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ANALYTICAL INVESTIGATION OF INTERACTION BETWEEN HUMIC ACIDS AND COPPER

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CHAPTER 1

GENERAL INTRODUCTION

Humic acids are soil organic matter and weak-acid polyelectrolyte. These widely distribute in soil and natural waters [1]. It is well-known that humic acids play as the complexing agent in natural systems. The informations about the interaction between heavy metal ions and humic acids are important for biology, environmental chemistry, geochemistry and soil science. For example, in natural waters, the hydrated copper(II) ion interferes with the algae growing. However, by adding a chelating agent, this toxicity of the copper(II) ion became weaker [2, 3]. For lead ion, the same effect on algae was reported [4]. The iron (III) ion which is essential for the micro-organisms was not ingested in the colloidal form, while if they bind with a chelating agent as EDTA, it can be ingested [5]. In the soil environment, humic acids have cation exchange capacity, and are involved in the transportation and the accumulation of the toxic heavy metal ions [6]. Therefore, studies about the binding ability of humic acids to heavy metal ions are also very interesting for the environmental sciences.

The stability constants and the complexing capacities to heavy metals are quantitative indicators for the interaction between heavy metal ions and humic acids. To estimate these values, methods to determine the uncomplexed forms and the complexed forms of heavy metal ions are needed. Especially, in case of discussing the complexing formation between heavy metal ions and organic compounds such as the humic acids in natural water, more sensitive, precise and simple methods for the separation and the determination of heavy metal species are required.

The many method for measurement of the heavy metal species (free or complexed) have been reported. For example, ion selective electrodes (ISE)

[7], stripping voltammetry [8], liquid chromatography [9], bioassay [10], the method using dialysis membrane [11], using ion exchanger [12], and so on.

When the complexing equilibria between copper(II) ions and humic acids is discussed in the trace level, the use of the ion-exchange method or the anodic stripping voltammetry (ASV) may be suitable methods. However, these methods hardly was used for the studies about the complexing equilibria between heavy metal ions and humic acids. In an ion-exchange method, the change of complexing equilibria occurs during ion exchange [13,14]. In an ASV, humic substances are adsorbed on the electrode and the electron transfer to free copper(II) ion is interfered during deposition process and stripping process [15-17]. However, if these problems will settle, these become powerful means to evaluate the copper(II) complexing ability of humic acids.

On the other hands, humic acids are heterogeneous polyelectrolyte and have various functional groups and various molecular weight distribution [18,19]. It has been reported that humic substances contains various acids which are different acidity. In the potentiometric studies of humic substances, two functional groups were recognized in the humic molecules [20]. They were strong or weak acid such as carboxylate or phenolic hydroxyl groups. In chromatographic studies, these functional groups could be detected by the chromatographic methods in which two acidic fraction of humic substances were separated [21 - 23]. However, it has not been known how these functional groups contribute to the coordination of heavy metal ions. Especially, binding constants of heavy metal ions in each binding-site are very interesting in the stability of heavy metal-humic complex to the variation of ionic strength, pH, competitive ions, and so on. Furthermore, calculation of thermodynamic constants is very important to evaluate the heavy metal complexing abilities of humic substances in various conditions. Such studies

will contribute to the prediction of variation of heavy metal species in the natural systems.

Still more, humic substances have molecular weight distribution [24]. This is mainly attribute to the heterogeneity of humic substances. Therefore, it is required that we must evaluate heavy metal complexing abilities of humic substances considering molecular weight heterogeneity. In order to evaluate acid-base equilibria of humic substances, ligand distribution was assumed [25-27]. These approaches are useful to evaluate the relation between amounts of protonated site and dissociation constants. This can be expanded to the complexing equilibria between humic substances and heavy metal ions and be useful to evaluate the complexing abilities considering heterogeneity of humic acids.

On the other hands, humic substances have hydrophobic domain such as alkyl hydrocarbon and aromatic rings [28]. These domains contribute to solubilize the insoluble matter such as pesticides, fundicides, herbicides, PCBs and so on [29-31]. The solubility enhancement of hydrophobic matter occurs in the presence of humic substances in natural waters system, and this is the fate of environmental potentiality. Metal chelating agents which are mainly used as fundicides are insoluble to water. However, it can be predicted that solubility of metal chelate will enhance in the presence of humic substances. However, solubilization of metal chelate occurs the dissociation of the metal chelate and treatment of the interaction between humic substances and metal chelate will be complicated. The evaluation of thermodynamic parameter between humic substances and the metal chelate is important to predict the amount of metal chelate flowed from soil environment to natural water.

From these points of view, the complexing equilibria between copper(II) and humic acids and the interaction between copper(II)-oxinate and humic

acids were investigated. The purpose of this work are as follows: (1) development of the method to determine the copper(II) species in the presence of humic acids, (2) evaluation of conditional stability constants and copper(II) complexing abilities, (3) relation between copper(II)-binding sites and functional groups, (4) evaluation of copper(II) complexing equilibria considering molecular weight heterogeneity, (5) the interaction between copper(II)-oxinate and humic acids.

The outlines in the present work is described as follows.

Chapter 1 is general introduction. In this chapter, the importance of investigations of complexing equilibria between heavy metal ions and humic substances in environments is discussed, and then the problems of the equilibrium chemistry of humic acids were described.

In chapter 2, the classification of humic substances and the preparation method of humic acids is described. In this chapter, the basic data such as elemental analysis, the UV-VIS spectra and the FTIR spectra are shown.

In chapter 3, determination of copper(II) complexing species or free copper(II) ions is performed by macroreticular dextran gel anion exchanger, diethylaminoethyl Sephadex A-25 (A-25) or sulfopropyl Sephadex C-25 (C-25). And then, interpretation mode to evaluate copper(II) complexing abilities (conditional stability constants and copper(II) complexing capacities) is described.

In chapter 4, a determination method of free copper(II) ion by anodic stripping voltammetry is described. In this chapter, to prevent the adsorption of humic acid on the electrode, surfactants is added in the solution and could eliminate the interference of humic acid. Still more, the mechanism of surfactant effect is also discussed.

In chapter 5, the relation between copper(II) complexing site and functional groups in humic acid is investigated by the potentiometric and the

conductimetric acid-base titration.

In chapter 6, evaluation of thermodynamic stability constant of copper(II)-humic acid complex is performed by extrapolating conditional stability constants at ionic strength to zero. Furthermore, effect of ionic strength on copper(II) complexing ability is discussed.

In chapter 7, the continuous stability distribution model is proposed to evaluate copper(II) complexing ability considering the heterogeneity of humic acids. The molecular weight fraction of humic acid is prepared, and then the stability distribution of the fraction is compared with each other.

In chapter 8, interactions between copper(II)-oxinate and humic acids are investigated. The partition of copper(II)-oxinate into humic acid was assumed and the partition coefficients of copper(II)-oxinate were evaluated.

In chapter 9, the summary of this work is described.

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CHAPTER 2

PREPARATION OF HUMIC ACIDS

2.1 Introduction

The organic matter of soils consists of a mixture of plant and animals products in various stages of decomposition, of substances synthesized biologically and/or chemically from the breakdown products and of microorganisms and small animals and their decomposing residues [1]. To simplify this very complex system, organic matter is usually divided into two groups: (a) nonhumic substances, and (b) humic substances.

The fractionation scheme of humic substances by Schnitzer et al. is shown in Fig. 2.1. The soil organic matter can be extracted by alkali such as sodium hydroxide or potassium hydroxide. The soluble fraction by alkali is classified two fractions: (1) precipitated by acid; humic acid (HA) that is high molecular weight fraction, (2) soluble fraction by acid; fulvic acid (FA) that is lower molecular weight fraction than HA. The insoluble fraction by alkali is denoted "humine" that consists of aliphatic hydrocarbon.

Humic substances generally exist as the bound form with iron(III), aluminum(III) and clays in natural systems. Therefore, the methods to obtain the substances free from these metals would be required. As the methods for the extraction of humic substances, the quantitative and unchangeable methods are desirable. However, these have not been satisfied yet [2]. Sodium hydroxide, sodium pyrophosphate or the mixed solution of alkali (for example, NaOH + NaF, NaOH + $Na_4P_2O_7$, NaOH + Na_2CO_3 and so on) were used as the reagent for the extraction [3, 4]. In the case of sodium hydroxide, the most nitrogen content of the all reagents was obtained [5]. On the other hands, Haye et al. used dioxane as the reagent for the extraction [6]. However, this brought about the positive error for the carbon content of

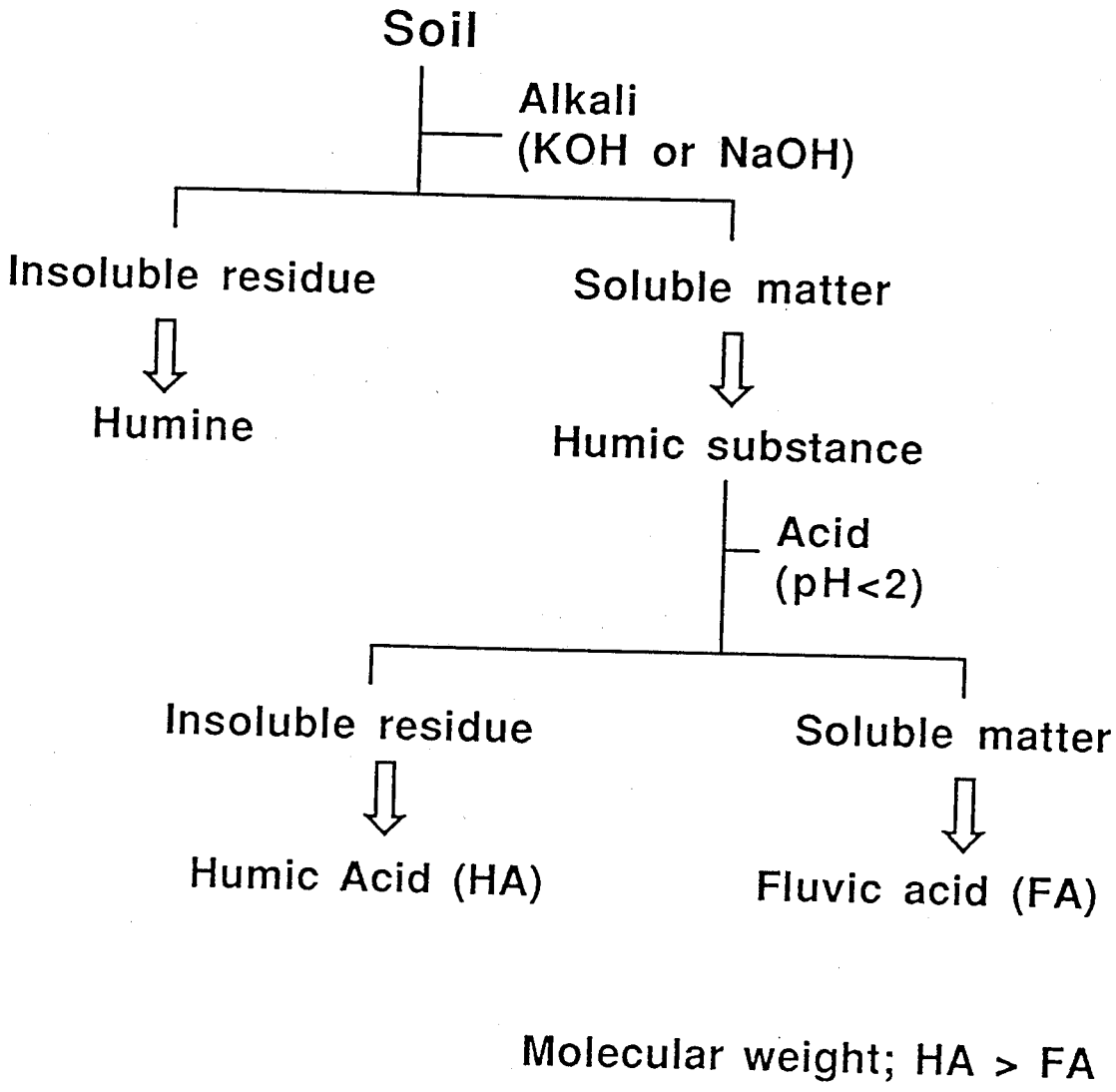

Fig. 2.1 Fractionation of humic substances.

humic substances. Still more, they examined the several methods and reagents for extraction, sodium hydroxide was the best reagent of the all.

The methods for the preparation of humic substances have been reported. Especially, the standard method was proposed by International Humic Substances Society (IHHS) [7]. Moreover, the standard humic materials, which were from Swanee river and Laurentian podzol, prepared by U.S.Geochemical Survey and Canada Soil Survey Committee [8, 9].

On the other hand, in order to characterize the structure of humic substances, the UV-VIS spectra have been measured [10-12]. It has been known that the absorbance in UV-VIS spectra of humic substances gradually increase with decreasing wavelength. Kumada et al. defined the ratio of absorptivity at 400 nm to 600nm (e_{400}/e_{600}) for the degree of humidity [11]. This was the indicator for the amount of aromatic groups, functional groups and free radical [13, 14].

In this chapter, the extraction of humic substances were performed by using sodium hydroxide. In order to obtain the protonated humic acid, humic acid fraction was separated as the precipitation in acidified solution. Subsequently, this was purified by dialysis, and then the powder of humic acid was obtained. The elemental analysis and the measurement of the UV-VIS spectra were performed for the each humic acid.

2.2 Preparation of Humic Acids

The scheme for preparation of humic acid is shown in Fig. 2.2. Peat is dried at room temperature and then 0.5 g of peat is leached by 300 ml of 1M NaOH for a week. The solution was separated by centrifugation at 3500 rpm for 15 min. The supernatant was transferred into 500 ml of beaker. The pH of this solution was adjusted below pH 2 by concentrated hydrochloric acid, and then stand for 2 days. This solution was centrifuged at 3500 rpm for 15 min. The precipitation (humic acid fraction) was transferred into dialysis tube. The dialysis was performed for 3 weeks versus distilled water to remove Cl^- ions and other ions. Subsequently, the material in the dialysis tube was transferred into the eggplant type flask. This was evaporated at 40°C. The residue was lyophilized for 3 days, and then the humic aid powder was obtained.

2.3 Elemental Analysis

The humic acid were extracted from a commercial humic acid, a peat soil and a marine sediment in Hokkaido. The humic acids used in the present work are as follows; commercial: Fluka Chemie. A.G. (FHA), Wako Pure Chemicals (WHA_1; Lot.TSH 1267, WHA_2; Lot.TLM 6304), peat: Shinshinotsu (SHHA), Sarobetsu (SAHA), Bibai (BHA), marine sediment: Funka bay (FBHA). The results of elemental analysis are shown in Table 2.1. Carbon, hydrogen and nitrogen were determined in all the humic acids. However, sulfur was only detected in FBHA.

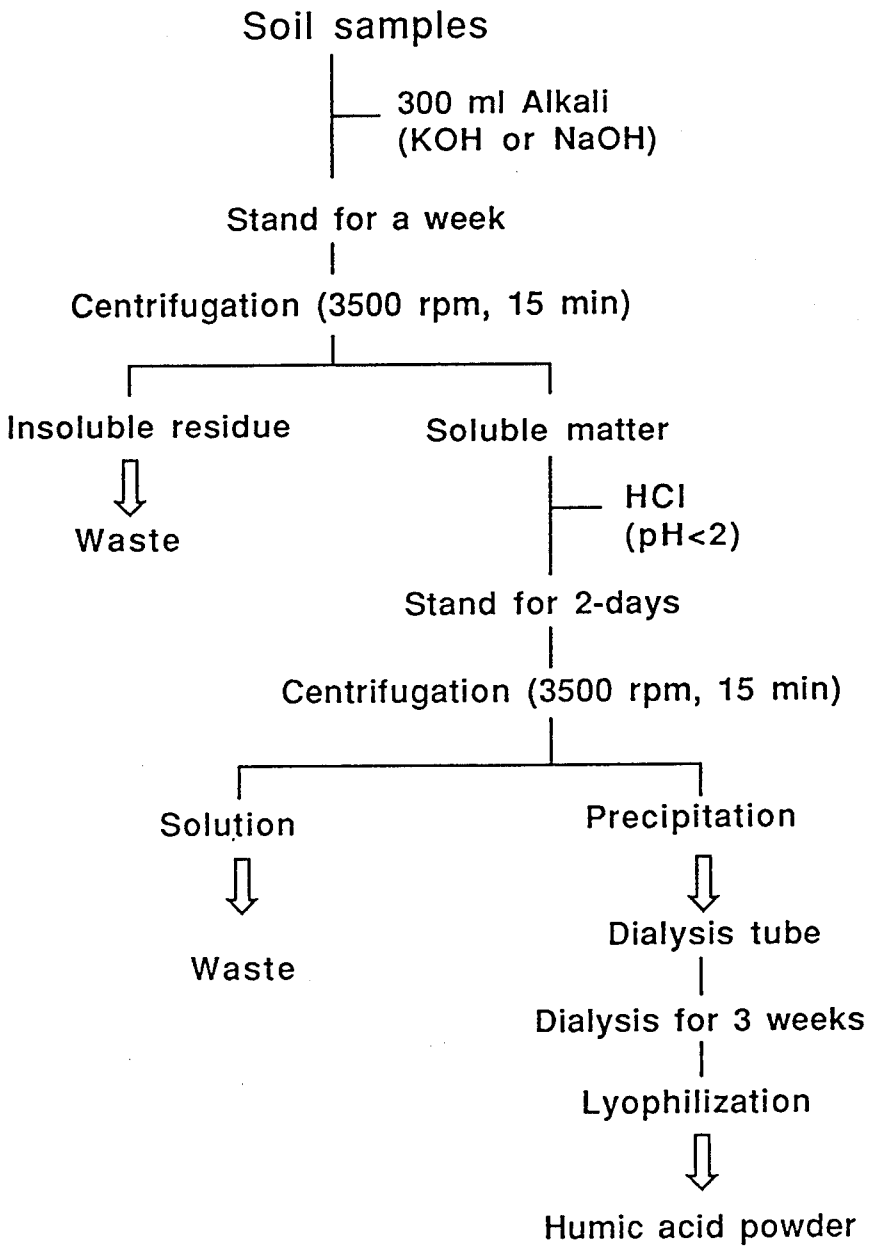

Fig. 2.2 Preparation procedure of humic acids powder.

Table 2.1 Elemental analysis of humic acids.

Humic acids	%C	%H	%N	%O*	%S	%ASH
Commercial humic acids						
FHA	47.0	4.30	0.60	46.0	—	2.06
WHA_1	52.0	4.85	1.21	38.6	—	3.34
WHA_2	54.1	5.90	1.77	37.0	—	1.23
Extracted from peat						
SHHA	51.2	4.72	1.74	40.0	—	2.44
SAHA	52.9	4.40	3.10	37.9	—	1.66
BHA	55.1	3.70	1.93	36.8	—	2.44
Extracted from marine sediment						
FBHA	49.1	6.10	5.48	33.5	2.91	2.87

* %O = 100 - %(C + H + N + S + ASH).

2.4 UV-vis spectra

The UV-vis spectra of humic acids are shown in Fig. 2.3 in which the solution containing 40 mg l^{-1} humic acid is adjusted pH 8 by HEPES buffer. The absorbance of each humic acid increases with a decrease of wavelength as described in the previous work [11]. However, soil humic acids (WHAs, FHA, SHHA, SAHA and BHA) have shoulders at about 250 ~ 300 nm. These seem to cause the decomposition of lignin which is premonition of humic substances [15]. Therefore, these absorbance decrease with proceeding the decomposition of humic materials. The BHA has a strong absorption at about 280 nm. Therefore, this seems to be close to lignin. On the other hand, FBHA which is extracted from marine sediment has a strong shoulder at about 400 nm. This seems to be due to the pheopigment which is decomposed from chlorophyl [16]. In order to certify this fact, the fraction of humine from marine sediment was extracted by acetone, and then the absorption of the extracted solution was measured (Fig. 2.4). The peaks at 660 nm and 400 nm are the same peaks as those of chlorophyls [17]. Therefore, the FBHA is produced by the decomposition of chlorophyls from phytoplanktons in the marine environment.

Still more, the values of e_{400}/e_{600} were calculated by each spectrum, and then summarized in Table 2.2. The aromatic contents were evaluated by using these values as following chapter.

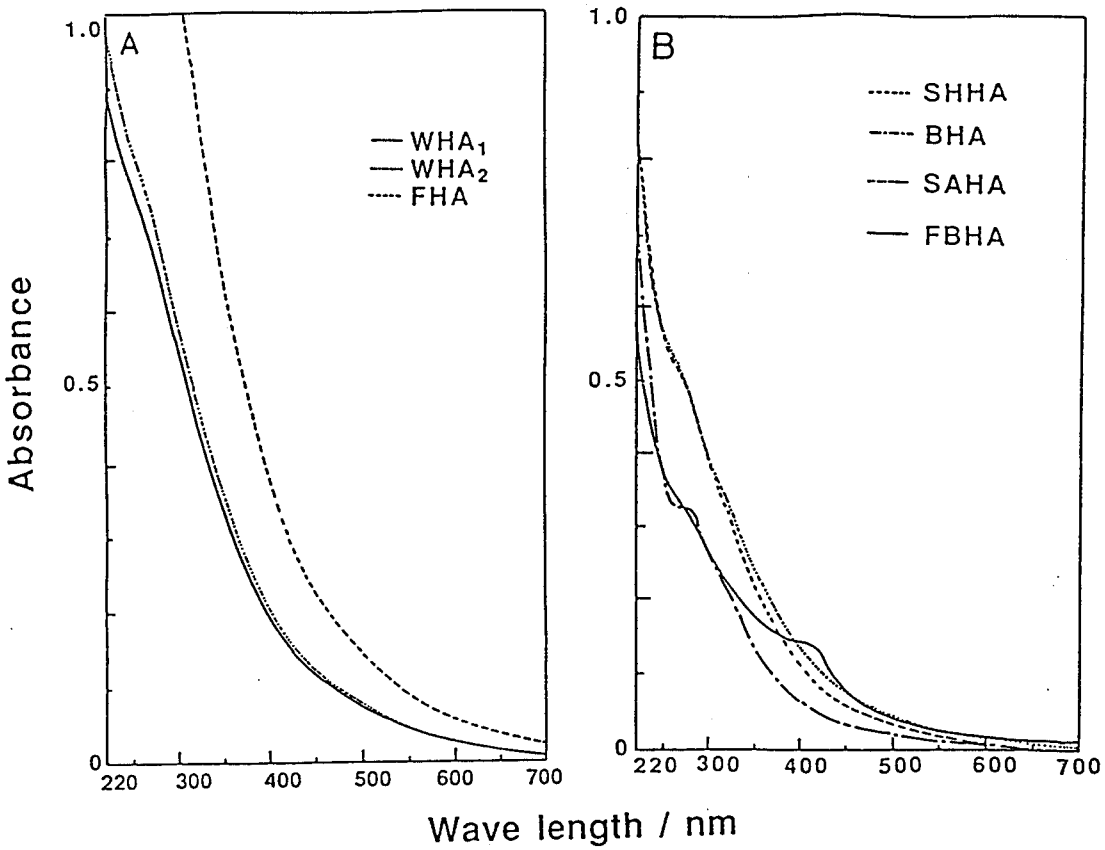

Fig. 2.3 UV-VIS spectra of humic acids.

0.05 M HEPES buffer (pH 8), A: commercial humic acids; [humic acid]: WHAs; 20 mg l^{-1}, FHA; 40 mg l^{-1}, B: humic acids from soil samples; [Humic acid]; 20 mg l^{-1}.

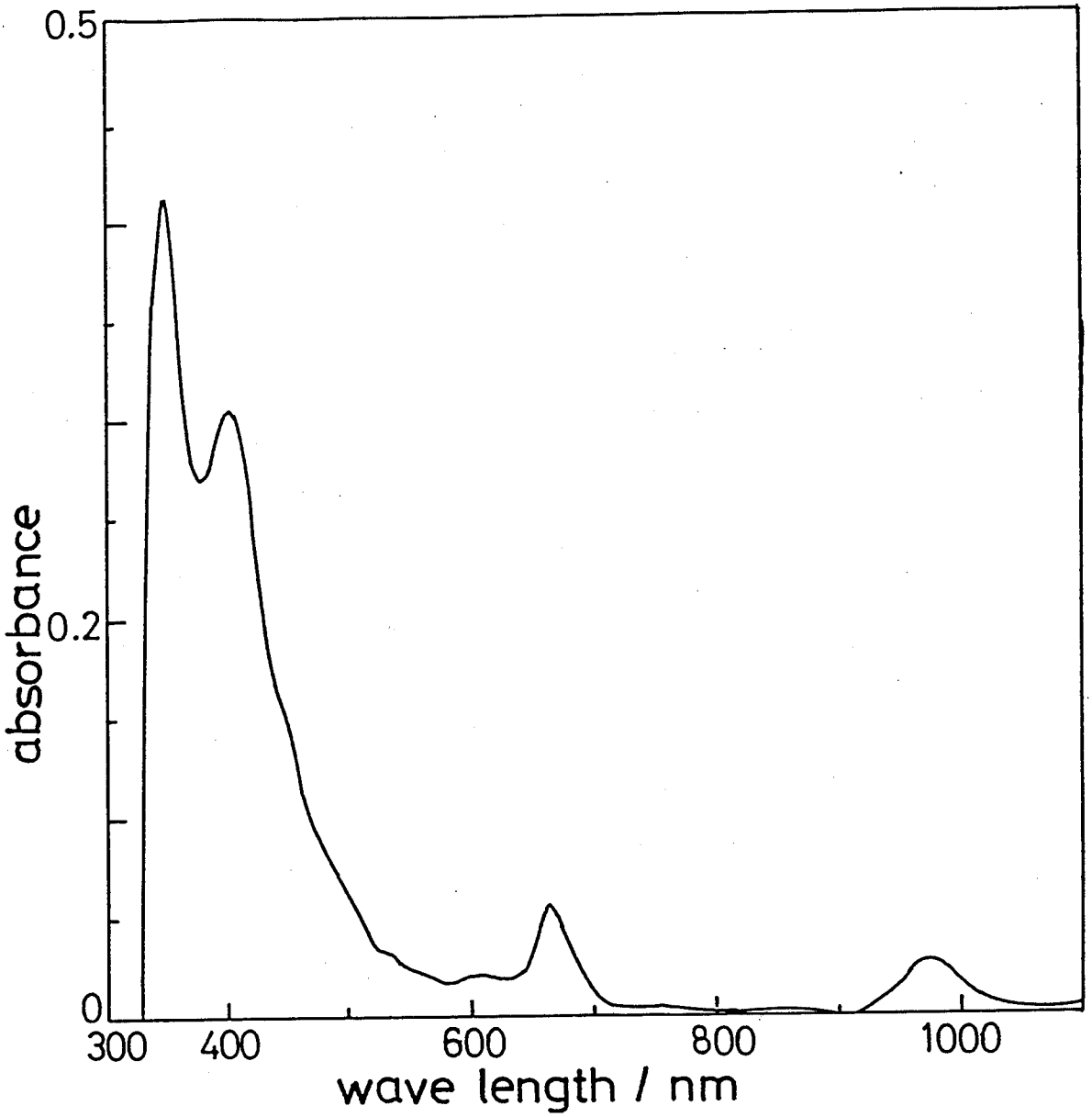

Fig. 2.4 UV-vis spectrum of humine in FBHA.

Humine was extracted by acetone solution.

Table 2.2 e_{400}/e_{600} values of humic acids.

