparison, the dissociation kinetics of the Fe(II)-La complex were determined using a RSP-1000-02-type stopped-flow spectroscopy system (UNISOKU, Co. Ltd.).

Results and Discussion

Kinetic stability of Fe(II)-HA complex

In our preliminary experiments, the absorbance value for Fe(II)-(*o*-phen) complex at $[\text{Fe}(\text{II})] = 50 \mu\text{M}$ and pH 3.6 (0.560) was much larger than that in the presence of HA (0.465). In addition, the absorbance values for the Fe(II)-(*o*-phen) complex decreased with increasing concentrations of HA. These results may be attributed to the fact that *o*-phen produces a color only in the presence of the free-Fe(II) species, and that the Fe(II)-HA complex contributes on negligibly to color formation. The following equilibria in the aqueous solution including Fe^{2+} , HA and *o*-phen can be considered as: (i) complex formation between Fe(II) and HA, (ii) complex formation between Fe(II) and *o*-phen, and (iii) a ligand-exchange reaction between HA and *o*-phen. These reactions are expressed as follows:



If eq (3) were very fast, all of the Fe(II) species in a solution containing Fe(II) and HA would contribute to color formation with *o*-phen. In this case, a speciation analysis of free-Fe(II) using *o*-phen would be impossible in an aqueous mixture of Fe(II) and HA (Fukushima and Taga, 1993). To evaluate the kinetic stability of the Fe(II)-HA complex, *o*-phen was added to aqueous solutions containing HA and Fe(II), and the variation in absorbance for Fe(II)-(*o*-phen) complex at 510 nm were monitored. In this experiment, the concentration of free-Fe(II) ($[\text{Fe}^{2+}]$) was determined from absorbance values at 510 nm. Therefore, the concentration of the Fe(II)-HA complex ($[\text{Fe}(\text{II})\text{-L}]$) can be calculated as:

$$[\text{Fe}(\text{II})\text{-L}] = C_{\text{Fe}(\text{II})} - [\text{Fe}^{2+}] \quad (4),$$

where $C_{\text{Fe}(\text{II})}$ represents the total concentration of Fe(II) added initially. The rate equation with respect to the dissociation of Fe(II)-HA complex can be expressed as:

$$-\frac{d[\text{Fe}(\text{II})\text{-L}]}{dt} = k[\text{Fe}(\text{II})\text{-L}] \quad (5),$$

where t and k denote the reaction time and pseudo-first-order rate constant, respectively. The integrated form of eq (5) can be expressed as:

$$\ln \frac{[\text{Fe}(\text{II})\text{-L}]_t}{[\text{Fe}(\text{II})\text{-L}]_0} = -kt \quad (6),$$

where $[\text{Fe}(\text{II})\text{-L}]_0$ and $[\text{Fe}(\text{II})\text{-L}]_t$ represent $[\text{Fe}(\text{II})\text{-L}]$ before and after the addition of *o*-phen, respectively. Figure 1 shows kinetic plots for the

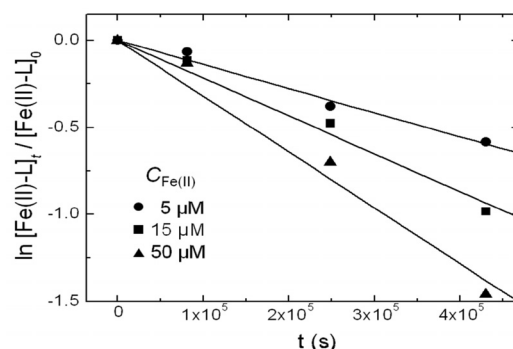


Fig.1 Kinetic curves for the dissociation of Fe(II)-HA complex at $C_{\text{Fe}(\text{II})}$ 5, 15 and 50 μM .

dissociation of Fe(II)-HA complex at $C_{\text{Fe(II)}} = 5, 15$ and $50 \mu\text{M}$, based on eq (6). The k values were calculated by the slopes of linear portion of the plots as follows: $(1.4 \pm 0.1) \times 10^{-6} \text{ s}^{-1}$ for $5 \mu\text{M}$; $(2.3 \pm 0.2) \times 10^{-6} \text{ s}^{-1}$ for $15 \mu\text{M}$; $(3.5 \pm 0.3) \times 10^{-6} \text{ s}^{-1}$ for $50 \mu\text{M}$, and slightly increased with an increase in $C_{\text{Fe(II)}}$. For comparison, the k values for the Fe(II)-La complex as an example of a readily dissociable complex were measured at $C_{\text{Fe(II)}} = 5$ and $50 \mu\text{M}$ using a stopped-flow spectroscopic system: $2.3 \pm 0.2 \text{ s}^{-1}$ for $5 \mu\text{M}$; $1.7 \pm 0.1 \text{ s}^{-1}$ for $50 \mu\text{M}$. Contrary to the Fe(II)-HA complex, the k values decreased somewhat at higher $C_{\text{Fe(II)}}$ for the case of Fe(II)-La, which is known to be a simple complex. An increase in $C_{\text{Fe(II)}}$ can increase the initial concentration of the Fe(II)-HA complex in eq (3), and this may lead to a lower k value, as in the case of the Fe(II)-La complex. Yapici et al. (2009) investigated the dissociation kinetics of a Cd(II)-fulvic acid complex in the presence of a chelating resin. The k values for the Cd(II)-fulvic acid complex increased with an increase in the initial concentration of the complex, and this is consistent with the trend for the Fe(II)-HA complex in our study. According to the report by Yapici et al. (2009), such a trend can be attributed to the heterogeneous nature of the ligands, in which the binding sites of humic substances can be classified as either weak or strong types. That is, metal ions bind to the strong sites first, and any remaining metal ions then bind to the weak binding sites (Buffle et al., 1990). Thus, the larger k value at higher $C_{\text{Fe(II)}}$ for the Fe(II)-HA complex can be attributed to the dissociation of Fe(II)-L that is bound to a weak binding site.

The k values for Fe(II)-La complex ($1.7 - 2.3 \text{ s}^{-1}$) were clearly much larger than those for Fe(II)-HA complex ($1.4 \times 10^{-6} - 3.5 \times 10^{-6} \text{ s}^{-1}$). These results emphasized that Fe(II)-HA complex is kinetically stable and ligand-exchange reaction in eq (3) is very slow. All analytical procedures for measuring free-Fe(II) species using *o*-phen were carried out within 60 min. Thus, the dissociation of the Fe(II)-HA complex by adding *o*-phen is negligible during the analytical procedure. These results show that free-Fe(II) species in aqueous solutions including Fe(II) and HA can selectively be determined by a colorimetric method using *o*-phen.

If dissociation of Fe(II)-HA complex for eq (3) would be reached to equilibrium, the K_b value might be estimated from the concentration of free-Fe(II)

species, determined by absorbance of Fe(II)-(*o*-phen) complex, and the concentration of free *o*-phen that can be analyzed using HPLC. However, the period for reaching constant absorbance values of Fe(II)-(*o*-phen) complex was more than 10 days. Because of rapid analysis and simplicity for measuring absorbance only, we employed the complexometric titration using colorimetry.

Evaluation of Fe(II) binding abilities

In ISEs and ASV, metal binding abilities (conditional binding constant, K_b , and binding capacity, N) can be evaluated by the changes in free-metal ion species at a variety of total concentrations of metal ions (Taga et al., 1989 and 1991; Town and Powell, 1993; Fukushima et al., 1992 and 1996). Thus, the colorimetric method using *o*-phen is applicable for use in a complexometric titration to evaluate the Fe(II)-binding abilities of HAs. Assuming a 1:1 complexation between Fe^{2+} and an arbitrary binding site in HA (L_i), K_b can be defined as:

$$K_b = \frac{[\text{Fe(II)}-L_i]}{[\text{Fe}^{2+}][L_i]} \quad (7)$$

The total concentration of ligand (C_{L_i}) can be expressed as below:

$$C_{L_i} = [L_i] + [\text{Fe(II)}-L_i] \quad (8)$$

From the measured $[\text{Fe}^{2+}]$ and $C_{\text{Fe(II)}}$, $[\text{Fe(II)}-L_i]$ can be calculated using eq (4). However, the calculated value for $[\text{Fe(II)}-L_i]$ should be considered as total concentration of Fe(II)- L_i for all binding sites. The following equation can be derived by combining eqs (7) and (8),

$$[\text{Fe(II)}-L_i] = \frac{C_{L_i} K_b [\text{Fe}^{2+}]}{1 + K_b [\text{Fe}^{2+}]} \quad (9)$$

Figure 2a shows an example of a colorimetric titration curve obtained for an HA sample. The absorbance values for the y-axis correspond to $[\text{Fe}^{2+}]$, and $[\text{Fe(II)}-L_i]$ can be calculated from eq (4). Thus, the relationship between $[\text{Fe}^{2+}]$ and $[\text{Fe(II)}-L_i]$ can be obtained, as shown in Fig. 2b. K_b and C_{L_i} were calculated, based on the nonlinear least square regression analysis of a data set for $[\text{Fe}^{2+}]$ and $[\text{Fe(II)}-L_i]$ to eq (9). The N values represent the numbers of moles of Fe(II)-binding sites per gram of