Humic acids	e_{400}/e_{600}
WHA_1	7.55
WHA_2	6.77
FHA	7.02
SHHA	9.00
SAHA	7.47
BHA	7.44
FBHA	6.55

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CHAPTER 3

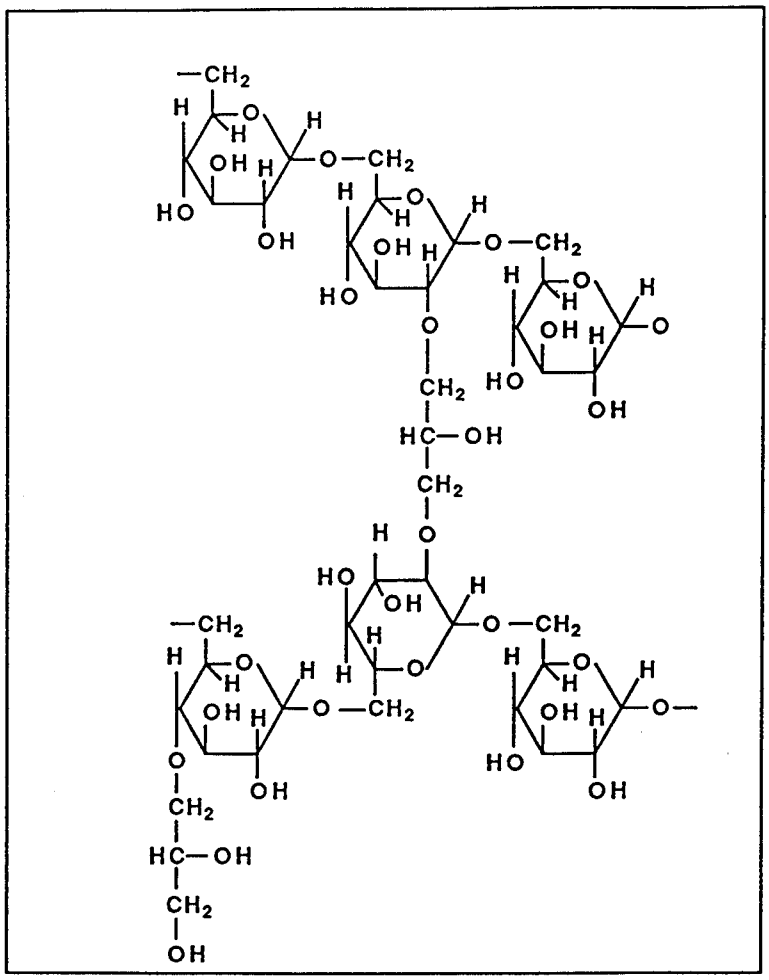
EVALUATION OF COPPER(II) COMPLEXING ABILITY OF HUMIC ACID BY USING ION-EXCHANGER

3.1 Introduction

Recently, a macroreticular dextran gel ion-exchanger is used for the measurement of heavy metal species in natural waters [1]. The structures of these gels are shown in Fig. 3.1. A weak-base anion exchanger, diethylaminoethyl Sephadex A-25 (A-25) is a cross-linked dextran gel with diethylaminoethyl group. This A-25 resin has a larger total exchange capacity (50 meq/100 ml gel) than any other gel ion-exchanger (e.g. DEAE-Sepharose, QAE-Sephadex etc.). Especially, this is suitable for retaining and separating nucleotide [2], peptide and proteins with molecular weights lower than 10^5. The A-25 resin could adsorb the metal-humic acids complex, but not the free metal ions [3, 4]. Using this property of A-25, Hiraide et al. evaluated the copper(II) complexing capacities of humic acids in river waters after concentration by coprecipitation and flotation [5].

On the other hand, a macroreticular dextran gel, strong-acid cation-exchanger, sulfopropyl Sephadex C-25 (C-25) was also used for the speciation of copper in river waters [6, 7]. The cationic free copper(II) ion was retained on the C-25 and the anionic copper(II)-humic acid complexes remained in the supernatant.

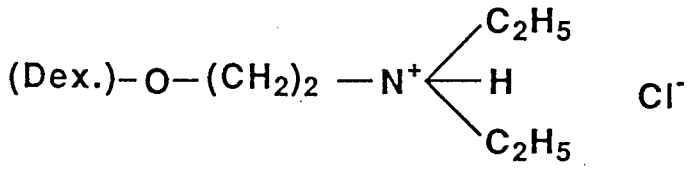
Still more, in some interpretation mode of the complexation with copper(II) ions for humic acids and organic matters in natural waters, the interpretation modes by Scatchard [8] and van den Berg [9, 10] have been frequently used for calculation of the conditional stability constants and complexing capacities. The adoption of the two-site model has been a reasonable success for measurement of heavy metal complexing capacities of organic



Dextran gel (Dex.)

Dextran gel ion-exchanger

1. *Anion-Exchanger:* Diethylaminoethyl Sephadex A-25 (A-25)



2. *Cation-Exchanger:* Sulphopropyl Sephadex C-25 (C-25)

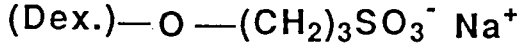


Fig. 3.1 Dextran gel ion-exchanger.

ligands in natural waters [11, 12].

In the present work, the fraction of the uncomplexed free copper(II) ion and that of the copper(II)-humic acid complexes were separated by using an A-25 or a C-25. The free copper(II) species or complex species could be determined by AAS, and then the copper(II) complexing abilities (conditional stability constants and copper complexing capacities) of humic acids from peat were estimated by Scatchard plot adopting the two-site model.

3.2 Method for calculation

To estimate conditional stability constants and the copper(II) complexing capacities of humic acids, a Scatchard plot was used. The equilibrium between copper(II) and humic acid in the sample solution was written as follows:

$$Cu^{2+} + L_i^{n-} \rightleftharpoons CuL_i^{-(n-2)} \qquad (3.1)$$

where L_i is the binding site of humic acid and n is the number of charges. If it is assumed that there are m sites concerned with copper(II) complex formation, which are independent of each other, in the sample solution, the mass balance can be described by Eqns. (3.2)-(3.4)

$$[Cu^{2+}]_t = [Cu^{2+}] + \sum^{m} [CuL_i] \qquad (3.2)$$

$$c_{Li} = [L_i] + [CuL_i] \qquad (3.3)$$

$$K'_i = \frac{[CuL_i]}{[Cu^{2+}][L_i]} \qquad (3.4)$$

where i is the number of binding site, $[Cu^{2+}]_t$ is the total copper(II) concentration in the sample solution, $[Cu^{2+}]$ is the concentration of free copper(II) ions, K'_i and c_{Li} show the conditional stability constants and the concentration of each binding site, respectively. Subtraction and rearrangement of these equations give,

$$\Sigma[CuL_i] = \frac{\Sigma K'_i[Cu^{2+}]c_{Li}}{1 + K'_i[Cu^{2+}]} \qquad (3.5)$$

In case of one-site, from Eqns. (3.4) and (3.5),

$$\frac{[CuL]}{[Cu^{2+}]} = -K'[CuL] + K'c_L \qquad (3.6)$$

If $[CuL]/[Cu^{2+}]$ is plotted against [CuL], Scatchard plot becomes linear and the conditional stability constant, K', and copper(II) complexing capacity, c_L, can be estimated from its slope and intercept. On the other hand, in the case of a two-site model assuming two binding sites independent of each other, we can obtain the following equation from Eqn. (3.5),

$$\Sigma[CuL_i] = \frac{K'_1 c_{L1}}{1+K'_1[Cu^{2+}]} + \frac{K'_2 c_{L2}}{1+K'_2[Cu^{2+}]} \qquad (3.7)$$

In this case, Scatchard plot is the lower projected curve. The two lines can be extrapolated for this curve and their slopes and intercepts can be related to the conditional stability constants (K'_1, K'_2) and copper complexing capacities (c_{L1}, c_{L2}) [8]. The regression analysis was used for the two lines. Where, copper(II) binding capacity, N could be defined as follows,

$$N = \frac{c_L \text{ (mol/l)}}{\text{HA (g/l)}} \qquad (3.8)$$

We defined "strong site" for the higher stability site (K'_1, N_1) and "weak site" for the lower stability site (K'_2, N_2).

3.3 Evaluation of copper(II) complexing ability using anion exchanger diethylaminoethyl Sephadex A-25

3.3.1 Experimental Section

Reagents and materials

The humic acids were obtained from Fluka Chemie AG (FHA) and peat soil (SHHA). The stock solutions of humic acids were prepared by dissolving 0.1 g of the humic acid powder in 0.1M potassium hydroxide solution and diluting to 100 ml. The diethylaminoethyl Sephadex A-25 (Pharmacia LKB Biochemistry) was washed by ultrasonic irradiation for 20 min in 0.1M hydrochloric acid solution, washed with water and then swollen in the 0.05M potassium chloride solution after adjusting pH to 6-7 by potassium hydroxide solution. The standard copper solution (1 g l^{-1}) was prepared by dissolving electrolytic copper (purity 99.999%) in concentrated nitric acid and diluting to 0.01M nitric acid solution. The ethylenediaminetetraacetic acid (EDTA) solution was prepared by dissolving EDTA4H (Wako Pure Chemical Industries, Ltd.) in 0.1M potassium hydroxide solution. The citric acid was of analytical reagent grade.

Apparatus

A polypropylene column (Bio-Rad) was used for the preparation of A-25 column. The concentration of copper in effluent was measured by a HITACHI 170-50 atomic absorption spectrometer with a GA-2 graphite-furnace atomizer under the following conditions: wavelength 324.7 nm; drying for 40 s at 300 °C; ashing for 20 s at 750 °C; atomization for 10 s at 2650 °C; background correction with D_2 lamp.

Procedure

After the pH of the sample solution containing copper(II) ions and humic acid was adjusted at 6.0, and then 25 ml of sample solution was passed through the column with 2 ml of A-25 resin at a flow rate of 5 ml min^{-1}. Then the A-25 column was washed with water. By this procedure, copper(II)-humic acid complex was adsorbed on A-25 resin, and only free copper(II) ion was eluted down. The column system is shown in Fig. 3.2. The effluent was enriched by heating it on the hotplate and was diluted to 25 ml by 0.2 M nitric acid solution. A 20 µl aliquot was injected into the graphite-furnace and the copper was determined by a graphite-furnace atomic absorption spectrometry. The copper(II) adsorbed on A-25 resin was determined as follows. The A-25 resin with copper(II)-humic acid complex was transferred to a centrifuge tube and 8 ml of 4 M nitric acid solution was added. The copper(II) adsorbed on the A-25 resin was desorbed by ultrasonic irradiation for 10 min. The solution and the A-25 resin was separated by centrifugation for 5 min and the A-25 was transferred to a 50-ml beaker. Eight milliliters of 4 M nitric acid solution was again added to the centrifuge tube, and the same procedure was repeated. The supernatant was introduced to a 50-ml beaker and dried up on the hotplate. The residue was decomposed by adding 0.5 ml of concentrated nitric acid and diluting this solution to 25 ml. The 20 ml aliquot was injected into the graphite-furnace.

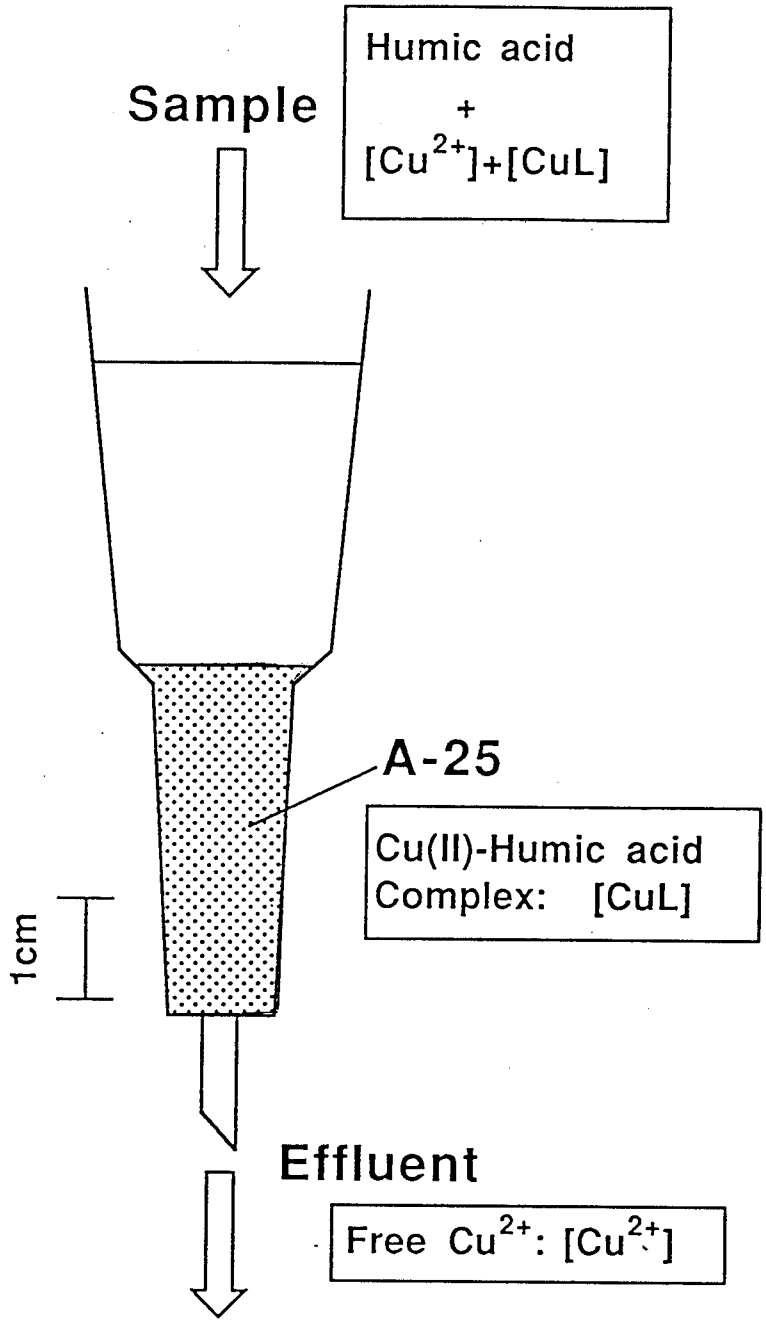


Fig. 3.2 A-25 column.

3.3.2 Adsorption of copper(II)-humic acids complexes on A-25

Fig. 3.3 shows the adsorption behavior of copper(II)-humic acids complexes to A-25 resin at various pHs. Citric acid was also investigated as the model ligand. In the case of citric acid, constant adsorption was shown in pH>6. Since the dissociation constants of the citric acid are pK_{a_1}=3.08, pK_{a_2}=4.74 and pK_{a_3}=5.40, the stable anionic complex was formed at the pH region beyond pK_{a_3}, and approximately quantitative adsorption was achieved. The amount of copper(II)-humic acids (FHA or SHHA) complexes adsorbing on A-25 column also decreased below pH 5 and was constant beyond pH 5. It was confirmed that non-labile complexes were formed at the pH region beyond pH 5.

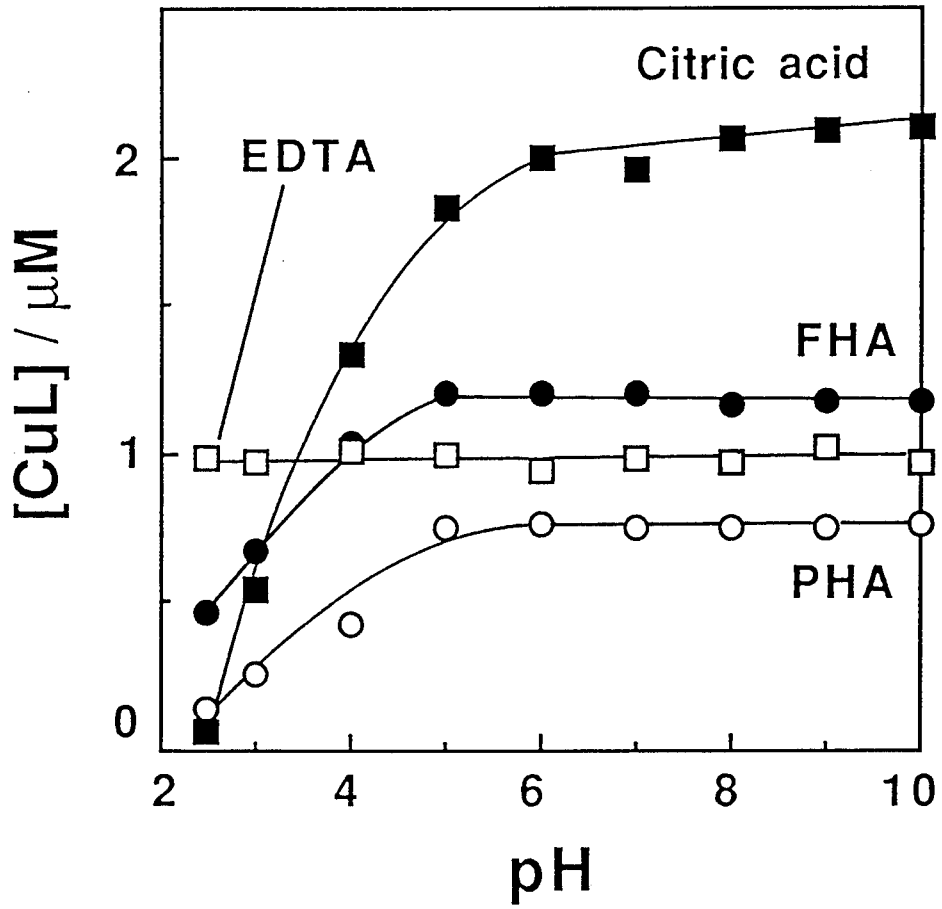


Fig. 3.3 Effect of pH on the adsorption of copper(II) complexes on the A-25.

The sample solution contained 60 nmol of copper(II) and the ligands.

3.3.3. $[Cu^{2+}]_t$ *vs* $[Cu^{2+}]$ plots

Fig.3.4 shows the $[Cu^{2+}]_t$ *vs* $[Cu^{2+}]$ plots, in which free copper(II) concentration, $[Cu^{2+}]$ is plotted against the total copper(II) concentration in sample solution, $[Cu^{2+}]_t$. When sample solution without the ligands was passed through an A-25 resin column, the $[Cu^{2+}]_t$ *vs* $[Cu^{2+}]$ plot was linear and the slope of this line (Fig.3.4 a) was 1. Therefore, it was confirmed that free copper(II) ions were not adsorbed on A-25 resin at all. Since it is known that humic acids contain carboxylic, phenolic hydroxyl and alcoholic hydroxyl groups [13] and that these functional groups contribute to complexing formation with metal ions. EDTA and citric acid were used as the model ligands in this work. When EDTA, which formed a stable chelate with copper at molar ratio 1:1, was used as the ligand, the copper(II)-EDTA complex was quantitatively adsorbed on A-25 resin (Fig. 3.4 d). The $[Cu^{2+}]_t$ *vs* $[Cu^{2+}]$ plots of FHA or SHHA were not linear (Fig. 3.4 b, c), since humic acids have many binding sites with different stability in the structure.

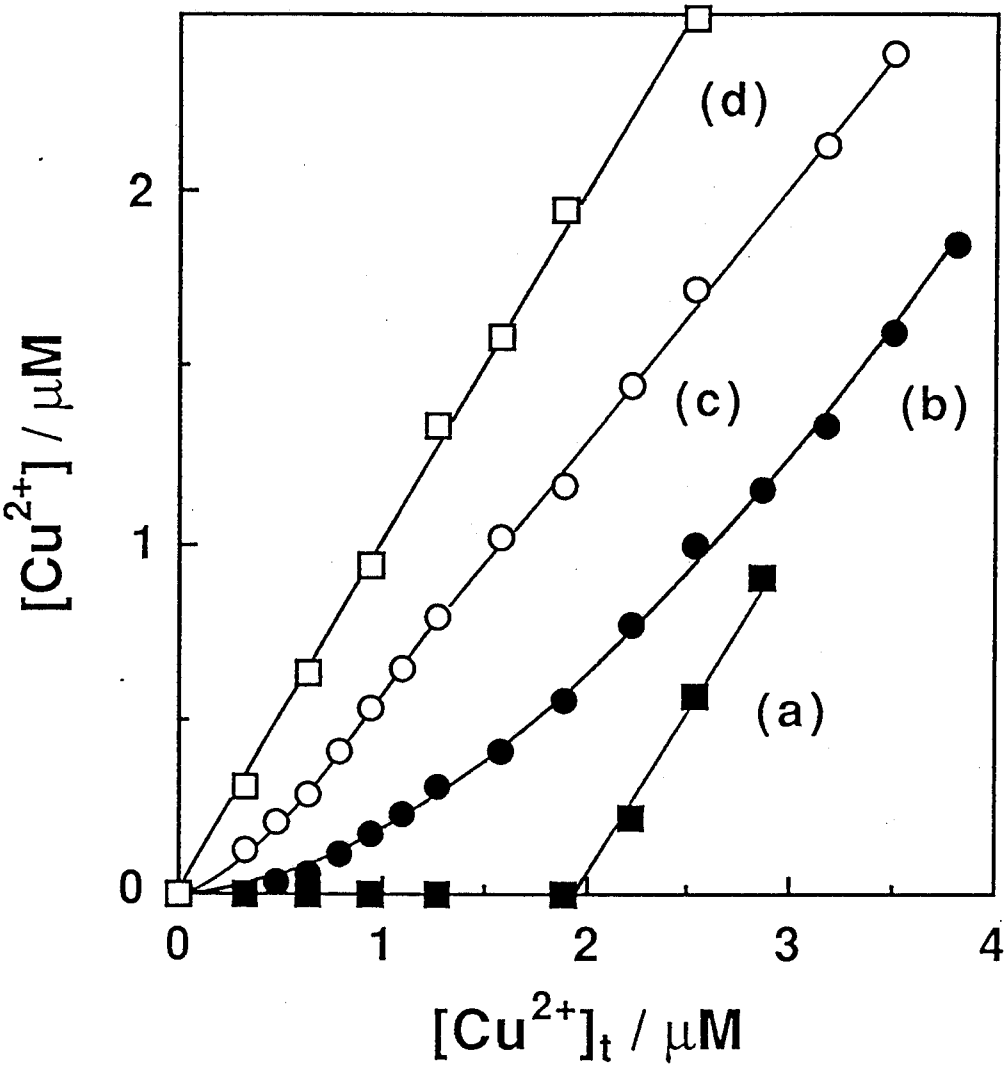


Fig. 3.4 $[Cu^{2+}]_t$ *vs* $[Cu^{2+}]$ plot of model ligands.

A 25 ml of sample solution (pH 6.0) containing 0 ~ 3.8 μM copper(II) was used. (a) 2 μM of EDTA, (b) 40 mg l^{-1} FHA, (c) 10 mg l^{-1} SHHA, (d) without ligands.

3.3.4. Copper(II) complexing ability of citric acid

The measurement for 3 μM of citric acid as a model ligand was performed by using A-25 resin column. The Scatchard plot at pH 7 was linear, as shown in Fig. 3.5. K' and c_L were calculated from the slope and intercept of this line. The values estimated were $\log K' = 5.7$ and $c_L = 2.2$ μM. The value for $\log K'$ was in good agreement with the literature values [14-16]. These results suggest that this method could be applied to the measurement of the complexing ability of humic acids.

3.3.5 Copper(II) complexing ability of humic acids

Fig. 3.6 shows a Scatchard plot for SHHA at pH 6. It was the lower projected curve because humic acids have many binding sites with different stability constants in their structure. The two lines were extrapolated for this curve by adaptation to the two-site model described previously. The conditional stability constants (K'_1, K'_2) and the concentration of binding site (c_{L1}, c_{L2}) could be estimated from these slopes and intercepts. The Scatchard plot for HA also showed the same shaped curve. These results are summarized in Table 3.1. The obtained conditional stability constants between copper(II) and humic acids were within the range of that reported by Bresnahan et al.[17] and Montoura et al.[18] (log K'_1; 6.0 ~ 8.8, log K'_2; 3.8 ~ 8.1, at pH 6). The measurement could be achieved under smaller ligand concentration (10 mg l^{-1}, 40 mg l^{-1}) than for other methods (over 1 g l^{-1}).

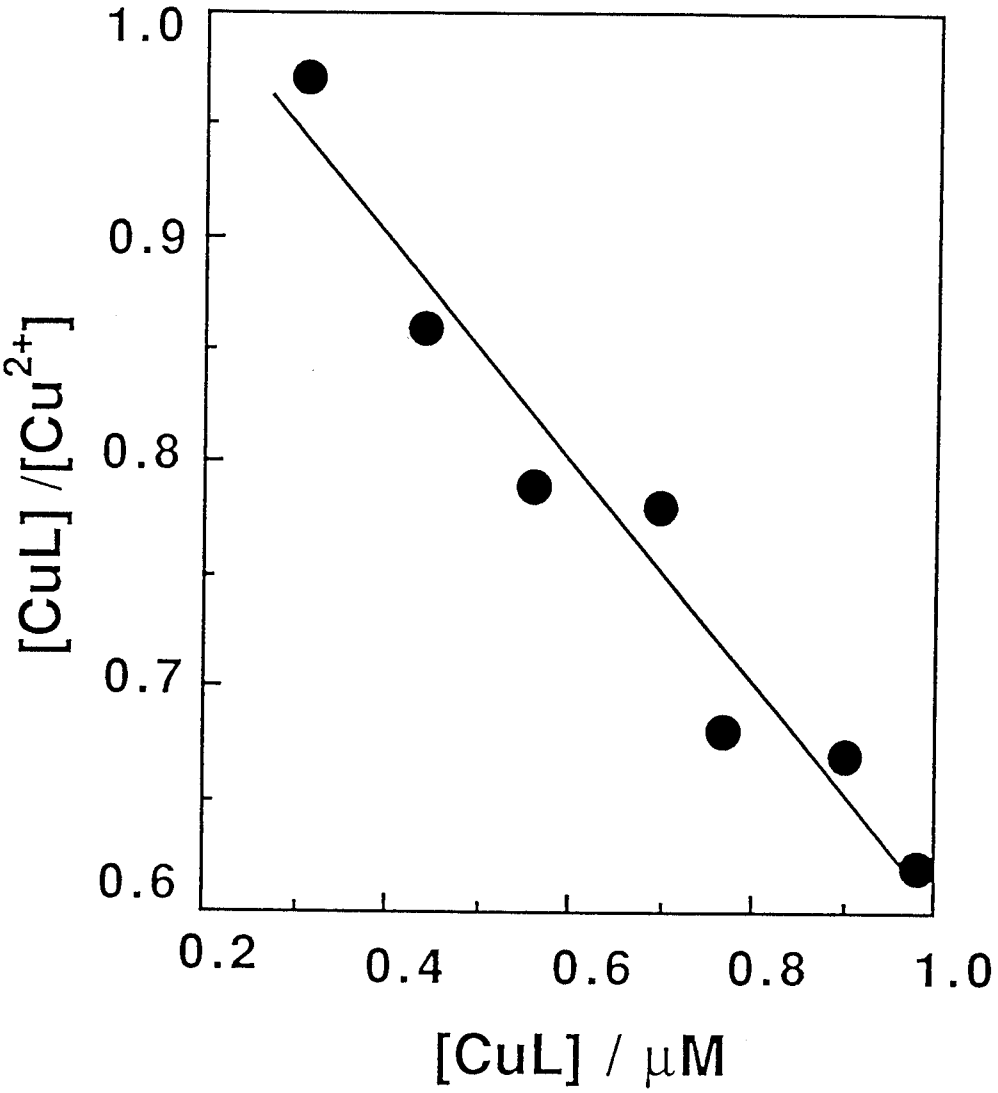


Fig. 3.5 Scatchard plot for citric acid.

Sample solution (pH 7.0); 25 ml, citric acid: 3 μM.

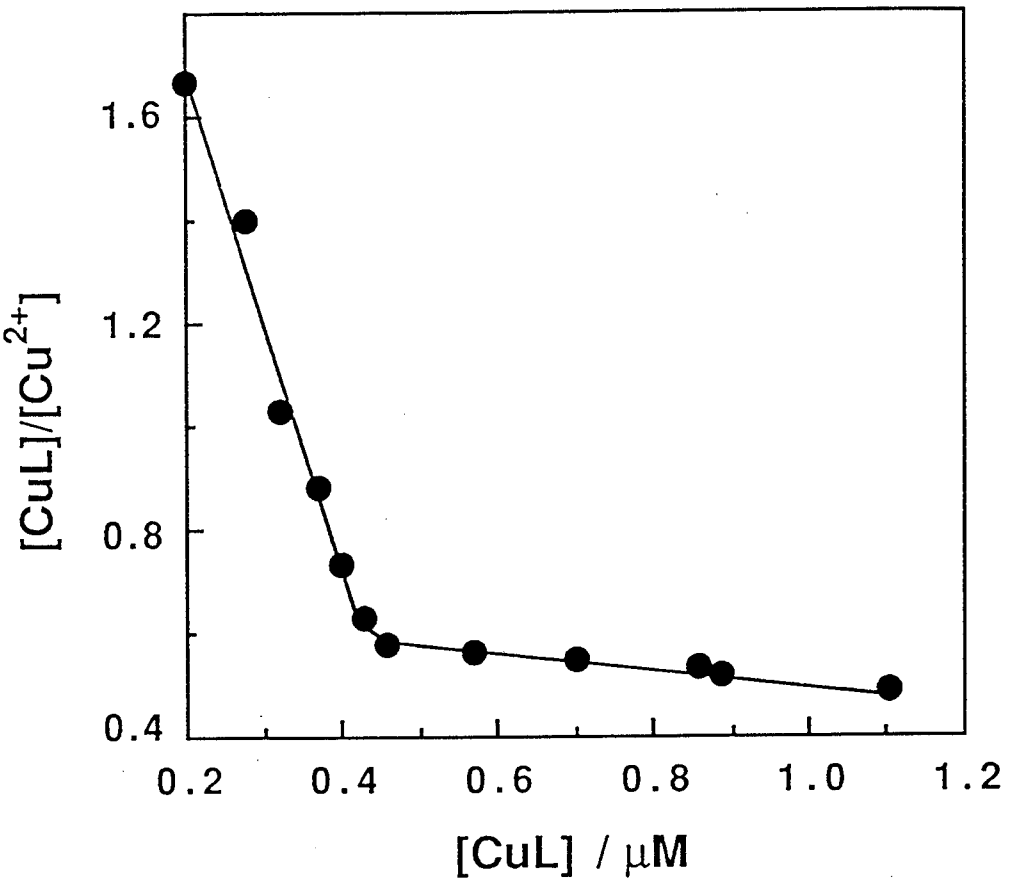


Fig. 3.6 Scatchard plot of humic acid extracted from peat soil (SHHA) and adopting to a 2-site model.

SHHA: 40 mg l^{-1}, pH: 6.

Table 3.1 Conditional stability constants (K'_1, K'_2) and copper(II) complexing capacities (c_{L1}, c_{L2}) for the humic acids at pH 6.

Humic acids	strong site		weak site	
/mg l^{-1}	log K'_1	c_{L1} /μM	log K'_2	c_{L2} /μM
FHA (40 mg l^{-1})	7.6	2.0	5.6	0.8
SHHA (10 mg l^{-1})	6.8	1.5	5.1	1.7

3.4 Evaluation of copper(II) complexing ability using cation exchanger sulfopropyl Sephadex C-25

3.4.1 Experimental Section

Reagents and materials

The humic acid powders were as follows: FHA, SHHA, SAHA and BHA. The method for preparation of the humic acids was described in chapter 2. The stock solutions of humic acids were prepared by dissolving these powders to 0.1 M potassium hydroxide. A copper(II) stock solution was prepared by dissolving electrolytic copper (99.999 % purity, Mitsuwa Pure Chemicals) to nitric acid. A nitrilotriacetic acid (NTA) was used as a model ligand (Wako Pure Chemicals). A strong-acid cation-exchanger sulfopropyl Sephadex C-25 (Pharmacia Biochemistry LKB, Na-form) was used. The other reagents were analytical grade (Wako Pure Chemicals).

Conditioning of C-25

The C-25 was converted in the K-form by the following procedure: the C-25 powder was put into a centrifuge tube and leached with 0.1 M of hydrochloric acid by the application of ultrasonic irradiation. The C-25 was decanted from water several times. Potassium acetate solution (1M) was added and the centrifuge tube was shaken for 5 min. This procedure was repeated 5 times. The C-25 was rinsed with water and stored in water.

Procedure

A 0.4 ml of the C-25 was packed into a polypropylene column which was shown in Fig. 3.7 (40 x 8 mm i.d., Bio Rad). The acetate buffer solution (potassium acetate/acetic acid) of experimental pH was passed through the column and then the water between the C-25 gel particles was excluded by suction. Five milliliters of the solution containing copper(II) ions and humic acids of adjusted pH was pipetted into the column prepared as described above. This column was shaken for 2 min and kept for about 30 min. By measuring the copper(II) concentration of supernatant by an AAS, the concentration of the copper(II)-humic acids complexes were determined.

Apparatus

A HITACHI 170-50 type atomic absorption spectrometer with an air/acetylene flame was used, and the copper was measured at 324.7 nm.

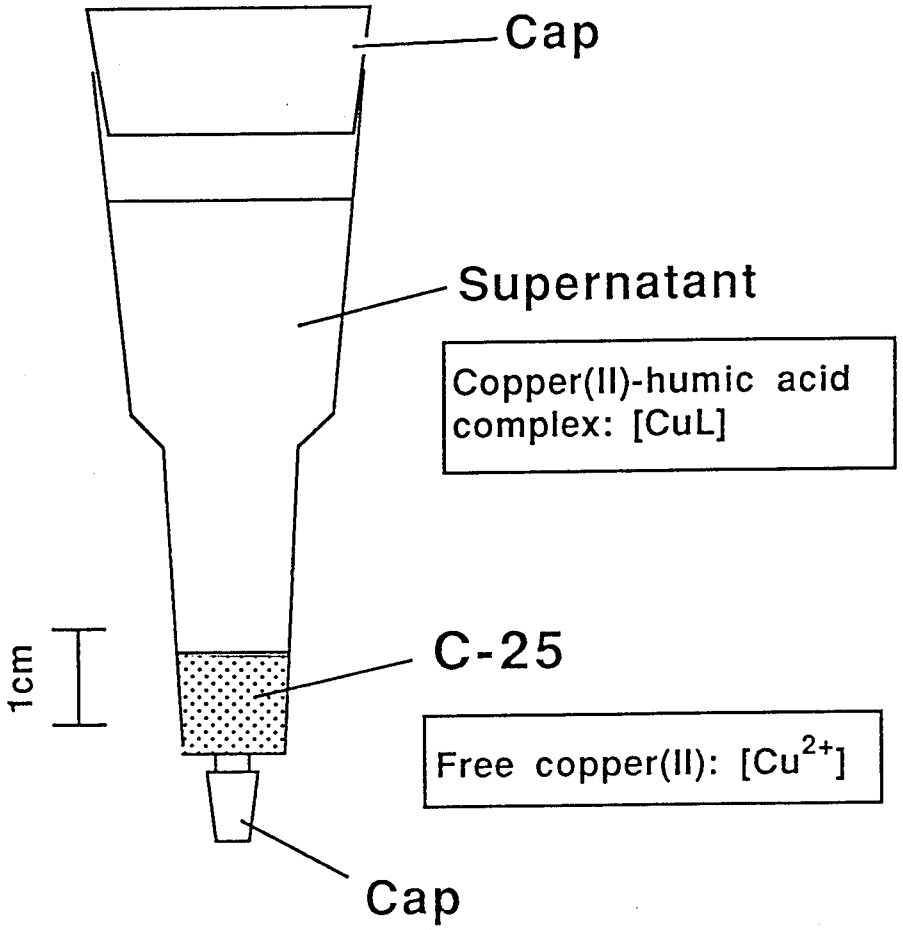


Fig. 3.7 C-25 batch operation system.

3.4.2 Adsorption behavior of copper(II) ion on C-25

In the present procedure, the cationic copper(II) ion was retained on the C-25 and the anionic copper(II)-humic acid complexes remained in the supernatant. When the solution contained only copper(II) ion adjusted to pH: 4.5 ~ 6.5 was shaken with the C-25 for 1 min, the copper(II) in the solution was completely retained on C-25 (Fig.3.8 a). It was found that the cation exchange rate was rapid. The cation exchange equilibria between the copper(II) ions and the potassium ion in the C-25 can be written as follows,

$$Cu^{2+} + 2K^+R^- \rightleftharpoons 2K^+ + CuR_2 \qquad (3.9)$$

where R means the sulfopropyl group in C-25. The cation exchange equilibria constant, K_{CuK} is given by:

$$K_{Cu}{}^{K} = \frac{[K^+]_w^2[Cu^{2+}]_r}{[K^+]_r^2[Cu^{2+}]_w} \qquad (3.10)$$

where $[K^+]_w$, $[Cu^{2+}]_w$ are the concentration of potassium and copper in aqueous phase, and $[K^+]_r$, $[Cu^{2+}]_r$ are the amount of potassium and copper(II) per milliliters of C-25 gel in the solid phase, respectively. The value of the exchange equilibrium constant was estimated to be 8.9 at pH = 4.5 or 6 by measuring $[Cu^{2+}]_w$ and the exchange capacity.

On the other hand, the partition ratio (D_0) in the absence of ligands can be written as,

$$D_0 = \frac{[Cu^{2+}]_r}{[Cu^{2+}]_w} = \frac{[K^+]_w^2}{[K^+]_r^2} K_{Cu}{}^{K} \qquad (3.11)$$

When ligands are present in the solution, the partition ratio, D, represents as follows,

$$[Cu^{2+}]_t = [Cu^{2+}] + [CuL] = [Cu^{2+}]_r/D \qquad (3.12)$$

Eqn. (3.12) can be rearranged,

$$[CuL] = [Cu^{2+}]_r - [Cu^{2+}] = [Cu^{2+}]_r/D - [Cu^{2+}]_r/D_0 \qquad (3.13)$$

Therefore, [CuL] can be determined by measuring the concentration of

copper(II) remained in the supernatant in the presence or absence of ligands. However, in C-25, copper(II) was mostly adsorbed on C-25, and then copper(II) was not remained in the supernatant (Fig. 3.8 a). Therefore, $[Cu^{2+}]_w \approx 0$, and then $D \longrightarrow \infty$. In this C-25 method, Eqn. (3.13) can be appropriated as follows,

$$[CuL] \approx [Cu^{2+}]_f/D \approx [Cu^{2+}]_w \qquad (3.14)$$

Therefore, the concentration of copper(II)-humic acid complex can be determined by measuring copper(II) in the supernatant.

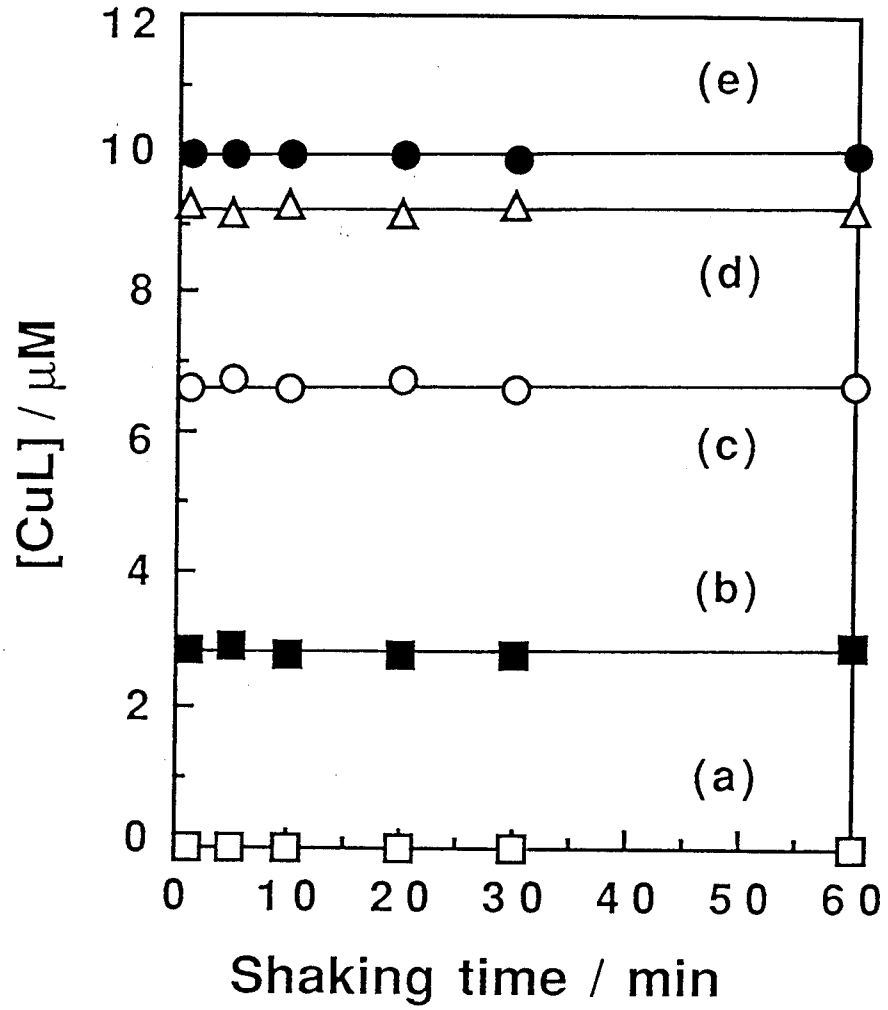


Fig.3.8 Effect of shaking time.

Humic acid (FHA): 35 mg l^{-1}, NTA: 10μM, Cu^{2+}: 16μM, (a)ligands-free; pH=4.5, 6 or 6.5, (b) humic acid; pH=4,5, (c)humic acid; pH=6, (d)NTA; pH=4.5, (e)NTA; pH=6 or 6.5.

3.4.3 Effect of shaking time and amount of C-25 gel

In case of using an ion-exchanger for the separation of the copper(II) species, it is a problem that the complexing equilibrium in the solution shifts during ion-exchange. Hence, it is needed to prove that the shift of the equilibria is negligible during batch operation. Therefore, to confirm this fact, the effect of the shaking time in the batch operation and the amount of C-25 gel was examined. When the solution containing copper(II) ions and humic acid (FHA) or NTA as a model ligand was shaken with C-25 for a shaking time varying from 1 to 60 min, the concentration of the copper(II)-humic acid complexes and the copper(II)-NTA complex in the supernatant were constant in spite of the shaking time as shown in Fig. 3.8 (b) ~ (e).

The effects of the amount of C-25 gel was also examined. For various amounts of C-25 (0.2, 0.4, 0.6, 0.8 and 1.0 ml), the concentrations of copper(II)-humic acid complexes and copper(II)-NTA complex were constant. From these results, it is obvious that the shift of the complexing equilibria was negligible during batch operation for cation exchange.

3.4.4 Determination of the copper(II)-humic acid complex

A free copper(II) ions were retained on the C-25. The copper(II) remained in the supernatant is attributed to the complexed copper(II) species with humic acid or NTA. The concentration of the free copper(II) ions could be estimated by subtracting the copper(II) concentration of the supernatant from the total copper(II) concentration of the original solution. The values were compared with those by a copper(II) ion selective electrode (ISE) method that responded to free copper(II) ions (Table 3.2). In the present method, the relative standard deviations (r.s.d.) of the measurement were less than 2 % (n=5). The values obtained by the C-25 method were in good agreement with the ISE method. However, in case of the lower copper(II) concentration in the sample solution, the free copper(II) ions hardly could be detected by the ISE, since the copper(II) mostly existed as the complex form with humic acid or NTA.

Table 3.2 Concentration of copper(II) species in the presence of humic acid (HA) or NTA.

[L][a]	pH	$[Cu^{2+}]_t$[b] /μM	C-25 method		ISE method
			[CuL][c]/μM	$[Cu^{2+}]$[d]/μM	$[Cu^{2+}]$/μM
NTA					
10 μM	4.5	15.9	8.4	7.5	6.7
	4.5	3.18	2.6	0.5	ND[e]
	4.5	1.59	1.4	0.2	ND
5 μM	6.0	15.9	5.3	11	11
	4.5	15.9	4.2	9.2	8.6
HA					
35.2	6.0	15.9	6.7	9.2	9.6
mg l^{-1}	6.0	2.38	1.9	0.5	ND
	6.0	1.59	1.4	0.2	ND
	5.0	15.9	4.9	11	11
	4.0	15.9	1.9	14	14
11.7	6.0	15.9	4.9	11	11
mg l^{-1}	4.5	15.9	0.9	15	15

a) NTA or HA concentration in the sample solution.

b) Copper(II) concentration in the sample solution.

c) Concentration of copper(II) species with NTA or HA.

d) Concentration of free copper(II) ions.

e) Not detected.

3.4.5 Evaluation of copper(II) binding-ability of NTA

The copper(II) binding-ability of NTA as a model ligand was evaluated. The relationship between the total copper(II) concentration $[Cu^{2+}]_t$ and the concentration of the copper(II)-NTA complex [CuL] was shown in Fig.3.9 a, b. At pH = 6.5, the copper(II) in the solution was mostly coordinated by NTA and the $[Cu^{2+}]_t$ *vs* [CuL] plot was linear with a slope of 1. At pH = 4.5, the complexation between copper(II) and NTA was weaker, and the $[Cu^{2+}]_t$ *vs* [CuL] plot has linear section in the range of $[Cu^{2+}]_t$ = 0 ~ 8 μM. The Scatchard plot of copper(II)-NTA complex was linear in present method (Fig. 3.10), and the copper(II) binding-ability of 10 mM NTA at pH 4.5 was estimated by the intercept and slope of the Scatchard plot. The log*K'* value and the concentration of NTA, c_L, were 5.8±0.4 and 10±1.0 μM, respectively. The log*K'* value obtained was in good agreement with a literature value of 5.87 that calculated with adjustment to the conditions (pH=4.5, 0.01M of acetate) [19]. The c_L value obtained was in good agreement with the original NTA concentration of 10 mM. From these results, it seems that the evaluation method for the copper(II)-binding ability using C-25 could also be used to evaluate the copper(II) binding-ability of the humic acids.

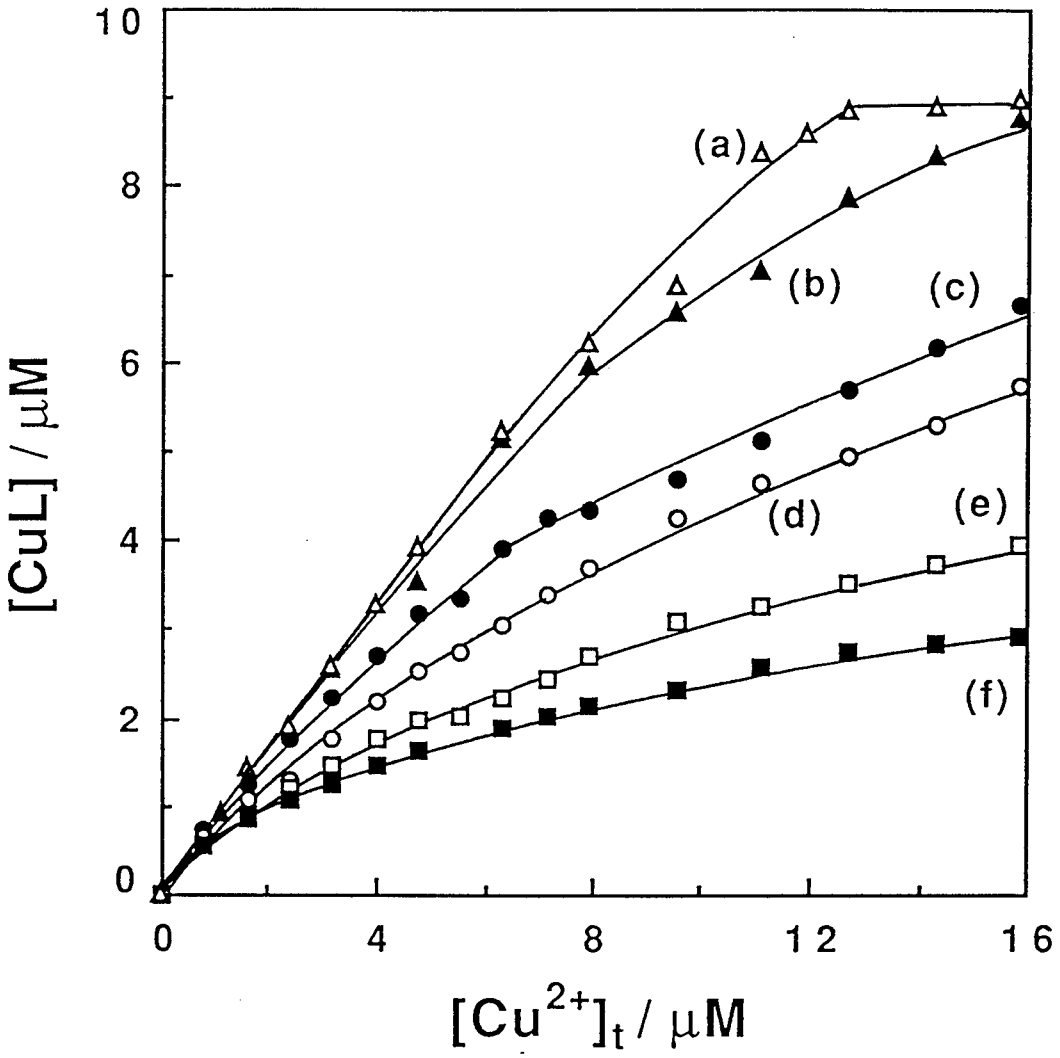


Fig.3.9 $[Cu^{2+}]_t$ *vs* [CuL] plot.

(a)NTA: 10 μM, pH=6, (b)NTA: 10 μM, pH=4.5, (c)FHA: 35 mg l^{-1}, (d)SHHA: 31 mg l^{-1}, (e)SAHA: 30 mg l^{-1}, (f)BHA: 37 mg l^{-1}; (c)-(f): pH=6.

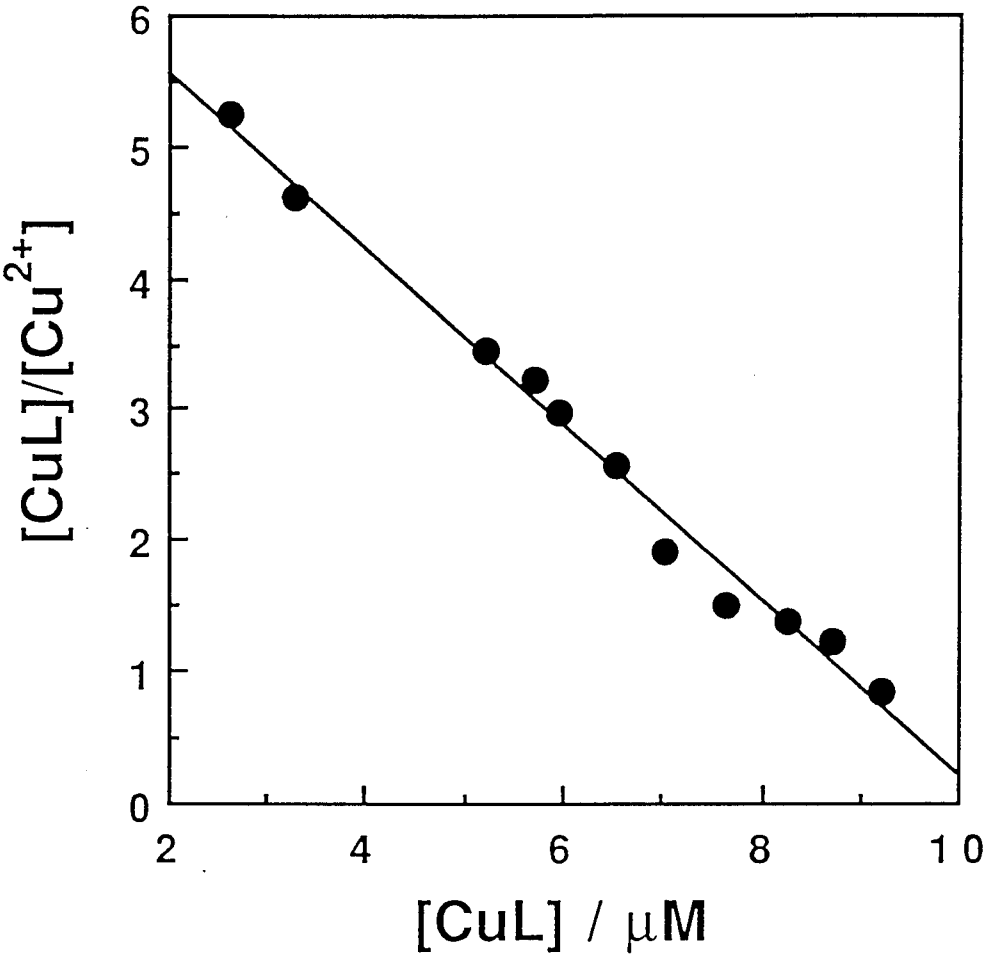


Fig. 3.10 Scatchard plot of NTA.

NTA: 10 μM, pH: 4.5 (0.01M HOAc/KOAc buffer).

3.4.6 Evaluation of the copper(II) binding-abilities of humic acids

The $[Cu^{2+}]_t$ *vs* [CuL] plots of the humic acids at pH=6 were shown in Fig. 3.9 (c) - (f). Since the humic acids have various binding sites to copper(II) ion, the $[Cu^{2+}]_t$ *vs* [CuL] plots did not have linear region. From the point of view of the soil chemistry, humic substances have the carboxylic groups, and the phenolic hydroxyl groups and it is known that the formers are stronger acid than the latters. It was predicted that copper(II) would be bound to these functional groups as a "strong" or a "weak" complex [20]. Moreover, it was proposed that a Scatchard plot adopting to a two-site model for the interpretation of copper(II) complexation by humic acids was a reasonable success [11]. The conditional stability constants and copper(II) complexing capacities, therefore, were calculated from a Scatchard plot adopting to a two-site model. A Scatchard plot of copper(II)-humic acid complex was shown in Fig. 3.11. In case of humic acid, a Scatchard plot could be divided into two linear sections. The conditional stability constants (K'_1, K'_2) and the concentration of each binding-site (c_{L1}, c_{L2}) were calculated by the slopes and intercepts of two lines.

On the other hand, using c_{L1} and c_{L2} that were estimated by a Scatchard plot, the copper(II) complexing capacities, N_1 and N_2 were calculated. The values of N_1 and N_2 were defined as the amounts of the copper(II) that bound to unit gram of humic acids (μmol Cu^{2+} per g humic acid). The logarithm of conditional stability constants ($\log K'_1$, $\log K'_2$) and the copper(II) complexing capacities (N_1, N_2) obtained of the four humic acid samples, were summarized in Table 3.3. The conditional stability constants which were obtained at 0.01 M acetate buffer solution (pH = 6), were in the range of $10^{4.9}$ to $10^{7.0}$. The conditional stability constants reported at pH=6 were in the range of $10^{3.5}$ to $10^{8.8}$ [11, 18, 21-23]. It therefore suggested that the conditional stability constants obtained by the present method would be in

good agreement. The copper(II) binding-abilities of each humic acid sample were different from each other. The order of the total copper(II) complexing capacities, $N_1 + N_2$ were as follows: BHA < SAHA < FHA < SHHA. The humic acid from Shinshinotsu (SHHA) showed the largest copper(II) binding capacity and that from Bibai (BHA) showed the smallest. It seems that such differences of the copper(II) complexing capacities in each humic acid were dependent on the kinds and the amounts of the functional groups in each humic acid.

3.5.Conclusions

The evaluation of the copper(II) binding-abilities (conditional stability constants and copper(II) complexing capacities) of humic acids from peat could be performed by using an anion-exchanger A-25 and a cation-exchanger C-25. The proposed methods would be a sensitive and simple one for the measurement of copper(II) complexing ability of humic acids in natural waters and soils.

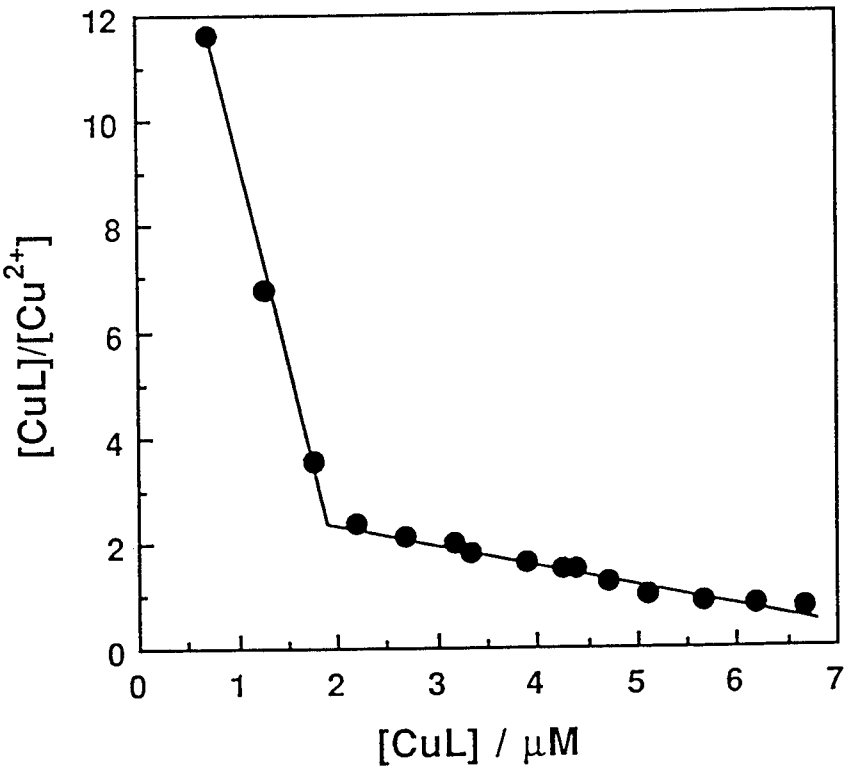


Fig. 3.11 Scatchard plot of FHA.