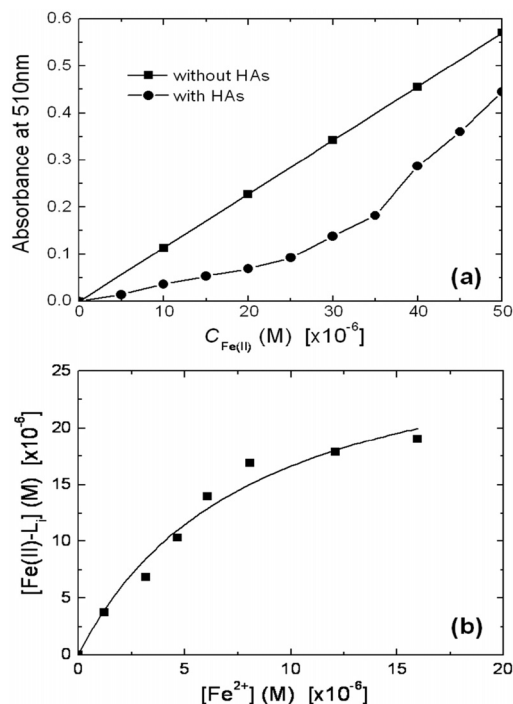


Fig.2 Complexometric titration curves for Fe(II) in the absence and presence of HA at pH 3.60 (a), and the relationship between $[Fe^{2+}]$ and $[Fe(II)-L_i]$ (b).

carbon in the HA sample, as expressed by the following equation:

$$N (\text{mol g}^{-1} \text{C}) = \frac{C_{Li}(\text{M})}{\text{HA} (\text{g L}^{-1}) \times \%C / 100} \quad (10)$$

The K_b and N values for HAs extracted from sediments are summarized in Table 2. Humic substances contain a variety of metal binding sites with different affinities for metal ions (Fukushima et al., 1996). Thus, the estimated K_b values in the present study represent the average binding constant for a variety of binding sites in HAs. The $\log K_b$ values were found to be in the range of 5.3 – 5.6, and

no significant differences were observed. These results indicate that the types and strengths of Fe(II)-binding sites are similar in HA samples, irrespective of their source.

On the other hand, the N values represent the numbers of total Fe(II)-binding sites in HAs. As shown in Table 2, significant differences were found in the N values. Oxygen-containing (e.g., acidic functional groups) and nitrogen-containing (e.g., amino and imidazole groups) functional groups can be considered to be potential Fe(II)-binding sites for Fe(II) in HAs. The O/C and N/C atomic ratios for the samples are summarized in Table 2. These can serve as indices for oxygen- and nitrogen-containing functional groups in HAs. However, no relationships were found between the O/C or N/C ratios and N values. The O/C ratio is related to ethers, esters and quinones as well as acidic functional groups that can serve as binding sites to Fe(II) in HAs. Thus, acidic functional groups (carboxylic and phenolic hydroxyl groups) were analyzed (Table 2). The values for total acidity (sum of carboxylic and phenolic hydroxyl groups) were relatively consistent with twice the N values, in which it was assumed that Fe(II) would bind to two acidic functional groups in HAs. Thus, it appears the Fe(II)-binding sites in HAs are mainly acidic functional groups. These results show that binding capacity can be used to determine the Fe(II)-binding abilities of HAs from sediments in dam reservoirs.

Conclusions

A colorimetric method using *o*-phen can be used to accurately determine the concentration of free-Fe(II) species in aqueous mixtures of Fe(II) and HA, and this can be applied to a complexometric titration to evaluate the Fe(II)-binding abilities (conditional binding constants and binding capacities) of HAs. A comparison between total acidity and Fe(II)-binding

Table 2 Fe(II)-binding abilities and acidic functional group content for HA samples.

HA samples	Fe(II)-binding abilities		Acidic functional groups (mmol g ⁻¹ C)		
	$\log K_b$	N (mmol g ⁻¹ C)	Total acidity	Carboxylic acid	Phenolic hydroxyl groups
Niiho 1	5.27±0.17	4.42±0.60	8.2±1.2	5.9±0.6	2.3±1.4
Niiho 2	5.27±0.21	6.54±1.29	17±2	4.1±0.1	13±2
Ebihara 1	5.47±0.29	3.75±0.73	7.8±0.7	4.6±0.3	3.2±0.8
Ebihara 2	5.63±0.09	5.23±0.35	9.8±0.1	4.3±0.2	5.5±0.2
Yoneoka 1	5.52±0.07	5.35±0.32	10±1	4.8±0.1	5.3±0.5
Yoneoka 2	5.26±0.08	5.64±0.46	13±2	4.8±1.3	7.8±2.2

capacities suggests that acidic functional groups in HAs serve as ligands to Fe(II) in HAs. For evaluating the quality of the unit required for using Fe(II)-HA complexes as fertilizers in coastal areas that contain barren ground, the Fe(II)-binding capacity can be considered to be a useful index. The colorimetric method using *o*-phen is a simple and practical method for evaluating the qualities of HAs in a unit.

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