Table 3.3 Conditional stability constants of copper(II)-humic acid complexes and copper(II) complexing capacities of humic acids at pH 6 and 0.01 M acetate buffer.

humic acids	strong site			weak site			N_1+N_2
	$\log K'_1$	N_1[a]	%[b]	$\log K'_2$	N_2[a]	%	
FHA	6.87	65.2	29	5.50	157	71	222
SHHA	6.85	69.2	30	5.57	163	70	232
SAHA	6.54	59.0	27	5.30	158	73	217
BHA	6.25	59.0	58	4.90	48.1	42	115

a) Cu^{2+} μmol per g of humic acids.

b) $\frac{N_1 \text{ or } N_2}{N_1+N_2}$ X 100 %

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CHAPTER 4

EFFECT OF SODIUM DODECYL SULFATE ON THE MEASUREMENT OF LABILE COPPER(II) SPECIES BY ANODIC STRIPPING VOLTAMMETRY IN THE PRESENCE OF HUMIC ACID

4.1 Introduction

An anodic stripping voltammetry (ASV) has been applied to the speciation analysis of heavy metal ions in the presence of natural organic ligands as humic acid (HA), in which uncomplexed species were measured as "ASV-labile" species [1-4]. This method is sensitive, simple and flexible.

Generally, in the presence of organic matter like HA, it was recognized that high concentration of organic matter brings about adsorption on the electrode and this adsorption lead to broadening anodic waves of labile heavy metal species [5,6]. Therefore, the adsorption of organic matter would interfere the determination of labile heavy metal species [7]. Moreover, dissociation of the metal complex in the diffusion layer occurs during deposition time [8]. This leads to positive error for the labile metal species.

Recently, in order to eliminate such interferences, glassy carbon electrodes with permselective membranes and polymer coatings have been developed, for example, using a dialysis membrane [9,10], a cellulose acetate membrane [11], and coating with Nafion [12]. These membranes and coatings could eliminate the adsorption of organic matter and prevent the dissociation of complexed species in the diffusion layer during the deposition time. Especially, a Nafion coated thin mercury film electrode [13] has been conveniently used for the measurement of labile species of heavy metal ions in the presence of natural organic ligands such as HA [14-16].

On the other hand, in spite of their organic characters, surfactants have been shown to accelerate the electrode reaction of analyte [17,18]. If the

surfactant can be more strongly adsorbed on the electrode surface than HA and the surfactant does not interfere with the electron-transfer reaction of the heavy metal ions, a reduction of the broadening of the anodic peak can be expected. The addition of surfactant is a simple method and a new approach for the measurement of labile heavy metal species in the presence of organic matter as HA by ASV. Also, it has been reported that the addition of sodium dodecylsulfate (SDS) can improve clarify the end point in the copper(II) titration of EDTA or nitrilotriacetic acid (NTA) by ASV with a hanging mercury drop electrode [19].

In this chapter, effects of SDS on the anodic waves of labile copper(II) species in the presence of HA are investigated by ASV with a thin mercury film glassy carbon electrode (TMFGCE). Moreover, this method is applied to the evaluation of the copper(II) complexing abilities (conditional stability constant and copper(II) complexing capacity) of HA.

4.2 Experimental Section

Reagents and materials

The humic acid (SHHA) was prepared according to chapter 2. A stock solution of 0.1M copper(II) was prepared by dissolving electrolytic copper (99.999% purity, Mitsuwa Pure Chemicals Co.) in nitric acid. Surfactants (analytical reagent grade, Wako Pure Chemicals Co.) were as follows: cationic type; dodecyltrimethylammonium chloride (DTAC), anionic type; sodium dodecylsulfate and sodium dodecylbenzenesulfonate (SDBS), nonionic type; poly(vinyl alcohol) (PVA) and Triton X-100. NTA (Wako Pure Chemicals Co.) was used as a model ligand. The water used was deionized twice.

Apparatus

A rotating ring-disk electrode system (RRDE-1, Nikko Keisoku) and a potentiostat (HA-501, Hokuto Denko) with a function generator (HB-105, Hokuto Denko) were used. A three electrode system was used: the working electrode was a glassy carbon rotating disk electrode with plating mercury film; the reference electrode was a saturated calomel electrode (SCE); the counter electrode was a platinum wire. The voltammograms were recorded with an X-Y plotter (RY-101, Rika Denki). A polarographic analyzer (P-1100, Yanagimoto Co.) with a dropping mercury electrode (DME) was used to measure the electrocapillary curve. All the measurements were carried out at 25±0.3°C.

Preparation of thin mercury film glassy carbon electrode

A glassy carbon disk electrode was polished by alumina slurry (particle size 1 μm) on a polishing cloth and rinsed with ethanol, 0.1M nitric acid, 0.1 M hydrochloric acid and then distilled water. Potential cycling between +0.2 V and -2.0 V vs. SCE at 0.5 Vs^{-1} was performed in 0.02 M hydrochloric acid. The TMFGCE was prepared by plating a mercury film at 750 rpm in a 2mM $Hg(NO_3)_2$ solution containing 0.02M nitric acid at -0.5V. The surface area and the thickness of mercury film of the electrode were 0.2 cm^2 and 0.4 μm, respectively.

Procedure

Into a glass cell, 50 ml of supporting electrolyte (0.1M KNO_3 containing 0.01M HOAc-KOAc buffer of pH 6) with 2 mg HA was pipetted and deaerated by nitrogen for 15 min. Twenty-five microliters of 1mM copper(II) standard solution was added to the supporting electrolyte by a micro-pipette and then the solution was stirred by rotating the TMFGCE (750 rpm) for 3 min. The conditioning potential (+0.05V) was applied for 30 s, and then the deposition potential at -0.5V was applied for 150 s with rotating the TMFGCE at 750 rpm. After the rest time (30 s at deposition potential, the rotation of electrode was stopped), the potential was linearly scanned at 75 mV s^{-1} to +0.2V and the anodic wave of copper(II) was recorded.

Electrocapillary curve

In electrocapillary curve, the interfacial tension, γ, was calculated according to the following equation,

$$\gamma = \frac{(\rho_{Hg} - \rho_{soln})m\tau}{2\pi r\rho_{Hg}} \qquad (4.1)$$

where m is the flow rate (mg s^{-1}) of Hg from the capillary, τ, ρ_{Hg} and ρ_{soln} are the drop time of mercury drop (s), densities of Hg and the solution, respectively. The radius, r, of the capillary is 0.052 mm. The mercury column height was 70 cm.

4.3 Stripping voltammograms

The effect of various surfactants on anodic waves of copper(II) by a cyclic voltammetry was investigated in the absence and presence of HA. From Table 4.1, it appears that the addition of SDS was useful, because (i) no interference with the electrode reaction of copper(II), (ii) no complexation with copper(II) and (iii) no interaction with HA is observed.

The stripping voltammograms of labile copper(II) species in the presence of HA are shown in Fig. 4.1. A broad anodic wave was obtained in the absence of SDS (Fig. 4.1 c). This was due to the adsorption of HA on the electrode and then the electrode reaction of copper(II) was interfered by the adsorption of HA. However, the sharp-shaped anodic wave was obtained in the presence of SDS (Fig. 4.1 d) together with an increase in peak current of about 20%. Therefore, adding SDS seems to be useful to eliminate the effect of HA for the measurement of labile copper(II) by ASV.

The anodic peak current for ASV with rotating disk electrode, i_p, is represented as follows [21],

$$i_p = k\omega^{1/2}tvC_B \qquad (4.2)$$

where k, ω, t, v and C_B are constant, rotating speed, deposition time, scan rate and bulk concentration of analyte, respectively. When the peak currents were measured in the absence or presence of HA, the anodic peak current was proportional to scan rate (v: 10 ~ 100 mV s^{-1}) and square root of rotating speed (ω: 200 ~ 2000 rpm), as is expected from Eqn. (4.2). Therefore, the peak current is based on a diffusion controlled process. In the present work, a rotation speed of 750 rpm was chosen because of the stability of the mercury film. Also, in the absence of HA, the peak current was proportional to concentration of copper(II) (0.1 ~ 10 μM) even if SDS was added. The concentration of labile copper(II) species was estimated by using this calibration curve.

Table 4.1 Effect of various surfactants on anodic peak of 0.1 mM copper(II).

Surfactants	i_p[a)]/μA	Anodic wave with HA[b)]	Interaction with HA[c)]	Complexation with Cu^{2+}
none	14	broad	____	____
Anionic surfactants				
SDS	14	sharp	no	no
SDBS	12	broad	no	no
Cationic surfactants				
DTAC	10	broad	yes	no
Non-ionic surfactants				
Triton X-100	9.3	broad	no	yes[16]
PVA	13	broad	no	yes[20]

a) anodic peak currents in the absence of HA; surfactants: 0.01%.

b) HA: 0.2g l^{-1}, surfactants: 0.01%.

c) clarified by comparing the absorbance of HA (at 400nm) in the presence of surfactants with that in the absence of surfactants; HA: 40 or 200 mg l^{-1}, surfactants: 0.01%.

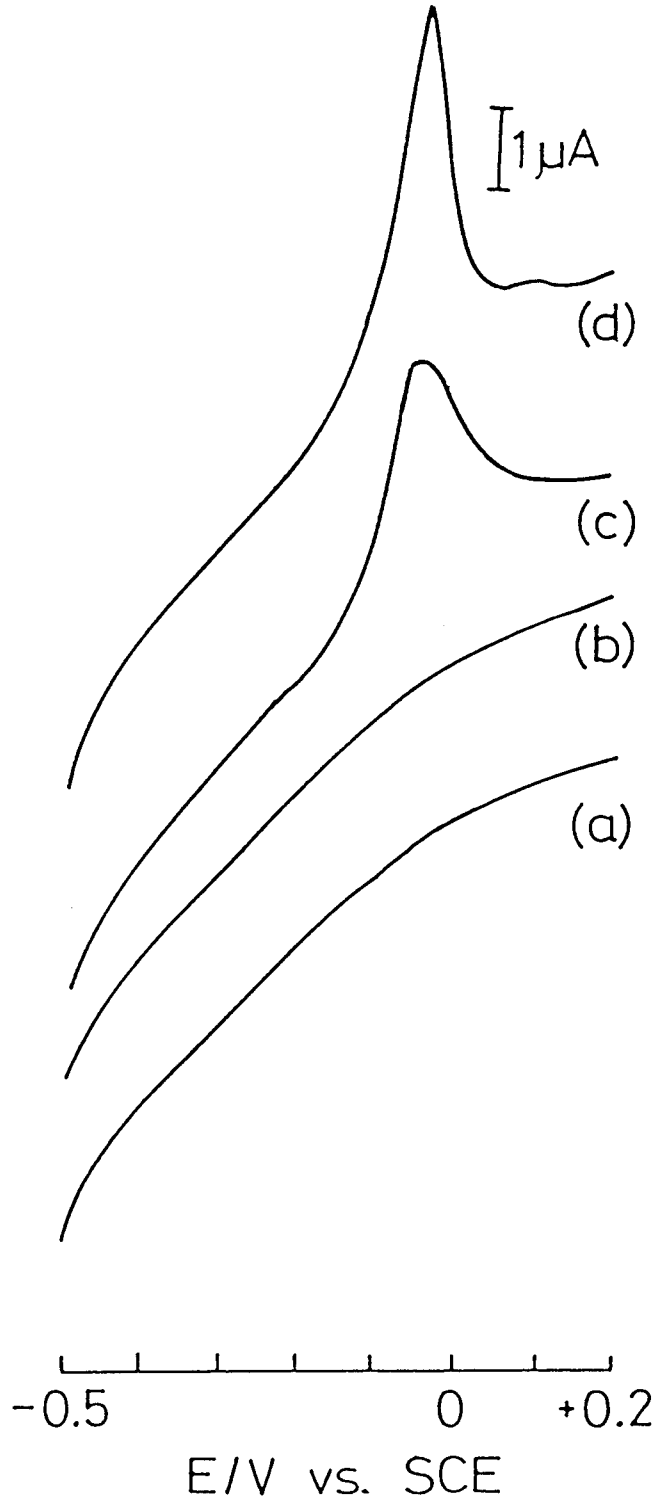

Fig. 4.1 Stripping voltammograms.

Cu^{2+}: 5μM, HA: 40mg l^{-1}, SDS: $2x10^{-3}$%, deposition time: 150 s, 75 mV s^{-1}, supporting electrolyte: 0.1M KNO_3 + 0.01M HOAc/KOAc buffer (pH 6), (a); supporting electrolyte + HA, (b); (a) + SDS, (c); (a) + Cu^{2+}, (d); (c) + SDS.

4.4 Pseudopolarograms of copper(II)

The pseudopolarograms of copper(II) in the absence of HA (Fig. 4.2 a, b) and those in the presence of HA (Fig. 4.2 c, d) are shown. From the limiting current, it can be estimated that about 20 ~ 30% of copper(II) exists as ASV-labile species in the presence of HA.

In the absence of HA (Fig. 4.2 a, b), the half-wave potential without SDS is in good agreement with that with SDS. From this result, it can be concluded that the adsorption of SDS on the TMFGCE does not interfere with the electron-transfer reaction of copper(II). However, in the presence of HA, the half-wave potential without SDS was 31 mV more negative than that with SDS. This suggests that the electron-transfer reaction of copper(II) was interfered by the adsorption of HA on the TMFGCE.

Moreover, in the presence of HA, the limiting current with SDS (Fig. 4.2 d) was higher than that without SDS (Fig. 4.2 c). The decrease in the limiting current without SDS would cause that amount of the copper(II) plating on the TMFGCE decreased during deposition time because of the adsorption of HA on the TMFGCE. These observations suggest that the adsorption of HA on the electrode could be eliminated by the addition of SDS.

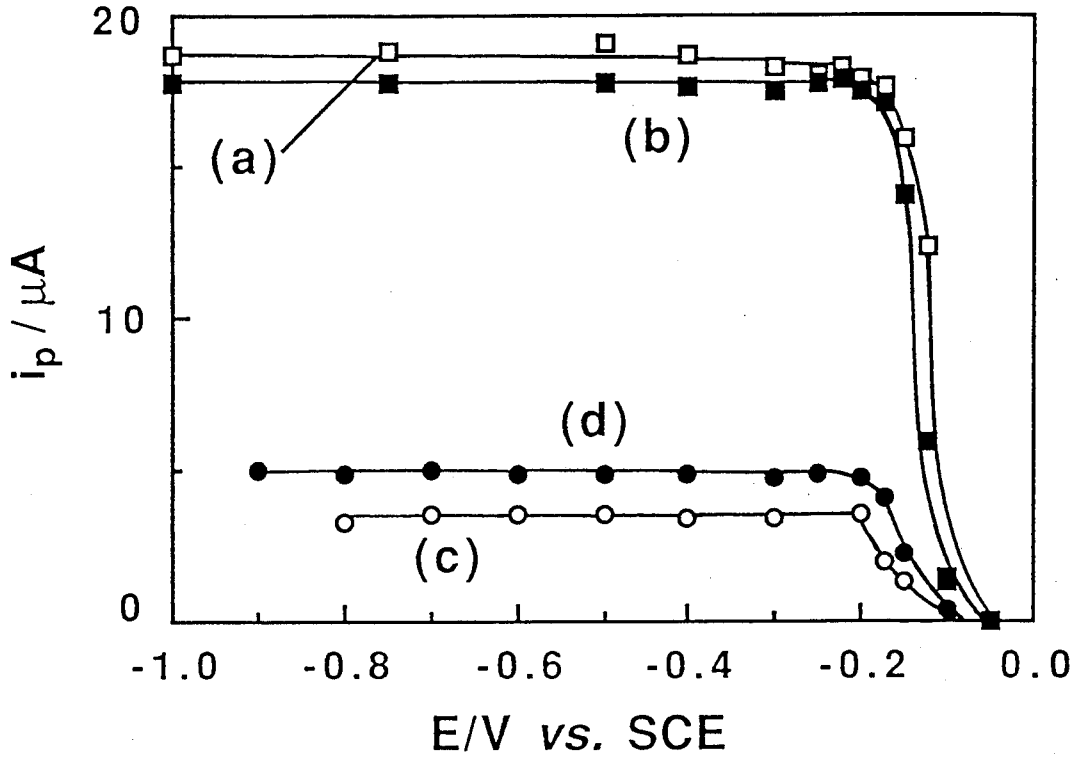

Fig. 4.2 Pseudopolarograms of copper(II).

HA: 40mg l^{-1}, SDS: $1x10^{-3}$%, deposition time: 150s, (a); Cu^{2+} 5μM, (b); (a)+SDS, (c); (a)+HA, (d); (b)+HA.

4.5 Effect of deposition time

The relationship between deposition time and peak current was investigated in the presence of HA, as shown in Fig.4.3. A non-linear relationship was obtained in the absence of SDS, and contrary to Eqn. (4.2). This may be due to the dissociation of copper(II)-HA complex in the diffusion layer during the deposition time. However, a linear relationship was obtained in the presence of SDS (Fig. 4.3 b). Therefore, addition of SDS seems to eliminate the interference of HA.

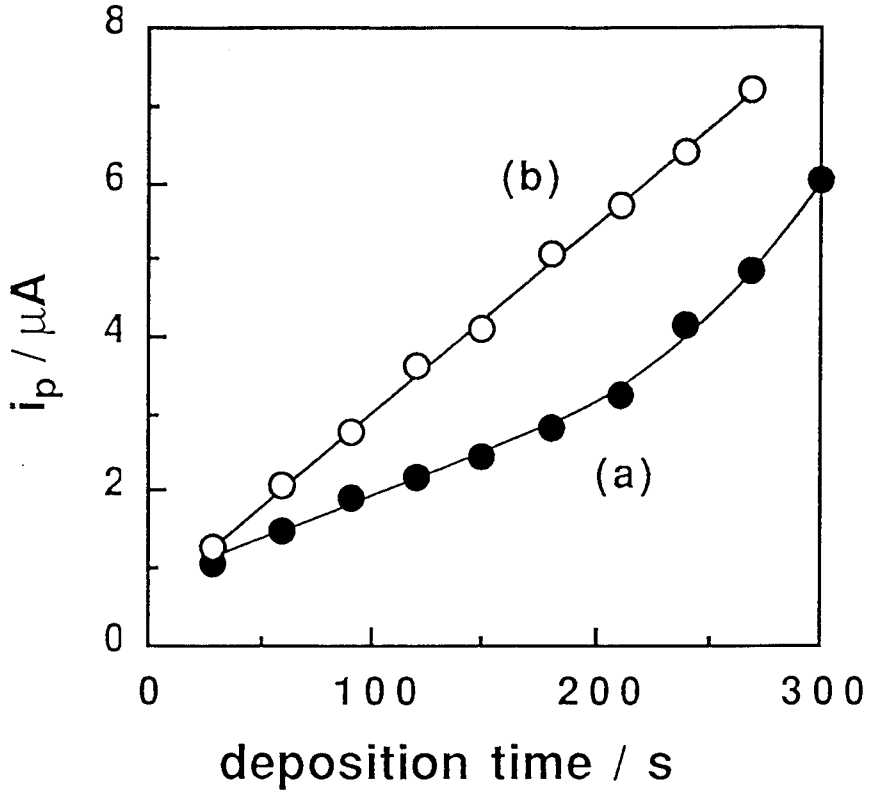

Fig. 4.3 Effect of deposition time.

Cu^{2+}: 5μM, HA: 40mg l^{-1}, pH 6, SDS: $1x10^{-3}$%, deposition potential: -0.5V, (a) without SDS, (b) with SDS.

4.6 Electrocapillary curves

As already pointed out, the problems were (i) adsorption of HA on the electrode, and (ii) dissociation of copper(II)-HA complex in the diffusion layer during deposition time. The addition of SDS seems to eliminate these interferences. To verify the effect of SDS, electrocapillary curves were investigated with a dropping mercury electrode (Fig. 4.4). The electrocapillary maximum (ecm) in the presence of SDS (Fig. 4.4 c) was almost the same as that in the presence of HA and SDS (Fig. 4.4 d) showing that the SDS could adsorb more strongly on the electrode rather than HA. Therefore, the adsorption of HA on the mercury electrode could be eliminated by adding SDS.

Furthermore, the derivative of the electrocapillary curve, $\partial\gamma/\partial E$, which is equivalent to the electrode surface charge density, q, as mentioned in a Lippmann's law: $\partial\gamma/\partial E = -q$. In the positive potential region from with respect to ecm, $\partial\gamma/\partial E$ is usually negative. So, the q is positive, and then anionic species in the solution are apparently gathered to the diffusion layer. That is, the higher $\partial\gamma/\partial E$, the more amount of anionic species are present in the diffusion layer. The $\partial\gamma/\partial E$ in the presence of SDS (Fig. 4.4 c,d) was lower than that in the absence of SDS (Fig. 4.4 a,b) beyond the ecm. This suggests that the amounts of anionic species such as HA in the diffusion layer decreased by the addition of SDS. So, it was found that SDS adsorbed on the electrode has an anion-exclusion effect. This effect of SDS would be useful to prevent the adsorption of anionic species such as copper(II)-HA complex or HA on the electrode.

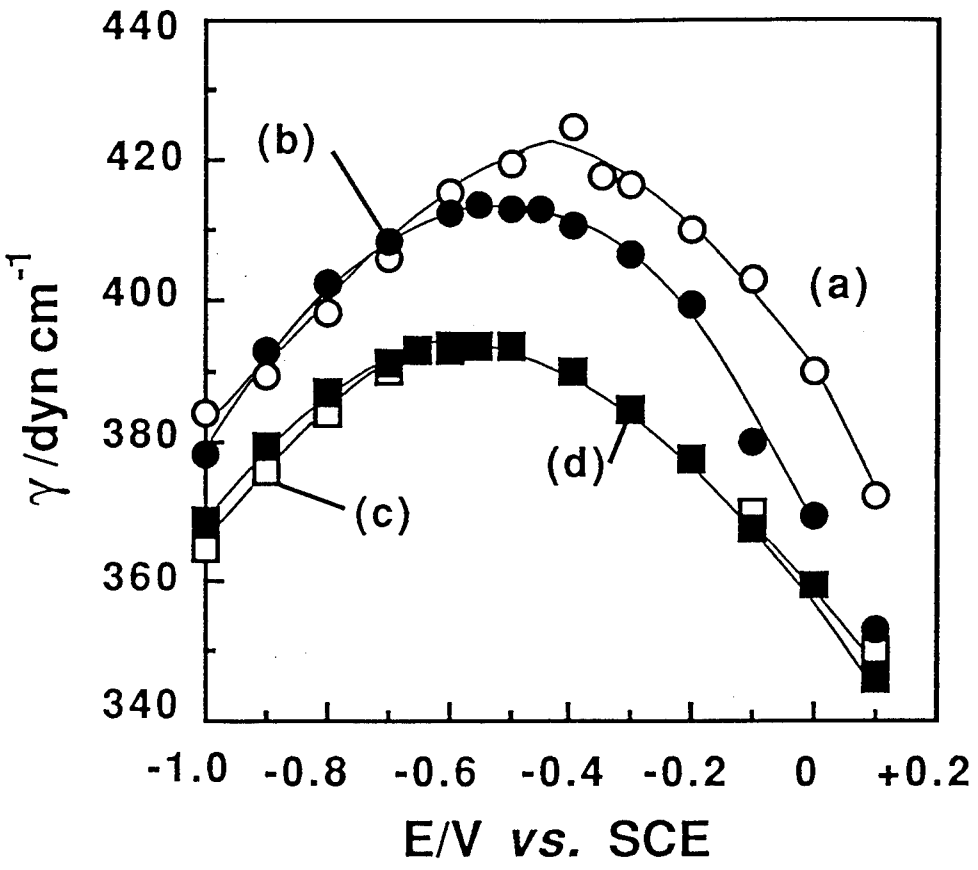

Fig.4.4 Electrocapillary curves.

HA: 40mg l^{-1}, SDS: $1x10^{-3}$%, supporting electrolyte: 0.1M KNO_3 + 0.01M HOAc/KOAc buffer (pH 6), (a): supporting electrolyte only, (b): (a)+HA, (c): (a)+SDS, (d): (c)+HA.

4.7 Effect of SDS concentration

Effect of the concentration of SDS on the peak currents was investigated in the range of 0 to $2x10^{-2}$% (Fig. 4.5). The anodic peak currents remained constant in the range of $5x10^{-4}$ to $2x10^{-2}$% (Fig. 4.5 a). In Fig. 4.5 b, the peak currents increase with increasing SDS concentration. This result suggests that the amounts of HA adsorbed on the electrode decrease with increasing SDS.

On the other hand, the effect of SDS was also studied by use of NTA as a model ligand. In Fig. 4.5 c, it is shown that the peak currents decrease with increasing SDS concentration. This can be attributed to the dissociation of copper(II)-NTA complex in the diffusion layer at the lower SDS concentration [23]. Moreover, from pseudopolarograms in the case of copper(II)-NTA complex, the half-wave potential with SDS shifted about 0.5V to more negative region than without SDS. Therefore, it found that SDS adsorbed on the electrode could prevents the direct reduction of the copper(II)-NTA complex.

Furthermore, the measurements of labile copper(II) species were made done with 10^{-3}% SDS in the presence of HA and with $5x10^{-3}$% SDS in the presence of NTA (Fig. 4.5 b and c).

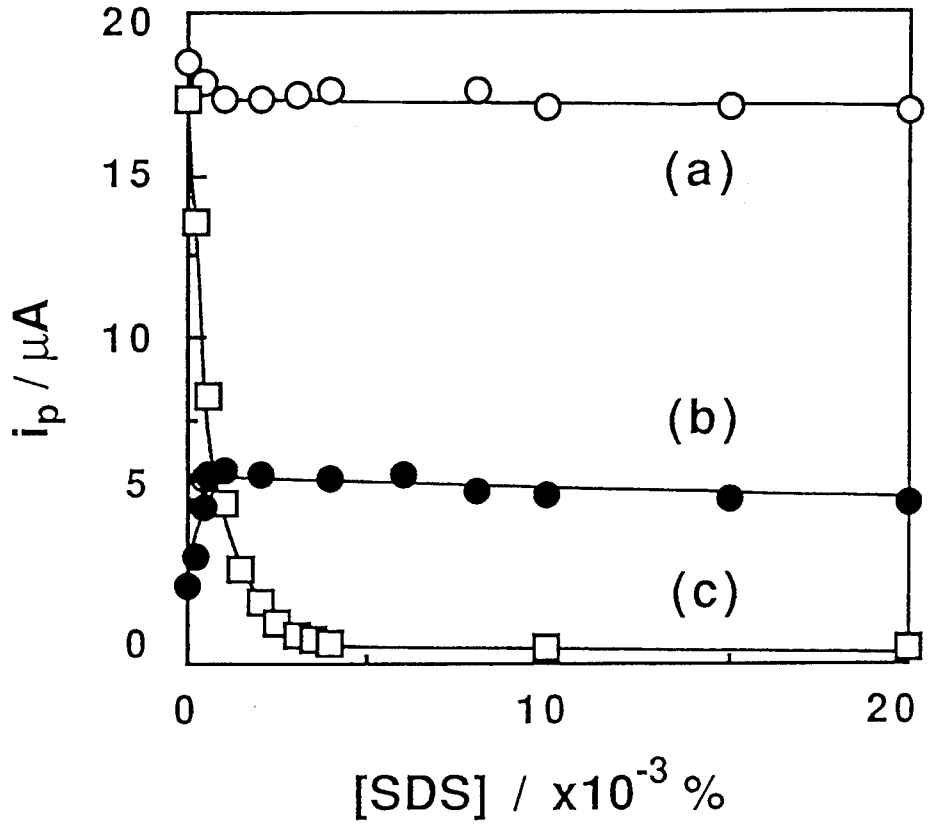

Fig.4.5 Effect of SDS concentration.

Deposition time: 150s, deposition potential: -0.5V, (a): Cu^{2+} 5μM, (b): (a) + HA 40 mg l^{-1}, (c): (a) + NTA 5μM.

4.8 Copper(II) titration curves of NTA

The copper(II) titration curves of NTA are shown in Fig.4.6. The titration curve in Fig. 4.6 (a) is linear and does not show an end point. In this case, most of copper(II) was ASV-labile because of the dissociation of the copper(II)-NTA complex in the electrode diffusion layer. However, in the presence of SDS, the titration curve shows a clear end point (Fig. 4.6 b). Moreover, the concentration of NTA added, 10μM, was in good agreement with the concentration evaluated by the titration curve, 9.7±0.4 μM (n=3). Therefore, the addition of SDS is useful for copper(II) titration of NTA.

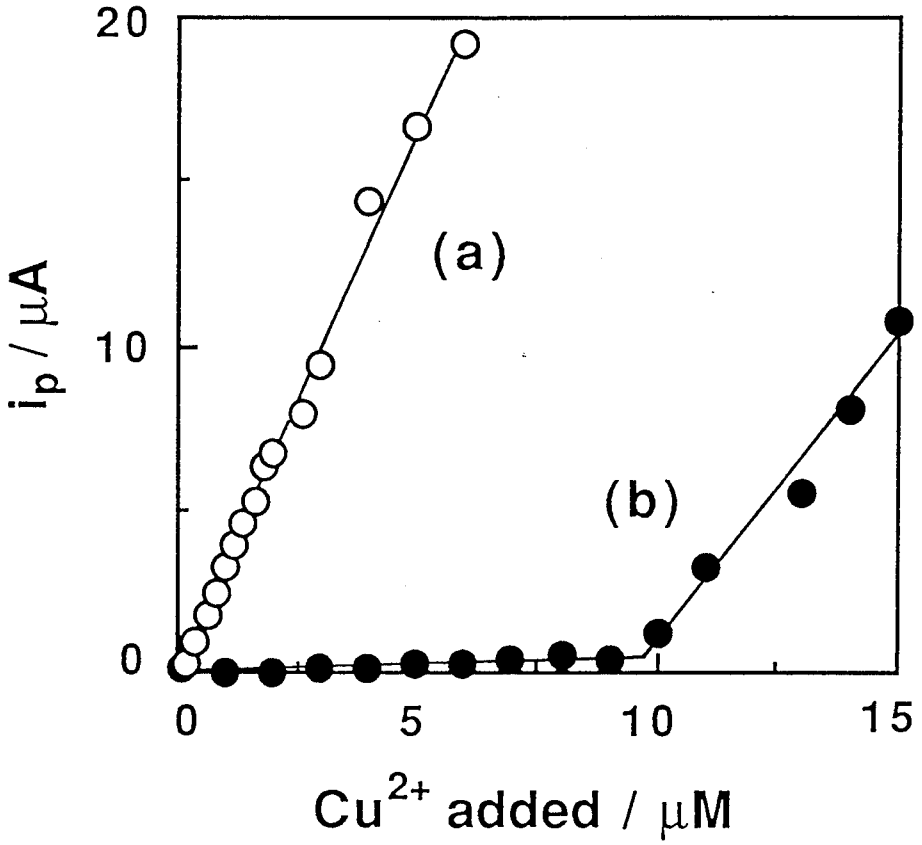

Fig.4.6 Copper(II) titration curves of NTA.

NTA: 10μM, pH 6, deposition time: 150s, deposition potential: -0.5V,

(a) without SDS, (b) with $5x10^{-3}$% SDS.

4.9 Evaluation of copper(II) complexing abilities of HA

The copper(II) complexing abilities of HA were evaluated by copper(II) titration method. The conditional stability constants of copper(II)-HA complex and the copper(II) complexing capacities were evaluated by a Scatchard approach as described in chapter 3 [22,23]. The relationship between conditional stability constant, K', and copper(II) complexing capacity, N, can be written as follows,

$$\frac{[Cu^{2+}]_t - [Cu^{2+}]_{labile}}{[Cu^{2+}]_{labile}} = -K'([Cu^{2+}]_t - [Cu^{2+}]_{labile}) + K'N[\text{HA added (g l}^{-1})] \qquad (4.3)$$

where $[Cu^{2+}]_t$ and $[Cu^{2+}]_{labile}$ represent total and labile copper(II) concentration, respectively. From the linear relationship between $([Cu^{2+}]_t - [Cu^{2+}]_{labile})/[Cu^{2+}]_{labile}$ and $[Cu^{2+}]_t - [Cu^{2+}]_{labile}$, K' and N can be evaluated. A Scatchard plot of HA is shown in Fig. 4.7. The Scatchard plot could be divided into two linear sections. Therefore, a two site model was adopted, and then the conditional stability constants (K'_1, K'_2) and the complexing capacities (N_1, N_2) with respect to each site were calculated by Eqn. 4.3 (Table 4.2). In the case without SDS, the logK' values were higher than those with SDS and the total complexing capacity, N_1+N_2, was about 16% higher than that with SDS. Therefore, the values in the absence of SDS are overestimated.

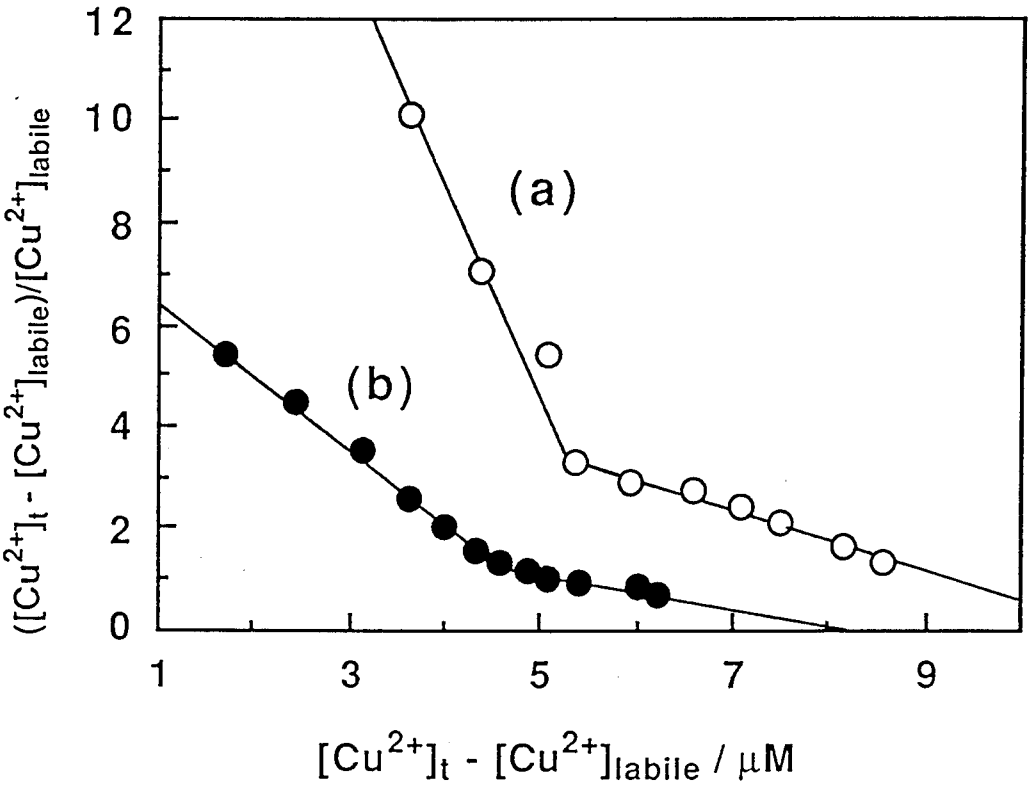

Fig.4.7 Scatchard plot of HA.

HA: 40 mg l^{-1}, pH 6, (a) without SDS, (b) with $1x10^{-3}$% SDS.

Table 4.2. Copper(II) complexing abilities at pH 6.

parameter	without SDS	with SDS
log K'_1	6.6	6.1
log K'_2	5.8	5.4
N_1 [a)]	160±8	135±5
N_2	118±6	85±3
N_1+N_2	278±14	220±8

a): Cu^{2+} µmol per g of HA.

4.10 Conclusions

Adding SDS could eliminate the adsorption of HA on the electrode. Therefore, the addition of SDS is a useful method for measuring the labile copper(II) species and subsequently for evaluating the copper(II) complexing ability of HA by ASV with mercury electrode. Moreover, the amount of HA (2 mg) consumed in the copper(II) titration when using ASV detection was much lower than those used in the other methods (about 20 mg) [22,23]. Also, the copper(II) titration by ASV will be useful for the evaluation of copper(II) complexing abilities of small amounts of HAs.

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CHAPTER 5

RELATIONSHIP BETWEEN COPPER(II) BINDING-SITE AND FUNCTIONAL GROUPS

5.1 Introduction

In the chapter 3, copper(II) complexing abilities of various humic acids from peat were evaluated by using a cation-exchanger [1,2]. However, clarifying the relationship between the copper(II)-complexing sites and the functional groups in humic acids may be important, since humic acids have various functional groups in their structure with different acidities [3].

On the other hand, the stability of copper(II)-humic acid complexes would need to be considered if the pH of aqueous solution would be lowered by acidification [4]. These would be comparable to the acidification of natural waters by acid rain. Increasing acidity of solution may cause the dissociation of the copper(II)-humic acid complexes to release toxic copper(II) ions. Therefore, the stability of copper(II)-humic acid complex should be investigated.

In this chapter, the dissociation behaviors of copper(II)-humic acid complexes were investigated by the pH or the conductimetric titration method. Moreover, the comparison of model ligands was performed in the view point of the acid dissociation of copper(II) complex. The relationship between the copper(II)-binding site and the functional groups in humic acid was discussed by the data from FTIR spectra and from the complexing abilities evaluated according to chapter 3.

5.2 Experimental Section

Humic acids used in this chapter were the same as those in the chapter 3, and then prepared according to the chapter 2. Thirty-ml of aliquot sample solution (dissolving humic acid by potassium hydroxide, [humic acid]: 50 mg l^{-1}) was pipetted into a titration cell. Subsequently, a 50 ml of the standard hydrochloric acid (0.05M) was dropped into the cell by an Eppendorf micro pipette, and then the pH and conductivity were simultaneously measured. In the present work, a conductimeter (CM-5b type, Toa Electronics Ltd. Co.) or a conductimetric cell (CG-201 pL, Toa Electronics Ltd. Co.) was used. A pH meter (M-8L type, Horiba Ltd. Co.) and a glass electrode (6328 type, Horiba Ltd. Co.) was used. A FTIR (Perkin Elmer, 1720-X type) was used for measuring the infrared spectra using a KBr disk method.

5.3 Comparison of amounts of the functional groups

The comparison of the copper(II) complexing capacities with the amounts of the functional groups in humic acids were investigated by conductimetric and pH titration. In this case, the humic acids were dissolved in the presence of an excess potassium hydroxide solution, and this solution was titrated by hydrochloric acid [5,6]. Conductimetric and pH measurements were simultaneously performed in the same solution. Both titration curves were shown in Fig. 5.1 (a) and (b). The conductimetric titration curve can be subdivided into three sections indicating that the humic acids probably have three kinds of the functional groups (Fig. 5.1 a, i ~ iii). The amounts of functional groups of each humic acid are summarized in Table 5.1 as well as the values of pK_as of the functional groups in each humic acid, which were calculated from both titration curves. The mean values of pK_as for each functional groups in four humic acids were 9.8: (i), 8.7: (ii), 6.1: (iii). It therefore suggests that the weaker functional groups of "i" or "ii" correspond to amino groups or phenolic hydroxyl groups, and the stronger functional groups of "iii" correspond to carboxylic groups. It probably seems that the former concerns with weak complexation with copper(II) and the latter concerns with strong complexation. The sequence of the total amounts of functional groups is the same as for the total copper(II) complexing capacities as shown in Table 5.1; BHA < SAHA < FHA < SAHA. This suggests that the copper(II) complexing capacities of the humic acids are related to the amount of each of the functional groups in the humic acids.

On the other hand, the percentages of copper(II) complexing capacities (N_1, N_2) to total N_1+N_2 and those of amounts for each functional group (i, ii and iii) to total amounts of functional groups were also shown in Fig. 5.2, respectively. It was obvious that, for BHA, (ii)+(iii) corresponded to N_1 and (i) corresponded to N_2. And then, it noted that, for BHA, the conditional stability

constants of copper(II) complexes with both strong and weak binding-sites were the lower values of all. It therefore suggested that the functional groups (ii) and (iii) related to the strong binding-site for copper(II), and (i) related to the weak binding-site in BHA. The functional groups of FHA, SHHA and SAHA, however, did not correspond to the copper(II) complexing capacities such as BHA. In these humic acids, the weaker binding-site dominated and the conditional stability constants both strong and weak binding-sites were relatively higher than that of BHA. It seems that the functional groups of the lower pK_as, (iii) contributed to the copper(II) complexation of FHA, SHHA and SAHA as the strong sites, and those of higher pK_as, (i) and (ii) contribute to the complexation of copper(II) as the weak binding-sites.

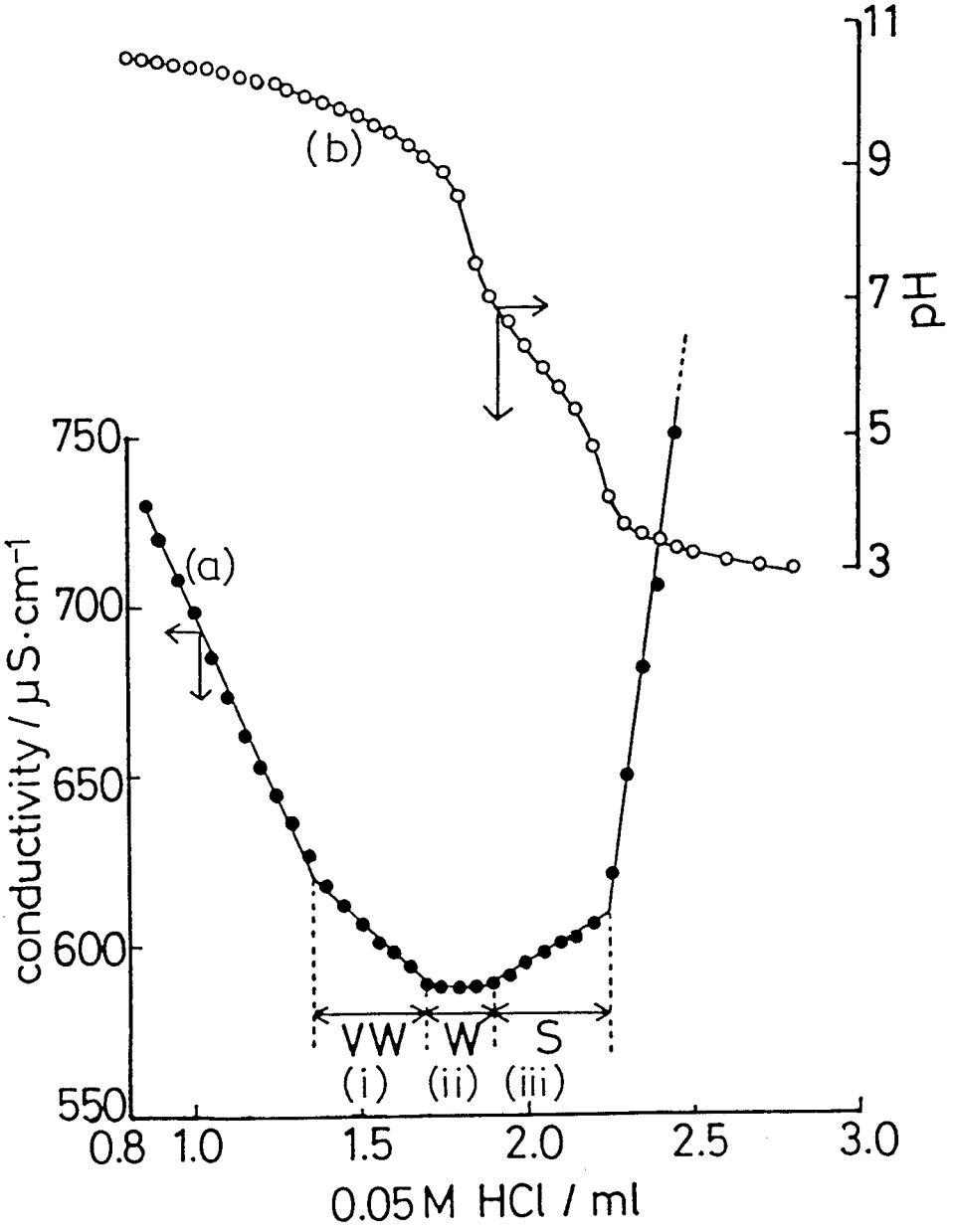

Fig. 5.1 Titration curves for humic acid.

(a) Conductimetric titration; (b) pH titration, Humic acid (BHA), 50 mg l^{-1}; sample volume, 30 ml.

Table 5.1 Amounts of functional groups in humic acids.

	FHA			SHHA			SAHA			BHA		
	meq	N_t[a)]	pK_a	*meq*	N_t	pK_a	*meq*	N_t	pK_a	*meq*	N_t	pK_a
i	14[b)]	—	10.0	15	—	9.8	14	—	9.7	10	—	9.8
ii	4.3	—	8.9	6.7	—	8.9	4.0	—	8.6	4.6	—	8.4
iii	20	—	6.0	20	—	6.0	16	—	6.3	9.8	—	6.1
total	38	222	—	42	232	—	34	217	—	24	115	—

a) Total copper(II) complexing capacity derived from Table 3.2: $N_t = N_1 + N_2$.

b) HCl *meq* / g of humic acid.

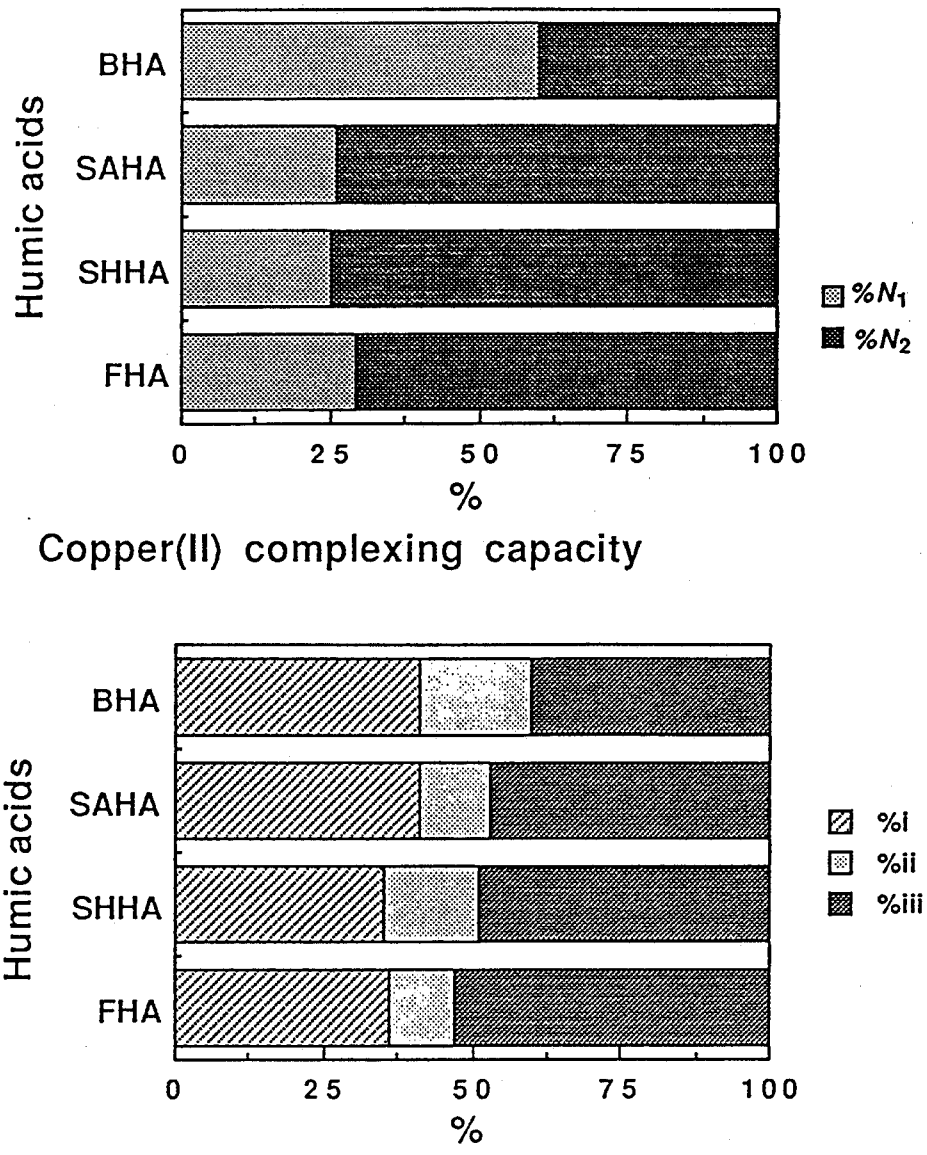

Fig. 5.2 Percentage of each functional group and copper(II) binding site.

5.4 Dissociation of copper(II)-humic acid complex

The pH titration curves in the case of model ligands, EDTA and hydroxyhydroquinone (HQ), were shown in Fig. 5.3 (a). In this titration, the excess potassium hydroxide was neutralized, and subsequently the protonation of functional groups in humic acid occurred. On the other hand, the titration curves in the presence of copper(II) were shown in Fig. 5.3 b. In these titration curves, lowering pH early occurred, and then the curves were shifted left. This is due to the copper(II) complexation of functional groups in humic acid. The shapes of the titration curves in the case of citric acid (CA) and phenylalanine (Phe) were the same as that in EDTA.

However, in the case of HQ, the titration curve has a plane region around pH 6.0. This is due to the dissociation of copper(II)-HQ complex. The pH titration curves of humic acids were shown in Fig. 5.4. Especially, the titration curve in Fig. 5.4 A-b, FHA, was the same shape as that of EDTA, and then the titration curve in Fig. 5.4 B-b, BHA, was the same as that of HQ. Moreover, the curve in Fig. 5.4 A-(a) was the same shape as that in SHHA, and then the curve in Fig. 5.4 B-(a) was the same as that in SAHA.

The conductimetric titration curves of humic acids were shown in Fig. 5.5. The V values in Fig. 5.5 mean the amounts of proton which was attached to the functional groups in humic acids, that is, the total amounts of the acidic functional groups in humic acids. When the copper(II) ions exists in the solution, the values of V correspond to the amounts of proton which attach to the free site in humic acid and correspond to proton which is consumed by the dissociation of copper(II)-humic acid complex and the formation of acidic complex as described following equation,

$$nH^+ + CuL^{m-} \rightleftharpoons Cu^{2+} + H_nL^{-(2+m-n)} \qquad (6.1)$$

$$nH^+ + CuL^{m-} \rightleftharpoons CuH_nL^{-(m-n)} \qquad (6.2)$$

where L means the humic acids or the model ligands. The V values

estimated by the conductimetric titration curves were summarized in Table 5.2. In FHA, SHHA, EDTA, CA and Phe, the v values decreased in the presence of copper(II) ions. However, in the case of SAHA, BHA and HQ, the V values did not change. These results suggest that the copper(II) complexes with FHA or SHHA are hardly dissociated in the acidified solution. However, the complexes with SAHA or BHA are easily dissociated by lowering pH.

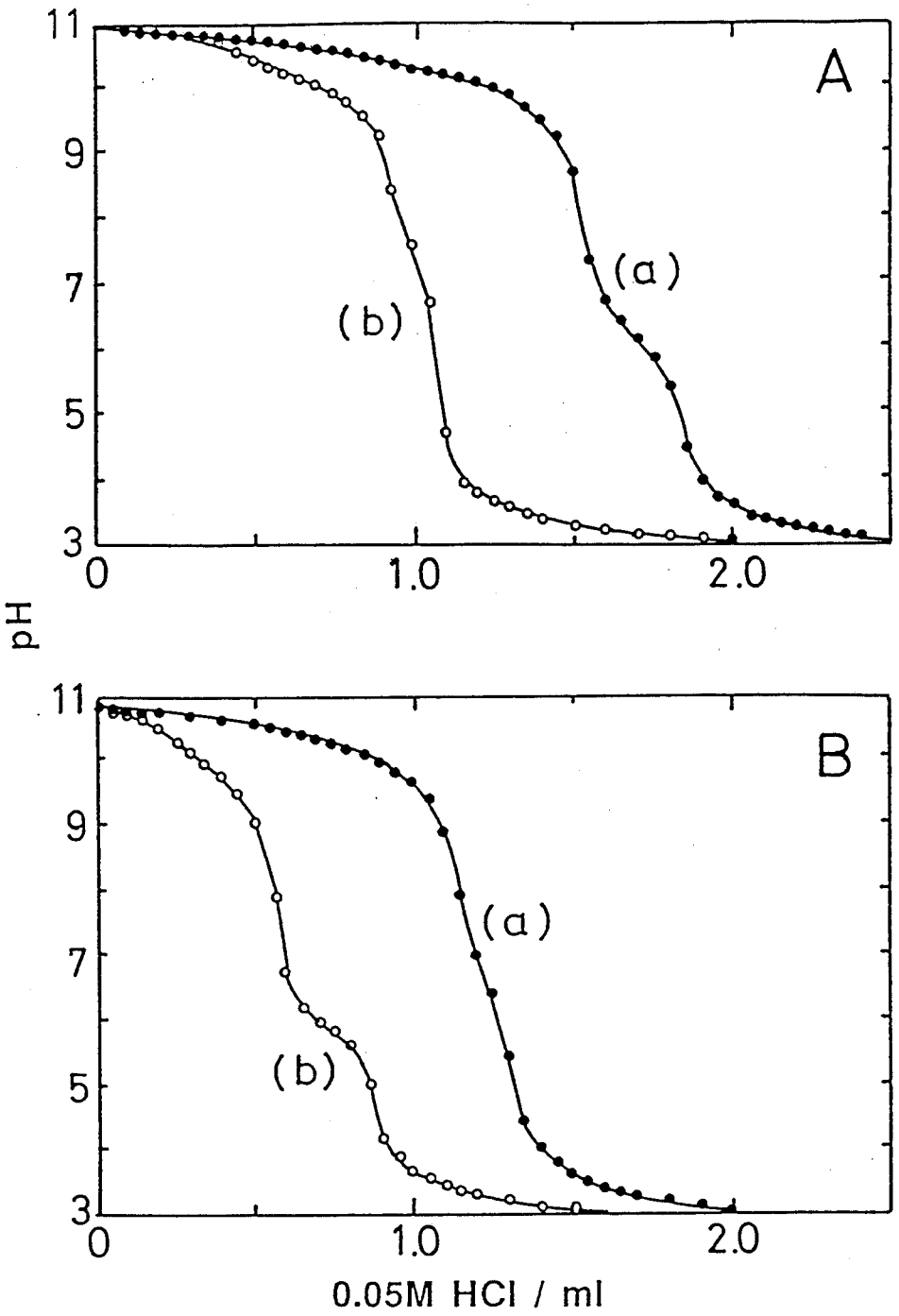

Fig. 5.3 pH titration curves of model ligands.

A: EDTA, B: Hydroxyhydroquinone (HQ),

(a) without Cu^{2+}, (b) with Cu^{2+}; 200 μM, EDTA or HQ; 200 μM.

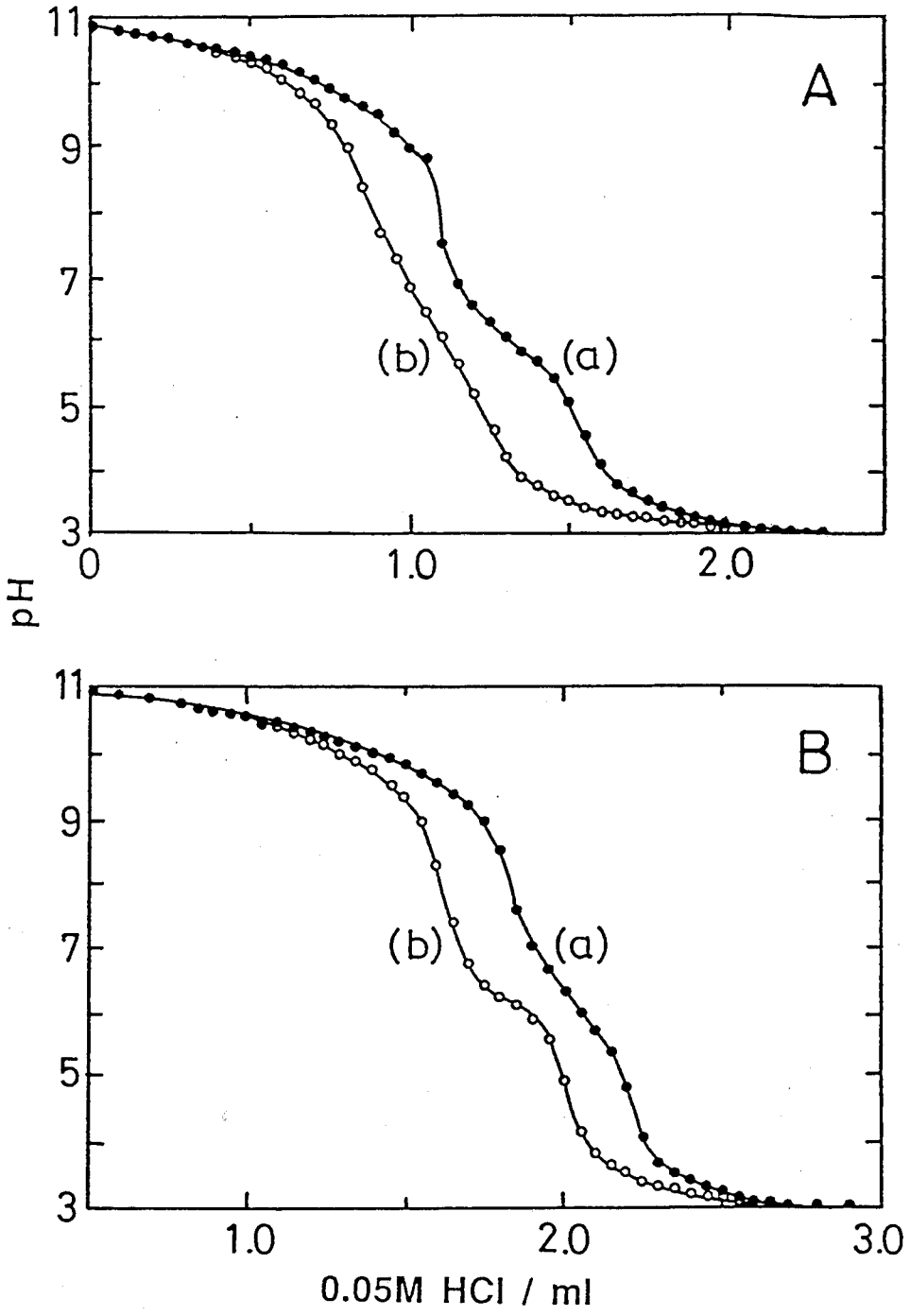

Fig. 5.4 pH titration curves of humic acids.

A: FHA, B: BHA, (a) without Cu^{2+}, (b) with Cu^{2+}; 100 μM,

KOH: 3.0 mM; FHA, 3.8 mM; BHA.

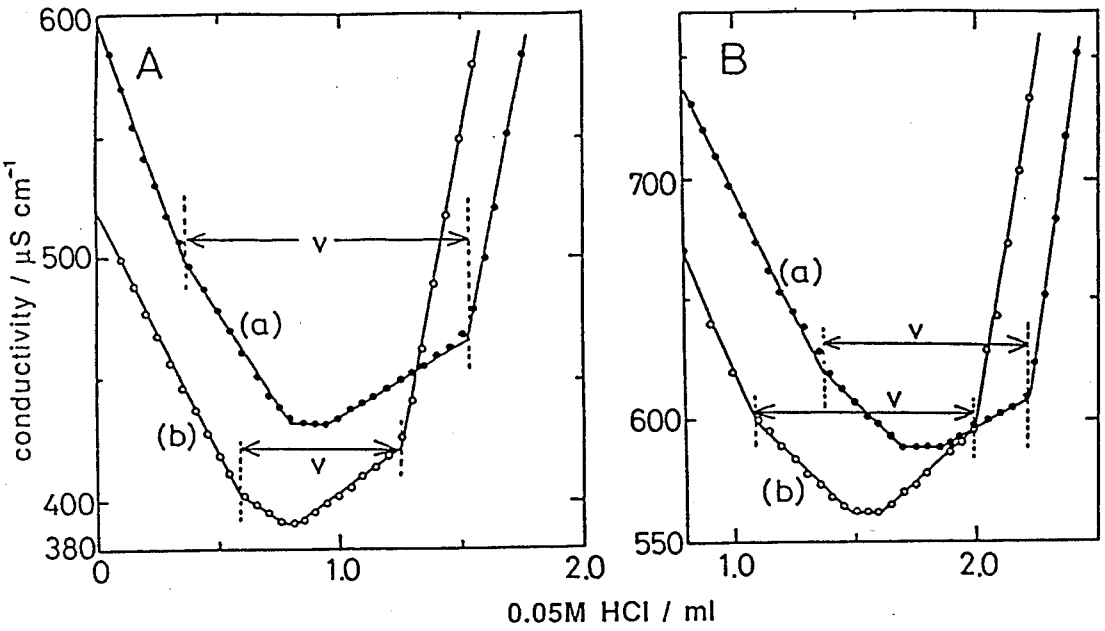

Fig. 5.5 Conductimetric titration curves of humic acids.

A: FHA, B: BHA, (a) without Cu^{2+}, (b) with Cu^{2+}; 100 μM.

Table 5.2 The V values of model ligands and humic acids.

	V/ *meq* HCl	
	without Cu^{2+}	with Cu^{2+}
Model ligands		
EDTA	0.42±0.03	0.22±0.02
CA	0.43±0.02	0.35±0.03
Phe	0.31±0.01	0.22±0.01
HQ	0.26±0.01	0.26±0.02
Humic acids		
FHA	0.57±0.02	0.33±0.01
SHHA	0.62±0.03	0.41±0.02
SAHA	0.51±0.01	0.51±0.03
BHA	0.39±0.01	0.40±0.01

5.5 Acidic functional groups in humic acid

The FTIR spectra of humic acids are shown in Fig. 5.6. From these spectra, the phenolic hydroxyl (Fig. 5.6 A) and the carboxyl (Fig. 5.6 B) groups seem to be contained in the humic acids. The peaks in Fig. 5.6 C were the C=O stretching by the carboxylate or quinones [7]. Quinones concern with the dark color of humic acids [8]. From these results and the pK_as of each functional group, i - iii, the phenolic hydroxyl and the carboxyl groups seem to mainly concern with the complexation with copper(II) in higher pH region, and then the carboxyl groups seem to concern with the complexation in lower pH region. Moreover, the peak areas of B in FHA and SHHA were higher than those of the peaks C. The peak areas of C in SAHA and BHA were higher than those of the peaks B. These results suggest that the content of carboxylic acid of FHHA or SHHA is larger than that of SAHA or BHA.

5.6 Conclusions

Since FHHA and SHHA contain the carboxyl groups which have the low pK_a values, the stable complexes can form in the acidified solution. However, since SAHA and BHA have small amounts of carboxyl groups and/or large amounts of phenolic hydroxyl groups such as hydroxyhydroquinone, the complexes can be easily dissociate in the acidified solution.

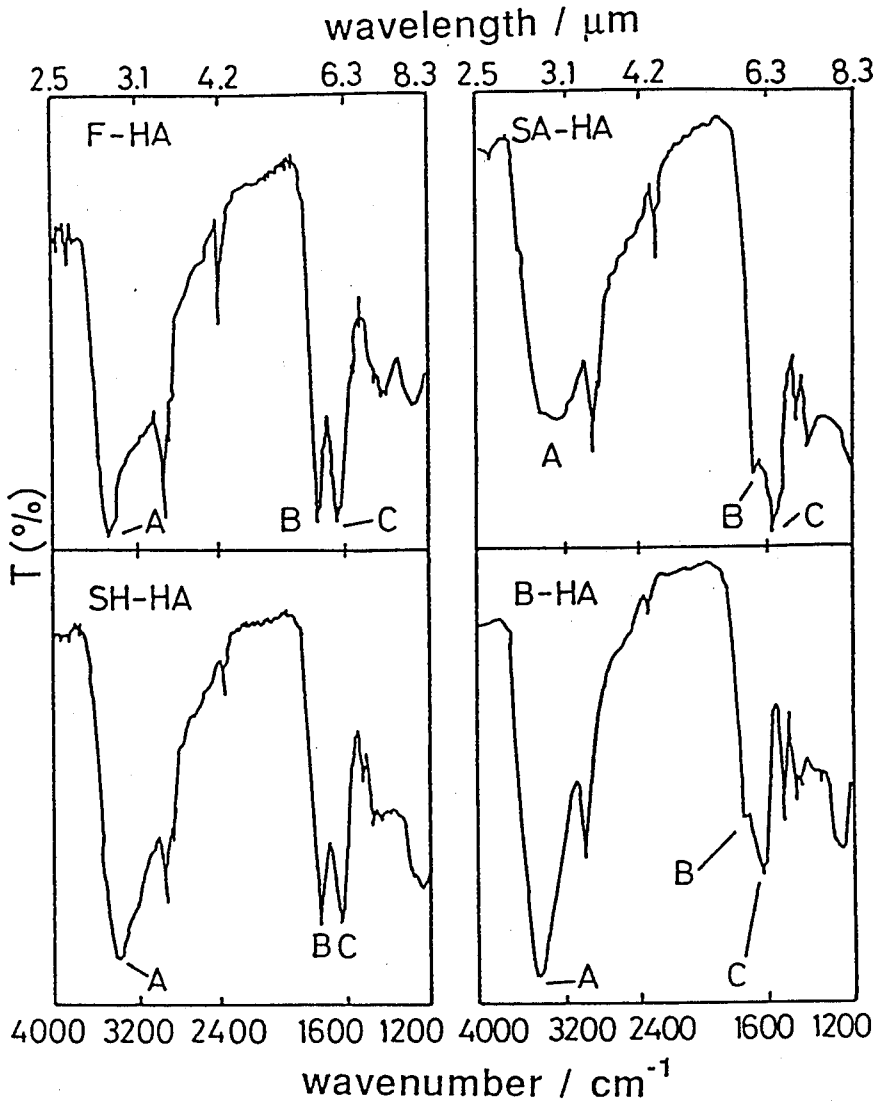

Fig. 5.6 FTIR spectra of humic acids.

FHA: A; 3451, B; 1718, C; 1627,

SHHA: A; 3407, B; 1718, C; 1617,

SAHA: A; 3386, B; 1718, C; 1615,

BHA; A; 3387, B; 1708, C; 1615, (cm^{-1}).

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CHAPTER 6

EFFECT OF IONIC STRENGTH ON COPPER(II) COMPLEXING ABILITY OF HUMIC ACID

6.1 Introduction

The complexing ability of humic acids (HAs) has been quantitatively evaluated by various methods. For example, the conditional stability constants and complexing capacities were calculated by the interpretation mode of a Scatchard et al. [1] and a Van den Berg et al. [2]. When the complexing abilities of HAs are evaluated by these interpretation modes, the free metal ions and the complex species must be individually determined. Several methods to measure the free and complex species separately have been reported such as ion selective electrode [3], voltammetry [4], ion-exchange method [5,6]. Especially, a macroreticular dextran gel, weak-base anion-exchanger, diethylaminoethyl Sephadex A-25 (A-25) has been useful for the speciation analysis of trace heavy metal ions in natural waters [7-9]. In these methods, anionic species such as heavy metal-HA complex and iron(III)-HA colloid could be retained on A-25, and then the other species such as cationic or non-ionic species could not be retained. Therefore, metal-HA complex species could be separated and determined by the A-25 method [10]. The copper(II) complexing abilities (conditional stability constants and complexing capacities) were also evaluated by a Scatchard plot [11]. However, the only conditional stability constants were evaluated, but not the thermodynamic stability constant in these investigation.

In this chapter, the thermodynamic stability constant of copper(II)-HA complex was evaluated by the method. At first, conditional stability constants and copper(II) complexing capacities at ionic strength, 0.005, 0.030, 0.055 and 0.105 were determined by using the Scatchard plot adopting a two-site

model. The thermodynamic stability constant could be evaluated by extrapolating these conditional stability constants to ionic strength zero. The effects of ionic strength on copper(II) complexing abilities were discussed from the viewpoints of the dissociation of acidic functional groups in HA and the conformational change of HA.

6.2 Experimental Section

The WHA_1 was used as HA, and then HA was extracted and purified according to the previous work [11]. The sodium salts of perchlorate, sulfate, nitrate and chloride were used to adjust ionic strength.

An A-25 (Pharmacia LKB Biochemistry, Sweden) was ultrasonically washed in 1M hydrochloric acid solution, and then decanted from distilled water for several times. The A-25 was stocked in distilled water. A 1.6 ml volume of A-25 was packed into a polypropylene column (40 x 8 mm i.d., Bio-Rad Labs., U.S.A.), and then washed with water. Subsequently, 10 ml of the buffer solution which is the same matrix as sample solution was passed through the A-25 column. An 8 ml of sample solution containing copper(II) and HA passed through the column at the flow rate of 10 ml min^{-1}. After desorbing copper(II)-HA complex retained on A-25 by 0.7 M nitric acid, the copper(II) in the solution was measured by an atomic absorption spectrometer (170-50 type, Hitachi LTD Co., Hitachi; Japan). The concentration of copper(II)-HA complex, [CuL], could be obtained from the amount of copper(II) retained as complex on A-25, and then the free copper(II) concentration, $[Cu^{2+}]$, could be calculated by subtracting [CuL] from the total copper(II) concentration, $[Cu^{2+}]_t$. The temperature of the sample solution was 22±1.0 °C.

The measurement of acidic functional groups in HA was performed by acid-base titration. A pH meter (M-13 type, Horiba LTD, Tokyo; Japan) and a glass electrode (6366 type, Horiba LTD) were used. The measurements were done under nitrogen atmosphere and at 25±0.5 °C. The evaluation of acidic functional groups in HA were according to the Cabaniss's report [12]. A ring method was used for the measurement of surface tension [13]. The measurement of the surface tension was done at 20±0.5 °C.

6.3 Effect of ionic strength on [CuL]

The relation between the square root of the ionic strength, $I^{1/2}$, and the concentration of the copper(II)-HA complex, [CuL], was shown in Fig.6.1 (a). The [CuL] increased with ionic strength. In the case of an anion complex with a low molecular weight ligand such as EDTA, the concentration of copper(II)-EDTA complex was constant up to the ionic strength of 0.080. However, the concentration rapidly decreased above the ionic strength 0.080 as shown in Fig. 6.1 (b). The decrease of copper(II)-EDTA complex would be due to reducing the ion-exchange capacity of A-25 [16]. On the other hand, HA could not be detected in the effluent at all even at the ionic strength of 0.105. Therefore, the increase of [CuL] with increasing ionic strength seems to be due to the increase of copper(II) complexing ability of HA itself.

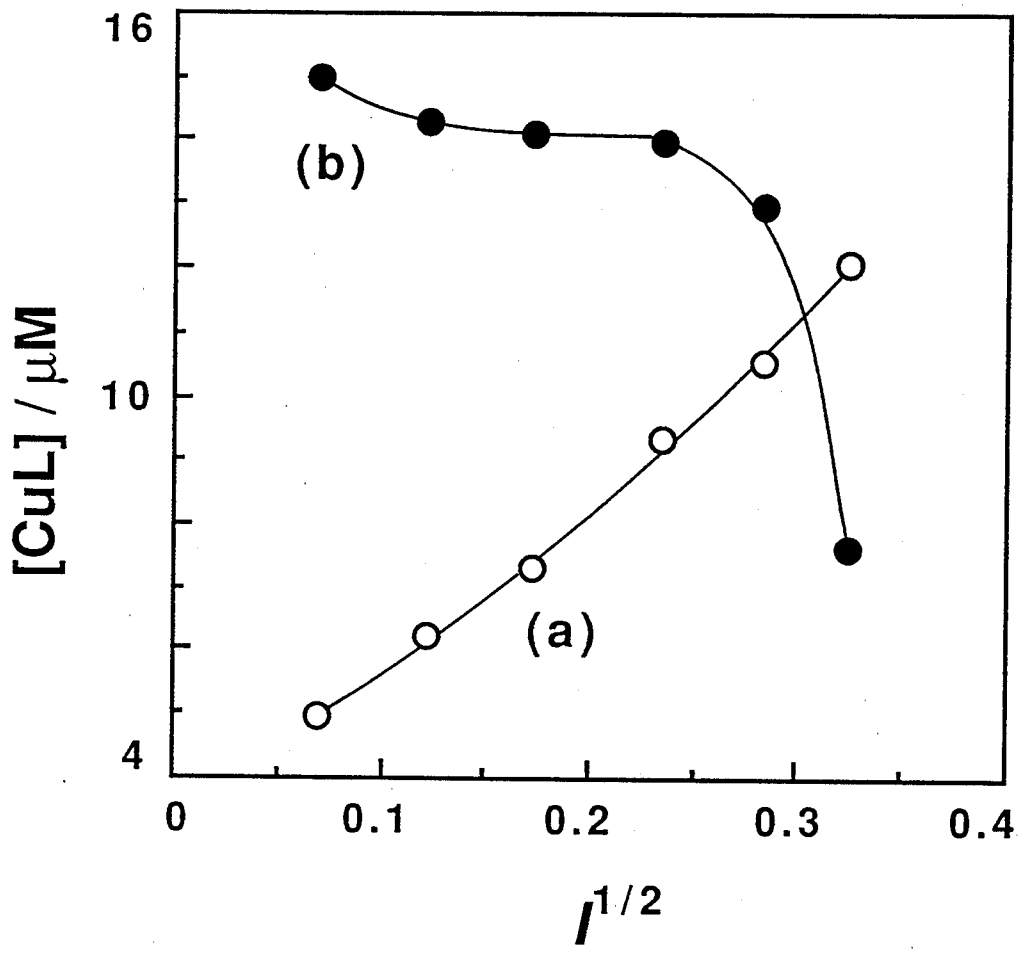

Fig.6.1 Effect of ionic strength on [CuL].

(a) HA; $[Cu^{2+}]_t$: 30μM, (b) EDTA; $[Cu^{2+}]_t$: 15μM, pH: 6, HA: 20mg l^{-1}, EDTA: 15μM.

6.4 Effect of ionic strength on *N* and *K* values

In order to quantitatively evaluate the copper(II) complexing abilities of HA, the conditional stability constants, K_1 and K_2, and the concentration of binding site, c_{L1} and c_{L2}, were calculated by the Scatchard approach adopting a two-site model [3]. From the mass balance for free copper(II) ions, $[Cu^{2+}]$, and copper(II)-HA complex, [CuL], the following equation was derived [13,17],

$$\frac{[CuL]}{[Cu^{2+}]} = -K_i[CuL] + K_i c_{Li} \qquad (3.6)'$$

where i means the kind of the binding sites, 1 for strong site and 2 for weak site. Copper(II) complexing capacity, N_i, was defined as moles number of copper(II) bound to 1g of HA (mol Cu^{2+} per g of HA), and then can be written as described in Eqn. (3.8)

$$N = \frac{c_L\ (\mu M)}{[HA\ (mg\ l^{-1})]} \qquad (3.8).$$

Therefore, the conditional stability constants and the copper(II) complexing capacities can be evaluated by a Scatchard plot in which the vertical axis is $[CuL]/[Cu^{2+}]$ and the horizontal axis is [CuL]. These results are summarized in Table 6.1. The K_0 in Table 6.1 means the average stability constant of copper(II) binding sites in the HA and this can be defined as follows [18],

$$K_0 = \sqrt{K_1 K_2} \qquad (6.1)$$

As shown in the results of Table 6.1, the average stability constants (K_0) and the total copper(II) complexing capacities ($N_t = N_1 + N_2$) increase with ionic strength. The increase of copper(II) complexing ability with ionic strength seems to be the reason that the dissociation of acidic functional groups is promoted by the electrostatic effect [14] and then the coordination of copper(II) to HA becomes easy. The interpretation was supported by the fact that the amounts of acidic functional groups, C_A, which is measured by acid-base titration, also increase with ionic strength.

Table 6.1 Copper(II) complexing abilities of humic acid in various ionic strength at pH 6, HA: 20 mg l^{-1}.

parameters	Ionic strength			
	0.005	0.030	0.055	0.105
$\log K_1$	5.64	5.68	5.71	5.92
$\log K_2$	5.00	5.05	5.19	5.55
$\log K_0$	5.32	5.34	5.45	5.74
N_1 [a)]	172	234	332	491
N_2	198	260	312	249
N_t	370	494	644	740
C_A [b)]	13.1	16.7	19.1	26.0
$-\log\Gamma_1$ [c)]	0.02	0.06	0.09	0.30
$-\log\Gamma_2$	0.01	0.06	0.20	0.56
$-\log\Gamma_0$	0.02	0.04	0.15	0.44

a) copper(II) complexing capacity; μmol per g of HA.

b) amount of acidic functional groups in humic acid; determined by potentiometry; meq per g of humic acid.

c) ratio of activity coefficient.

6.5 Evaluation of thermodynamic stability constant

The relation between square root of ionic strength, $I^{1/2}$, and logarithm of conditional stability constants, log$K$, is shown in Fig. 6.2. The thermodynamic stability constant, $K_0°$, is evaluated by extrapolating $I^{1/2} = 0$. The results were as follows: log$K_0°$; 5.30, log$K_1°$; 5.62, log$K_2°$; 4.99. From the Debye-Huckel's theory, the relationship between thermodynamic stability constant and conditional stability constant can be written as follows,

$$K° = K\Gamma = \frac{K\gamma_{CuL}}{\gamma_{Cu2+}\gamma_L} \qquad (6.2)$$

where the Γ and γ mean the ratio of activity coefficient and the activity coefficients of each species, respectively. The values of γ can be recognized as the function of the ionic strength, the charge of HA molecule and the radius of HA as polyion. Although it is difficult to measure the activity coefficients, γ, of each ion in practice, the value of Γ can be calculated from the relationship between square root of ionic strength and conditional stability constant. The results obtained by the least-square curve fitting were as follows,

$$-\log\Gamma_0 = 6.271\times10^{(5.662\sqrt{I}-3)} \quad (r=0.992) \qquad (6.3)$$

$$-\log\Gamma_1 = 8.430\times10^{(4.691\sqrt{I}-3)} \quad (r=0.989) \qquad (6.4)$$

$$-\log\Gamma_2 = 3.988\times10^{(6.891\sqrt{I}-3)} \quad (r=0.991) \qquad (6.5)$$

where the subscripts, 0, 1 and 2 are referred to K_0, K_1 and K_2.

On the other hand, the concentration of the free ligand can be represented by using the thermodynamic stability constant,

$$[L^-] = \frac{[CuL]}{K°/\Gamma_0([Cu^{2+}]_t - [CuL])} \qquad (6.6)$$

From Eqn. (6.6), the free ligand concentrations can be calculated by using [CuL], $K_0°$ and G_0 values obtained experimentally. The plots of [CuL] or [L^-] obtained by the calculation are shown as the function of total copper(II)

concentration in Fig. 6.3. These results show that both the [L^-] and [CuL] increase with ionic strength. This tendency is in good agreement with that in the amount of acidic functional groups and the copper(II) complexing capacities. The dissociation of functional groups in HA seems to be promoted with increasing ionic strength. Moreover, the total copper(II) complexing capacity, which can be calculated, also increases with ionic strength (Table 6.2). These results are in good agreement with the experimental values with respect to I=0.005, 0.030, 0.055 and 0.105 in Table 6.1.

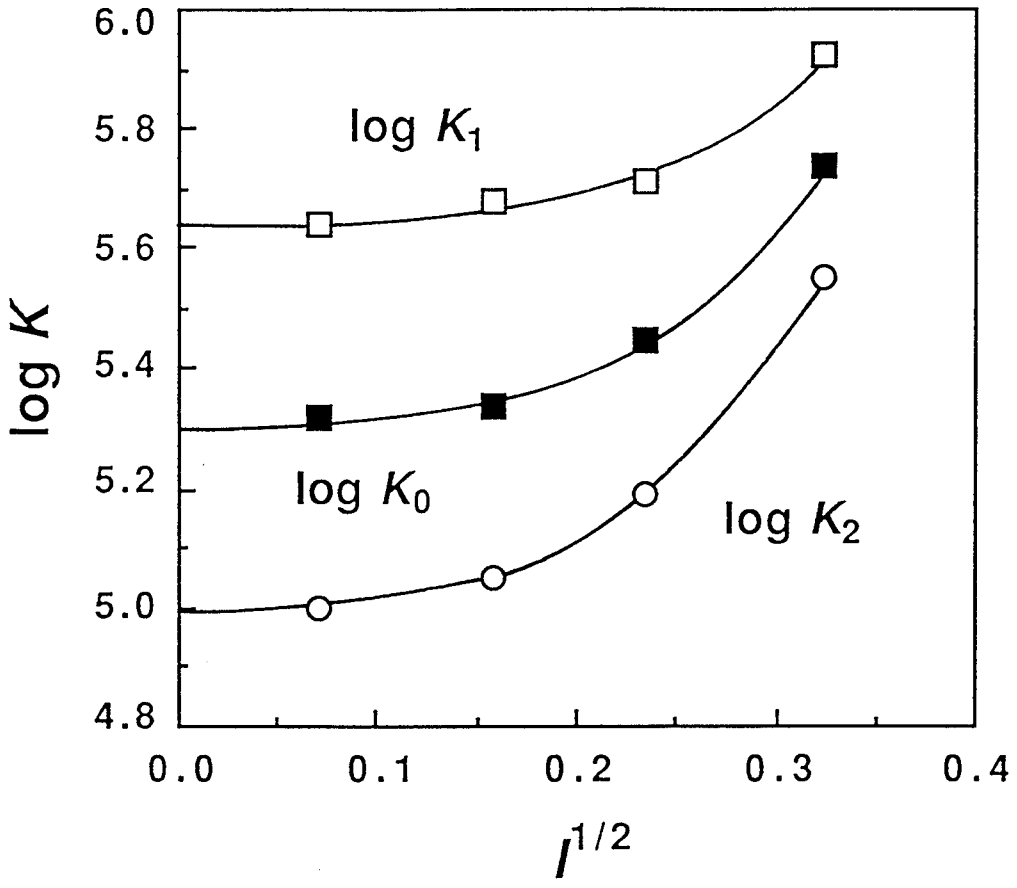

Fig. 6.2 Estimation of thermodynamic stability constants.

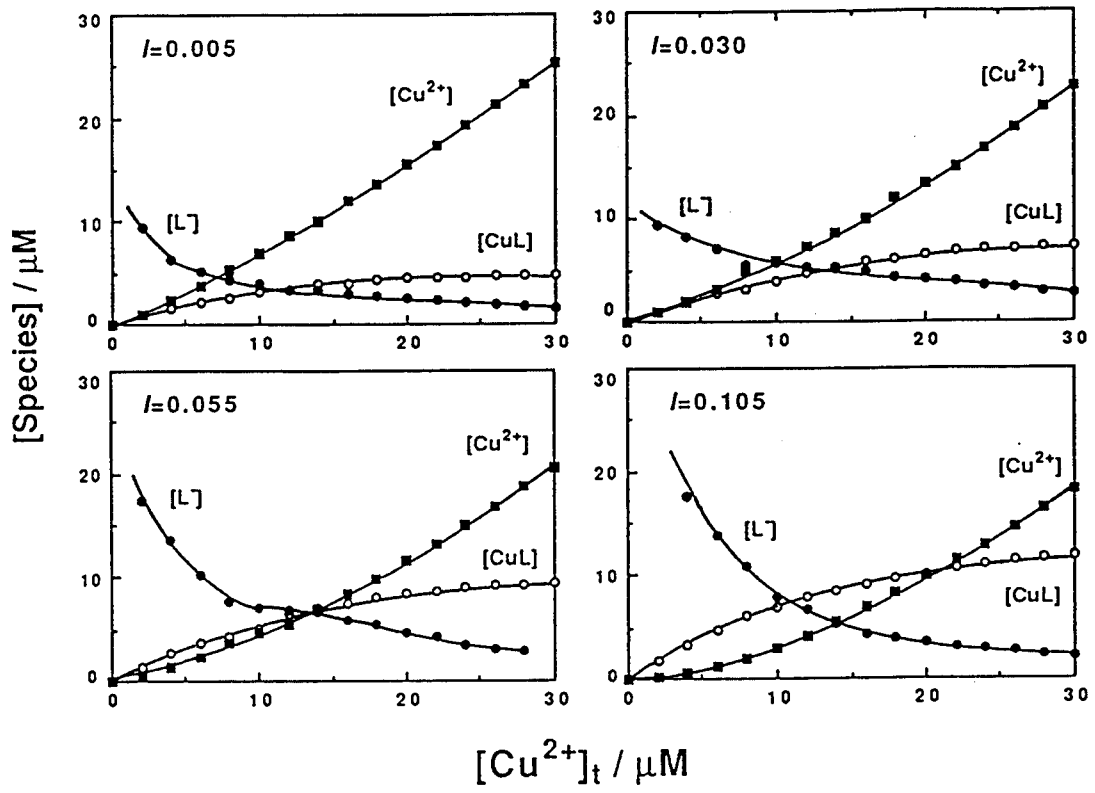

Fig. 6.3 Effect of ionic strength on [L$^-$].

logK_0°, Γ_0 and [CuL] measured were used to evaluate [L$^-$].

Table 6.2 Calculation of N_t using $K_0°$ and Γ_0 in various I.

I	$-\log\Gamma_0$	[CuL] / μM	N_t
0.005	0.964	4.98	338
0.015	0.931	6.21	423
0.030	0.871	7.30	495
0.055	0.736	9.34	621
0.080	0.562	10.6	670
0.105	0.373	12.1	719

$[Cu^{2+}]_t$: 30 μM, pH 6, HA: 20 mg l^{-1}.

The K_0 value calculated in Fig. 6.2 was used.

6.6 Effect of inorganic anions

It is generally known that as the ionic strength is higher, the complexing ability of organic ligand with low molecular weight becomes much low because of the competition between co-ions and metal ion. However, the copper(II) complexing ability of HA increased with ionic strength. It is thought that the conformational change of HA by the presence of ionic species affected the copper(II) complexing ability in various ionic strength. HA is random-copolymeric structure, and then the conformation of HA could be variable with the condition of solution (e.g. pH, ionic strength, temperature, polarity of solvent and so on). The higher ionic strength, the more spherical structure of HA in polyelectrolyte [19]. When HA is spherical structure, the acidic functional groups, which are hydrophilic groups, seem to have a tendency to orient themselves to the solvent (water). Therefore, the surface negative charges may be larger, and then the heavy metal ions will be accumulated around the HA sphere molecules. The surface tension of HA solution was measured in these points of view. The surface tensions of the solution containing 50 mg l^{-1} HA in various ionic strength are shown in Fig. 6.4. The surface tension decreased with increasing ionic strength. The larger surface charges of HA, the larger amounts of HA adsorbed on the surface. Therefore, the surface free energy (that is, surface tension) will decrease. On the other hand, the absolute viscosity of HA was also measured in the solution containing 0.1M NaOH and 0.1M $NaClO_4$, and then this was 0.02. This value is in agreement with the values by Kumada et al. (0.02 ~ 0.05) in which these values are those of the spherical polymer [20]. From these results, it is predicted that the HA molecule becomes more spherical with an increase of ionic strength.

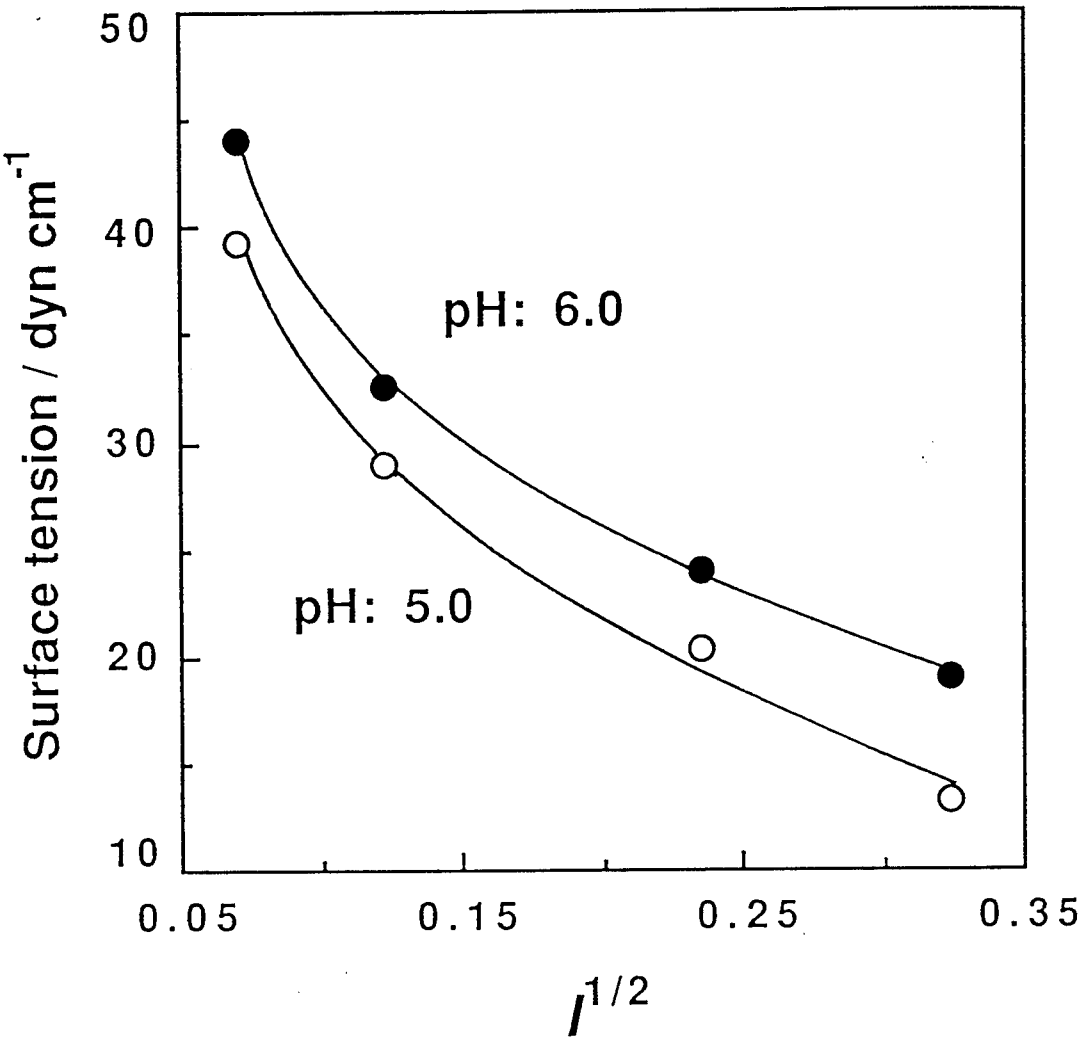

Fig. 6.4 Effect of ionic strength on surface tension at 50 mg l^{-1} of HA.

The conformation of HA in the ionic solution will be dramatically changed by the kinds of inorganic anions. Therefore, the copper(II) complexing ability of HA will be also affected by the kinds of anions. The copper(II) complexing abilities, in the presence of nitrate, sulfate and chloride, are summarized in Table 6.3. The orders of the average stability constants, K_0, and the copper(II) complexing capacities, N_t, were $SO_4^{2-} > NO_3^- > Cl^-$. This result was in good agreement with the decreasing effects of inorganic anion on the critical micellar concentration of anionic surfactant such as sodium dodecylsulfate [21]. This is due mainly to the decrease of the thickness of the ionic atmosphere surrounding the ionic head groups in the presence of the additional electrolyte. The same phenomenon must occur in the case of HA. Therefore, the largest value of copper(II) complexing ability in the case of SO_4^{2-} can be suggested that HA molecules prefer to make more spherical conformation in the presence of SO_4^{2-}.

Table 6.3 Copper(II) complexing abilities of humic acid in various anions.

	SO_4^{2-}	NO_3^-	Cl^-
$\log K_1$	5.90	5.72	5.62
$\log K_2$	5.43	5.23	5.07
$\log K_0$	5.67	5.48	5.35
N_1	392	342	238
N_2	369	254	181
N_t	761	596	419

6.7 Conclusions

It was found that the copper(II) complexing abilities of HA increased with an increment of ionic strength. This fact will gives an important information for the evaluation of the dynamic changes of heavy metal species in the environment such as an estuary where ionic strength dramatically change. This seems to be the reasons that (i) dissociation of the acidic functional groups in HA is promoted with increasing ionic strength, (ii) HA molecules occur the conformational change to become sphere, and then facilitate the coordination of copper(II) with an increase of ionic strength.

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CHAPTER 7

EVALUATION OF COPPER(II) COMPLEXING ABILITY OF HUMIC ACID IN VARIOUS MOLECULAR WEIGHT FRACTIONS

7.1 Introduction

Humic substances widely distribute in natural waters and soil environments. These have the polyelectrolytic characters and the heavy metal ions complexing abilities [1,2]. The complexations of heavy metal ions with humic substances concern with the toxicity for phytoplankton in natural waters and the accumulation of heavy metal ions in soil environments [3-6]. Therefore, the knowledges about complexation between heavy metal ions and humic substances are interesting in the point of view of environmental chemistry, biology and geochemistry.

It has been known that humic substances are heterogeneity containing various functional groups and molecular weight fractions [7,8]. Therefore, it was required to interpret the acid-base or the complexing equilibria of humic substances taking into account the heterogeneity such as the molecular weight distribution and various functional groups.

First, there are some discrete ligand models. These are assumed that there are several independent binding sites which have various acidity in the humic molecule, and then these individually bind with heavy metal ions. Especially, the copper(II) complexing capacities and metal binding constants were often evaluated by adopting the two or three site models which was assumed the existence of several different types of functional groups in humic molecules. For example, there are a few modes for interpretation by Scatchard et al. and Ruzic [9,10]. Secondary, there is the continuous distribution model. For example, the affinity spectrum and the continuous stability distribution models were used in the interpretation of acid-base and

the complexing equilibria of heterogeneous polyelectrolyte such as proteins and humic substances [11-16].

Especially, Dzomback and Fish et al. reviewed about the comparison of the discrete models with the continuous distribution models [17,18]. The discrete ligand models could not represent actual binding sites, and then fail to predict the effects of small quantities of strong binding sites. However, Fish et al. said that the discrete models are shown to be a simple and accurate means of predicting metal-humate binding capacities within the range of calibrating titration [18]. The continuous distribution models, however, reflect the state of titration and the interactions among sites. That is, the ligand distribution would be a function of free metal concentration as well as binding constants because of the variation of binding constants with free metal concentration [17]. In the continuous distribution models, the distribution function was assumed, and then the parameters (*e.g.* binding constant, ligand concentration, standard deviation of binding constant) could be calculated the non-linear least squares treatment of the experimental data.

On the other hand, humic substances have the aggregation properties such as surfactants [19,20]. From the investigation about spectroscopy of humic substances, the interaction between humic molecules would be attribute to the donor-acceptor charge-transfer mechanisms [21]. Power et al. showed the aggregate characters of humic substances by means of the thermal lens detection of absorbance using the Ar-ion laser [22]. Furthermore, Wang et al. confirmed the interaction between humic molecules by mixing the different molecular weight fractions and subsequently by the measurements of UV-vis and fluorescence spectra [23].

As described above, the humic substances in natural systems seem to be the polydisperse mixture of random polymeric materials which constructed

with various molecular weight fractions. It could be therefore predicted that the interactions between molecular weight fractions of humic substances such as the donor-accepter mechanisms were proceeded in natural system by mixing various molecular weight fractions. Moreover, the metal binding sites may be affected by such the interaction. Therefore, the complexing abilities of each molecular weight fractions of humic substances may be different from that of the mixture.

In this chapter, the acid precipitated humic acid (TOTAL) was fractionated by the size exclusion chromatography (s.e.c.). Moreover, the copper(II) complexing abilities of the molecular weight fractions and mixture were evaluated by the copper(II) complexing titration using an anodic stripping voltammetry (ASV). The discrete ligand model (the Scatchard approach) was used to evaluate the ligand concentration. And then, the continuous stability distribution model was used to evaluate the conditional stability constants between copper(II) ions and each binding site of humic acid. Furthermore, the relationship between the interaction of molecular weight fraction and the copper(II) complexing abilities were discussed by the data obtained from the UV-vis, fluorescence and FTIR spectra of each molecular weight fractions and the mixture of humic acids.

7.2 Experimental Section

Preparation of molecular weight fractions of humic acid

The humic acid (WHA_2) was used in the present chapter. The humic acid was extracted and purified according to chapter 2, and then this humic acid (acid precipitated humic acid fraction) was denoted "TOTAL". This was dissolved in 0.05M Na_2HPO_4 (1 g humic acid l^{-1}), and then 7 ml of aliquot was injected into a Sephadex G-50 column (50Ø X 460 mm). The mobile phase ($1X10^{-3}$ M phosphate buffer) was pumped by a peristaltic pump (Pharmacia LKB Biochemistry, P-1 type) at a flow rate of 4.5 ml l^{-1} [23]. The molecular weight fractions of the humic acid were sampled every 4 minutes by a fraction collector (RediFrac, Pharmacia LKB Biochemistry). The detection of humic acid was performed by using an UV-vis detector (Hitachi, L-4200 type). The wavelength of detection was at 254 nm. The chromatogram was recorded by a recorder (FBR-251A type, Toa Electronics Ltd.). The fractions were fractionated with three sections according to the chromatogram. Each fractions were concentrated by the evaporation, and acidified by 0.1M HCl. And then the humic acid was precipitated. In order to remove chloride ions, these were dialyzed against distilled water for a few weeks using a Spectra/Pore Memrane (Spectrum Medical Industries INC) that was a nominal molecular weight cut-off of 1000. Finally, the powder of humic acid was obtained by the liophylization.

ASV detection of Copper(II) ions

The concentrations of uncomplexed copper(II) species (the ASV-labile copper(II) species) were determined by an ASV. The experimental procedure, conditions and equipments were according to chapter 4. The 40 ml aliquot of supporting electrolyte containing 10 mg l^{-1} of humic acid (0.1M KNO_3 + 0.01M acetate buffer at pH 6) was taken into the cell, and then deaerated by nitrogen gas bubbling for 15 min. Subsequently, the standard solution of copper(II) ions was dropped into the solution by an Eppendorf pipette, deaerated by nitrogen gas for 3 min. Then the uncomplexed copper(II) species were measured by an ASV. The measurements were performed at 25±0.3 °C.

Spectroscopic measurements

The UV-vis spectra were measured by an UV-2200 type spectrophotometer (Shimadzu Co., Ltd.) in HEPES buffer (0.05M, pH 8). The fluorescence spectra were measured by a RF-510 type spectrofluorophotometer (Shimadzu Ltd. Co.). The FTIR spectra were measured by a Perkin Elmer 1720-X type FTIR spectrometer and a FTIR-5300 type FTIR spectrometer (Japan Spectroscopic Co., Ltd.) with the KBr disk method.

7.3 Molecular weight fractionation of humic acid

The gel chromatogram of humic acid is shown in Fig. 7.1. According to three peaks in the chromatogram, the humic acid could be grouped for three sections (F_a, F_b and F_c in Fig. 7.1). The results of elemental analysis of these molecular weight fractions are summarized in Table 7.1. Moreover, the ranges of molecular weight distribution and the peak areas are summarized in Table 7.2. Molecular weight of the fraction of humic acid was calculated by the following equation by Determann and Michel [24],

$$\log M = 5.415 - 0.864(V_e/V_0) \qquad (7.1)$$

where M, V_e and V_0 represent the molecular weight, the elution volume and the void volume, respectively. The void volume, V_0, was measured by using a Blue Dextran 2000, and then this value was 333 ml. Moreover, the A_{TOTAL} was assumed as the peak area from retention time of 60 to 220 min. The A_{Fa}/A_{TOTAL}, A_{Fb}/A_{TOTAL}, and A_{Fc}/A_{TOTAL} in Table 7.2 represent the ratio of peak areas of each molecular weight fractions to that of TOTAL. Furthermore, the absorptivity of each molecular weight fractions were not different from each other at 254 nm. Therefore, the ratio of amounts (g) of each humic acid (n_{Fa}:n_{Fb}:n_{Fc}) is about 1:5:2.

Table 7.1 Elemental analysis of humic acids.

Humic acids	%C	%H	%N	%O	%ASH
Total	54.1	5.90	1.77	37.0	1.23
F_a	51.2	4.72	1.74	40.0	2.44
F_b	52.9	4.40	3.10	37.9	1.66
F_c	55.1	3.70	1.93	36.8	2.44

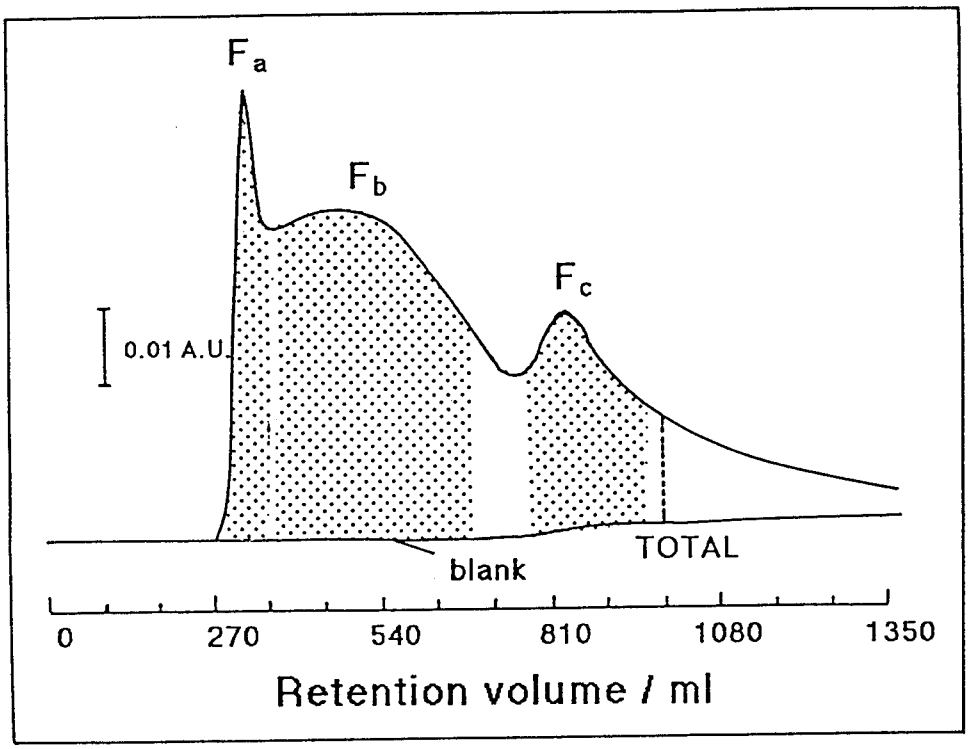

Fig. 7.1 Chromatogram of humic acid.

Blank: 0.01 M Na_2HPO_4, sample volume: 7 ml.

Table 7.2 Evaluation of molecular weight distributions.

	F_a	F_b	F_c
M	>36000	27000 ~ 4200	2600>
V_e / ml	334±9.2	521±13	859±21
$A_{Fa,b,c}/A_{TOTAL}$ [a)]	0.111	0.503	0.194

a) A_{TOTAL}: Peak area of chromatogram for the retention time from 60 to 220 min. $A_{Fa,b,c}$: Peak area of chromatogram for F_a, F_b or F_c.

7.4 Evaluation of copper(II) complexing capacities

The copper(II) complexing capacities of each fractions and the mixture of fractions were evaluated by adopting to the discrete ligand model, a Scatchard approach. The Scatchard plot was shown in Fig. 7.2. The MIX in Fig. 7.2 means the mixture at the proportion of amounts of each fractions with $n_{Fa}:n_{Fb}:n_{Fc} = 1:5:2$. Since the two site in humic acid that has a different acidity was assumed, the Scatchard plot could be divided two linear sections. The linear relationship between $\Sigma[CuL_i]/[Cu^{2+}]$ for the vertical axis and $\Sigma[CuL_i]$ for the horizontal axis can be described as,

$$\frac{\Sigma[CuL_i]}{[Cu^{2+}]} = -K_i\Sigma[CuL_i] + K_i c_{L_i} \qquad (3.6).$$

The concentration of the copper(II) binding site, c_{Li}, can be evaluated by the intercept and slope of the Scatchard plot [9]. The total c_{Li} values, c_{Lt} ($= \Sigma c_{Li}$), obtained by a Scatchard's approach were summarized in Table 7.3. Moreover, the copper(II) complexing capacity, N_t, ($= \Sigma N_i$), which means the copper(II) ions bound with unit gram of humic acid can be expressed as,

$$N = \frac{c_L \text{ (mol l}^{-1})}{[\text{HA (g l}^{-1})]} \qquad (3.8)$$

where [HA (g l^{-1})] represents the concentration of humic acid in the solution (g l^{-1}).

On the other hand, the X_A and X_n represent the fraction obtained by the peak areas of chromatogram and the amounts of humic acid fractions, respectively. These are witten as,

$$X_{A_{Fa}} = \frac{A_{Fa}}{A_{TOTAL}}, \quad X_{A_{Fb}} = \frac{A_{Fb}}{A_{TOTAL}} \text{ and } X_{A_{Fc}} = \frac{A_{Fc}}{A_{TOTAL}} \qquad (7.2)$$

or

$$X_{n_{Fa}} = \frac{n_{Fa}}{n_{Fa}+n_{Fb}+n_{Fc}}, \quad X_{n_{Fb}} = \frac{n_{Fb}}{n_{Fa}+n_{Fb}+n_{Fc}} \text{ and } X_{n_{Fc}} = \frac{n_{Fc}}{n_{Fa}+n_{Fb}+n_{Fc}} \qquad (7.3).$$

It can be predicted that the values of $X_A N_t$ or $X_n N_t$ of TOTAL or MIX is agreement with the sum of $X_A N_t$ or $X_n N_t$ of each fractions, F_a, F_b and F_c. However, not that these sum were equal values of $X_A N_t$ and $X_n N_t$ of TOTAL

or MIX, but that the $X_A N_t$ or $X_n N_t$ of TOTAL or MIX was lower than those of each fractions. Therefore, the copper(II) complexing ability of mixture (TOTAL or MIX) was lower than those of each fractions. This result was contradiction to the prediction. In order to explain this reason, the metal binding constants were evaluated in the following section.

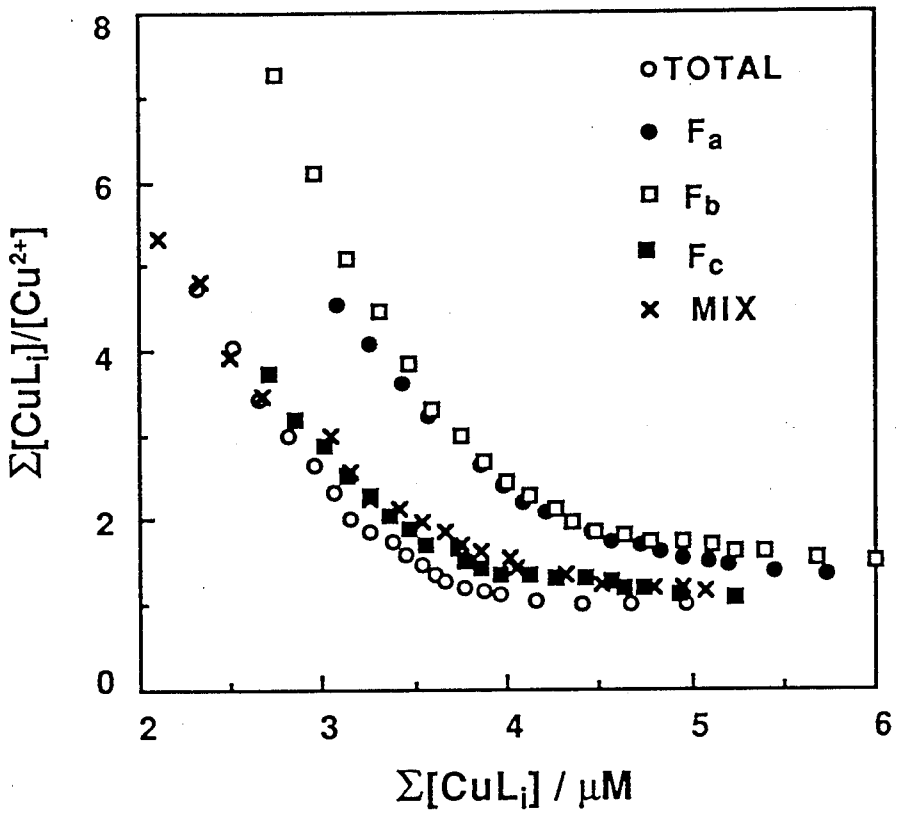

Fig. 7.2 Scatchard plot.

Table 7.3 Copper(II) Complexing Abilities of Humic Acids.

	TOTAL	MIX	F_a	F_b	F_c
X_A[a)]	1.000	___	0.111	0.503	0.194
	(0.834)[b)]				
$Xn_{Fa,b,c}$[a)]	___	1.000	0.125	0.625	0.250
$c_{L,t}$	9.03	11.7	9.23	12.0	9.85
N_t	903	965	923	1203	985
N_tX_A	903	___	104	605	191
	(757)			(900)	
$N_tX_{Fa,b,c}$	___	965	115	752	246
				(1110)	

Humic acid concentration: 10 mg l^{-1}.

a)$X_A = A_{Fa,b,c}/A_{TOTAL}$, $X_{Fa,b,c} = n_{Fa,b,c}/(n_{Fa}+n_{Fb}+n_{Fc})$

b)$X_{A_{Fa}} + X_{A_{Fb}} + X_{AFc}$; excepted for the unsampled fractions.

7.5 Evaluation of metal binding constants

The metal binding constants were evaluated by the continuous stability distribution model. The complexation between the arbitrary binding site in the humic molecule, i, and copper(II) ions can be represented as,

$$Cu^{2+} + L_i \rightleftharpoons CuL_i \qquad (7.4).$$

The metal binding constant between each binding sites and copper(II) ions is as follow,

$$K_i = \frac{[CuL_i]}{[Cu^{2+}][L_i]} \qquad (7.5).$$

From the mass balance in the solution, the concentration of the copper(II)-humate can be written as,

$$\Sigma[CuL_i] = [Cu^{2+}]_t - [Cu^{2+}] \qquad (7.6)$$

where $[Cu^{2+}]_t$ represents the concentration of total copper(II) ions. Therefore, the $\Sigma[CuL_i]$ means the concentration of copper(II)-humate which can be obtained from an experimental titration. Combining Eqn. (7.5) with (7.6), the following relationship can be derived,

$$\Sigma[CuL_i] = \frac{K_i c_{L_i}[Cu^{2+}]}{1+K_i[Cu^{2+}]} \qquad (7.7)$$

On the other hand, the Gaussian distribution function may be assumed as the distribution of stability of copper(II) binding site in the humic molecule. Therefore, the following distribution function can be written as,

$$c_{L_i}(K_i) = \frac{c_{Lt}}{\sigma\sqrt{2\pi}} \exp[-\frac{1}{2\sigma^2}(\mu - \log K_i)^2] \qquad (7.8)$$

where σ and μ represent one standard deviation of mean and the mean of metal binding constant, respectively. Combining Eqn. (7.7) with (7.8), the concentration of copper(II)-humic acid can be written as,

$$\sum_{i=1}^{m}[CuL_i] = \frac{c_{Lt}}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \frac{K_i[Cu^{2+}]}{1 + K_i[Cu^{2+}]} \exp[-\frac{1}{2\sigma^2}(\mu - \log K_i)^2]\, d\log K \qquad (7.9)$$

The values of σ and μ could be calculated by the nonlinear least-squares

technique of the experimental data such as $[Cu^{2+}]_t$ and $[Cu^{2+}]$.

The experimental titration curves and those obtained by the nonlinear least-squares technique are shown in Fig. 7.3. The experimental curves were good agreement with the calculated curves. Therefore, the Gaussian distribution function assumed above is evidently appropriate to describe the complexation between copper(II) and binding site in the humic molecule.

On the other hand, Eqn. (7.8) can be displaced by using N and X,

$$N_i(K_i) = \frac{X c_{Lt}}{[HA(g\ l^{-1})]\sigma\sqrt{2\pi}} \exp[-\frac{1}{2\sigma^2}(\mu - \log K_i)^2] \quad (7.10).$$

The μ and σ values evaluated were summarized in Table 7.4, and then the profiles of continuous stability distribution were drawn according to Eqn. (7.10) (Fig. 7.4). In Fig. 7.4-a, the profile of TOTAL compared with those of each fractions. It can be predicted that the μ value of TOTAL can be agreement with the average of μ values of each fractions. However, the μ values of each fractions were larger than that of TOTAL. Furthermore, the μ value of MIX was compared with the μ values of each fractions (Fig. 7.4-b). The μ value of MIX was also lower than the μ values of each fractions.

From these results, it found that the copper(II) complexing abilities of TOTAL and MIX were lower than those of each fractions. This may be caused by that in the case of TOTAL or MIX, the copper(II) binding sites are shielded by the interaction between each fractions.

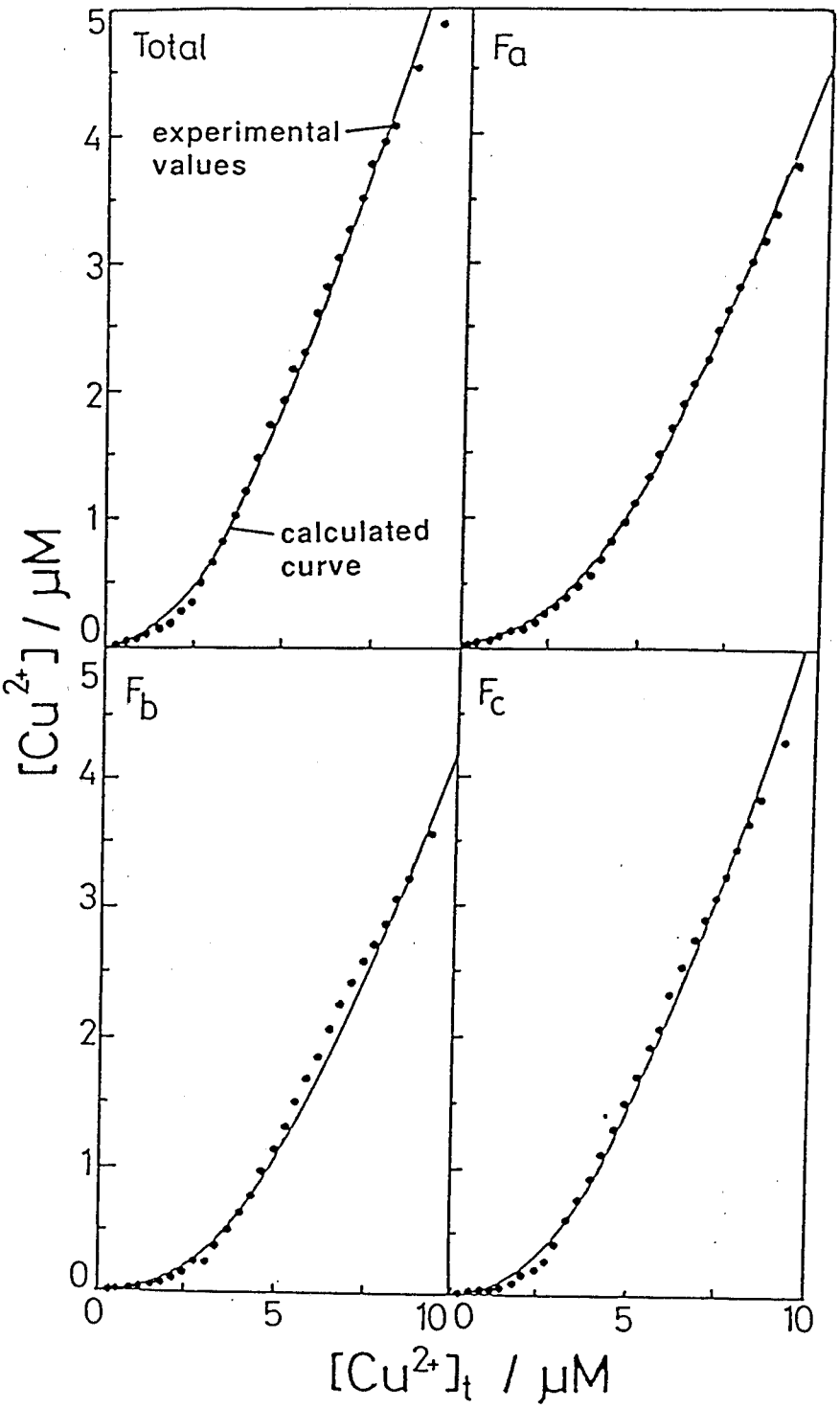

Fig. 7.3 Copper(II) titration curves of humic acids.

Humic acids: 10 mg l^{-1}, pH 6.

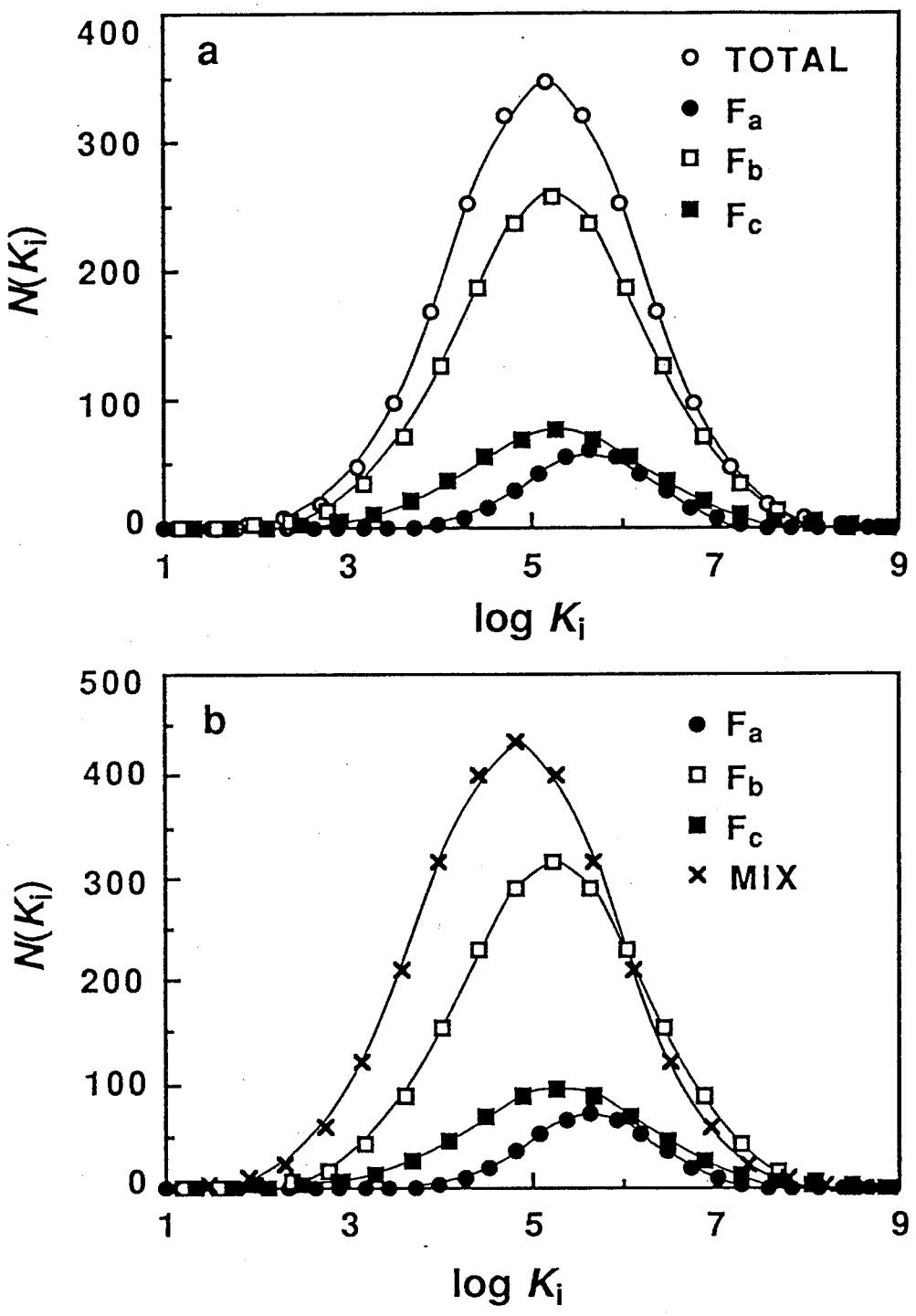

Fig. 7.4 Continuous stability distribution curves.

Comparison with TOTAL: a, MIX: b.

Table 7.4 The μ and σ values.

	TOTAL	MIX	F_a	F_b	F_c
μ	5.01	4.84	5.42	5.44	5.54
σ	1.35	1.05	1.10	0.98	1.00

7.6 UV-vis and fluorescence spectra

In order to ascertain the interaction between each fractions, the UV-vis and florescence spectra of humic acids were observed. The UV-vis spectra are shown in Fig. 7.5 where the vertical axis represents the absorptivity, e ($l\ g^{-1}$). A "MIX" in Fig. 7.5 represents the absorptivity of the mixture (n_{Fa}:n_{Fb}:n_{Fc} = 1:5:2). Moreover, a "SUM" in Fig. 7.5 represents the sum of the spectra of each fractions, and then the absorptivity of the SUM, e_{SUM}, can be written as bellow,

$$e_{SUM} = X_{nFa}e_{nFa} + X_{nFb}e_{nFb} + X_{nFc}e_{nFc} \qquad (7.11).$$

If there would be no interaction between each fractions, the absorption curve of MIX would trace that of SUM. The absorbance parameter, e_{400}/e_{600}, was often referred in the interpretation of UV-vis spectrum of humic substances [25]. This value concerns with the molecular weight of humic substances and the concentration of free radical. However, the values of e_{400}/e_{600} of SUM and MIX were 9.35 and 6.62, respectively. From these results, it found that the shape of the spectrum for SUM was different from that of MIX. Wang et al. reported that the sum spectrum of the molecular weight fractions of humic substances was not agreement with the spectrum of mixture. This suggests the interaction between each fractions such as the donor-accepter charge transfer mechanisms [22].

On the other hand, the fluorescence spectra of SUM and MIX were measured (Fig. 7.6). The intensity of SUM, I_{SUM}, can be written as,

$$I_{SUM} = X_{nFa}I_{nFa} + X_{nFb}I_{nFb} + X_{nFc}I_{nFc} \qquad (7.12).$$

Theoretically, the fluorescence intensity of MIX was expected to be the same as SUM. However, the experimental value of MIX was 20 % lower than the fluorescence intensity of SUM. This suggests the like an energy donor-acceptor interaction of humic molecules [22]. Therefore, the interaction between each fractions seems to occur in the case of MIX.

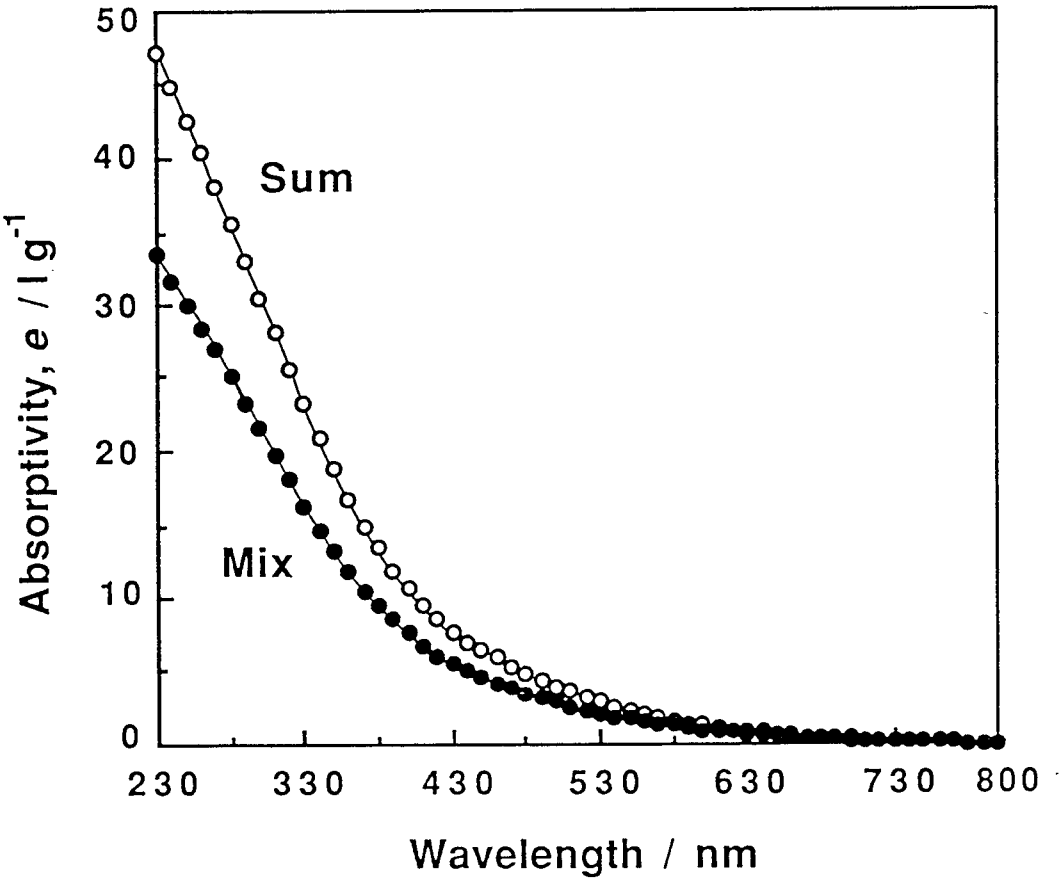

Fig. 7.5 UV-vis spectra of humic acid.

MIX: spectrum of mixed solution; $n_{Fa}:n_{Fb}:n_{Fc}$ = 1:5:2, SUM: sum spectrum of F_a, F_b and F_c.

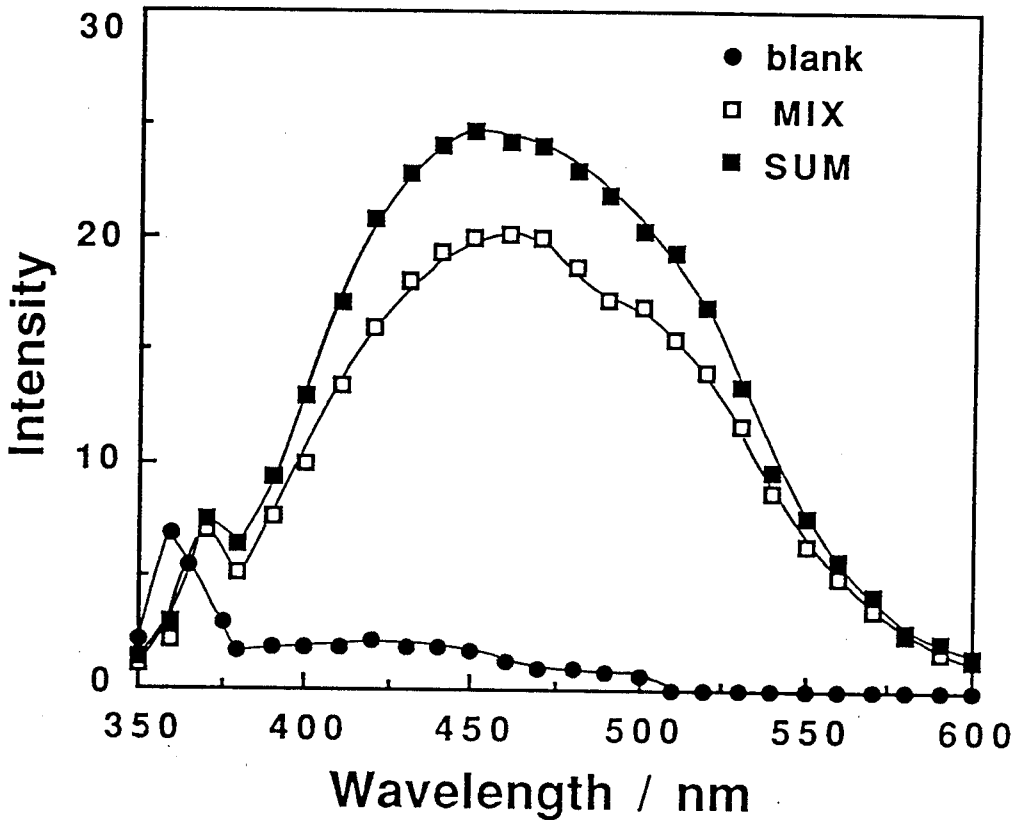

Fig. 7.6 Fluorescence spectra of humic acid.

MIX: spectrum of mixed solution; $n_{Fa}:n_{Fb}:n_{Fc}$ = 1:5:2, SUM: sum spectrum of F_a, F_b and F_c.

7.7 FTIR spectra

The part of FTIR spectra (2000 ~ 1200 cm^{-1}) are shown in Fig 7.7. The focus was the peak about 1750 ~ 1600 cm^{-1} region which showed C=O stretching of carboxylic acid and ketones. The peaks were separated two sections. The peak about 1710 cm^{-1} shows the protonated carboxylic acid, and then the peak about 1620 cm^{-1} shows the carboxylate anion [26]. Moreover, the peaks about 1710 cm^{-1} may also show the dimerization of carboxylic acid by hydrogen bond. When there are the interaction between the molecular weight fractions with the hydrogen bond of carboxylate, the peaks about 1710 cm^{-1} might be emphasized. Therefore, the peak areas about 1710 cm^{-1} (1800 ~ 1640 cm^{-1}) compared with those about 1620 cm^{-1} (1640 ~1520 cm^{-1}). The ratios of peak areas about 1710 cm^{-1} to those about 1620 cm^{-1} were as follows: 0.753; TOTAL, 1.249; MIX, 0.608; F_a, 0.453; F_b, 0.572; F_c. The peak areas about 1710 cm^{-1} of TOTAL and MIX were higher than those of each molecular weight fractions. Therefore, these results suggest that the hydrogen bond of carboxylic acid in humic molecules was proceeded in the mixture such as TOTAL and MIX.

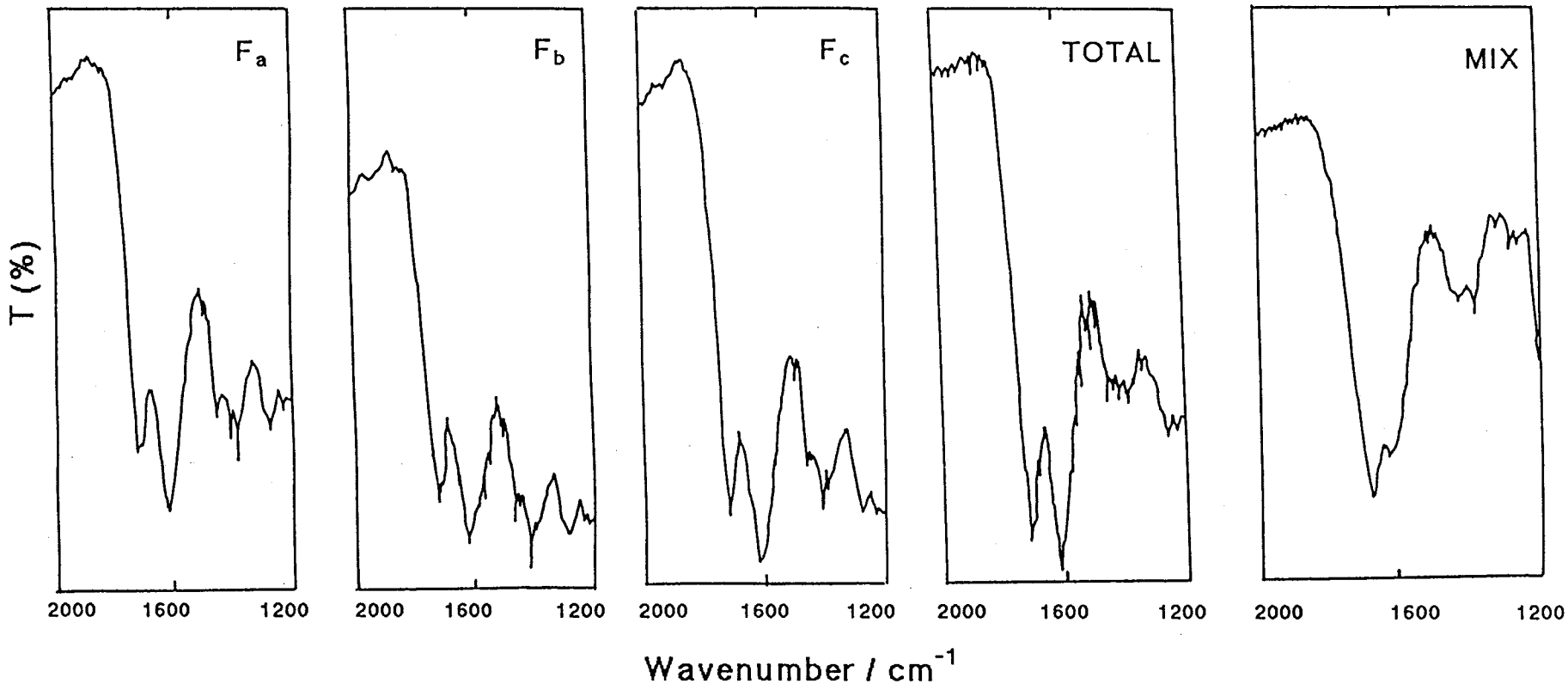

Fig. 7.7 FTIR spectra of humic acids.
The spectra of F_a, F_b, F_c and TOTAL were observed by a Perkin Elmer 1720-X type FTIR spectrometer, the spectrum of MIX was observed by a FTIR-5300 type FTIR spectrometer.

7.8 Conclusions

The molecular weight fractions of humic acid was prepared. The copper(II) complexing capacities were evaluated by a discrete model, and then the mean values of metal binding constants, μ, were evaluated by the continuous stability distribution function model. It was noted that the copper(II) complexing abilities of the mixture for TOTAL and MIX were smaller than those of the molecular weight fractions. This was due to occur the interaction between the molecular weight fractions. This mechanism was confirmed by the UV-vis, fluorescence and FTIR spectra. In the UV-vis and fluorescence spectra, the comparison of the MIX with SUM spectrum suggested the interaction between the molecular weight fractions. Moreover, it was suggested by the FTIR spectra, where the hydrogen bond was proceeded in the mixture of each fractions. Therefore, the copper(II) binding sites might be shielded by the hydrogen bond of carboxylic acid. These mechanism was shown in Fig. 7.8. This is the typical model of humic molecule by Schnitzer et. al [27]. The copper(II) binding sites mainly seem to be carboxylic acids which were free state in molecules (the arrows in Fig. 7.8). However, when the other molecular weight fraction would be added, the hydrogen bonds in the free state carboxyl groups would be proceeded by the donor-accepter interactions, hydrophobic interaction, van der Waals force, London force, and so on. The carboxylic sites for copper(II) binding seem to be shielded by the hydrogen bond of each carboxilic sites. Therefore, copper(II) ions may be hardly able to coordinate with the humic molecules.

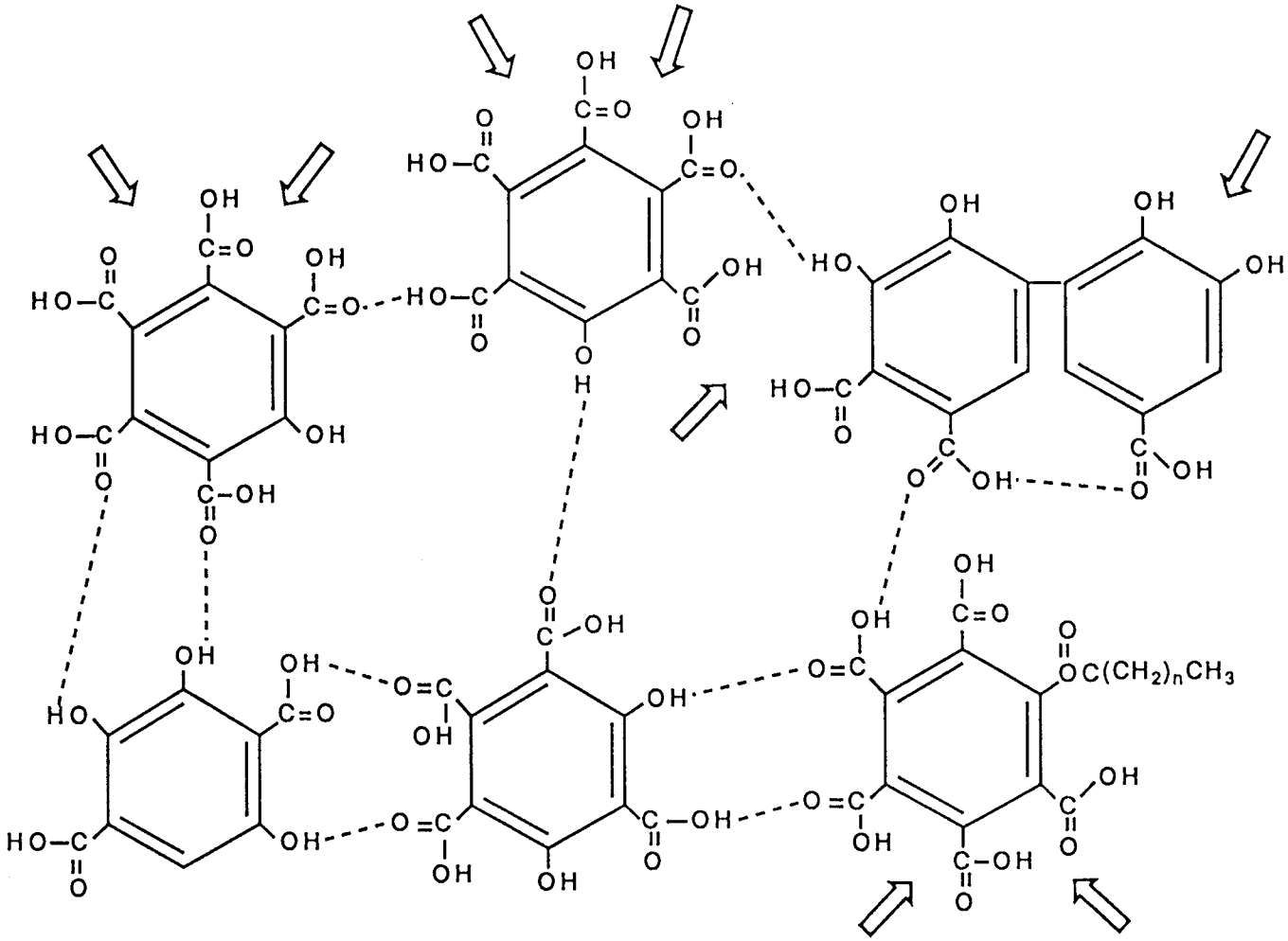

Fig. 7.8 Typical model of humic molecule presented by Schnitzer et al..
The arrows mean the copper(II) binding sites predicted.

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CHAPTER 8

INTERACTION BETWEEN COPPER(II) OXINATE AND HUMIC ACIDS

8.1 Introduction

Humic acids, which widely distribute in natural environments, are weak-acid polyelectrolyte, and then have the buffering actions and the cation exchange capacities in natural waters and soils [1-3]. Especially, the complexation between heavy metal ions and humic acids was studied in the points of view of the toxicity to phytoplanktons [4-6].

Still more, it was reported that humic acids had the aggregation points like a critical micellar concentration (CMC) and acted as the surface active agents [7-9]. Therefore, the non-polar domains such as aromatic compounds seem to exist in humic molecules. Hence, one interested in the interaction between hydrophobic domains in humic molecules and hydrophobic organic pollutants (HOPs) [10].

The knowledges about interaction between humic acids and HOPs were important because of the speciation of HOPs in natural waters. Although HOPs which are insoluble in water exist as the particle forms adsorbing on the clay minerals, the water solubility of HOPs increase in the presence of the dissolved organic carbon (DOC) such as humic acids [11,12]. For example, when the particles with phenanthrene and pyrene were suspended in the solution containing the surface active agents, the HOPs desorbed from the particles [13]. Therefore, it found that the water solubility of phenanthrene increased in the presence of DOC such as humic substances [14,15]. Also, the water solubility of HOPs such as DDT, PCB and lindane increased in the presence of humic substances [16,17]. These mechanisms were attribute to the "partition-like" interaction. Chiou et al.

concluded that strength of the interaction between HPOs and humic substances depend upon the polarity of humic substances. That is, this was explained by the experiment using the model compounds such as polyacrylic acid and the ratio of the polar elements (O and N) to the non-polar element (C) [18].

On the other hand, the metal chelate have been widely used as the fungicides [19]. Although the inorganic mixed copper(II) chemicals such as $CuSO_4$, CuO, $CuCl_2$ and so on were used for the same purpose, the metal chelate became to be used because of the excellent permeability for bio-membrane [20]. Therefore, the toxicity of the metal chelate for pytoplanktons would be stronger than that in the case of hydrate heavy metal ions in aqueous environments. It has been well-known that the toxicity of heavy metal ions is repressed by the complexation with humic substances [21-24]. However, it was reported that humic substances had the same effect in the metal chelate as that in heavy metal ions. For example, although copper(II)-oxinate, $Cu(OX)_2$, was strongly toxic for the *coliform bacilli* and the killifish, the toxicity was weakened in the presence of humic substances [25].

The $Cu(OX)_2$ recently used as the fungicide in the golf links, and then the outflow of the $Cu(OX)_2$ around there has been become a serious environmental pollution. Soil organic matter such as humic substances seems to concern with the outflow of $Cu(OX)_2$ to the aqueous environments. Therefore, the knowledges about the interactions between metal chelate and humic substances are required in the point of view of environmental ecology.

In this chapter, the partition of $Cu(OX)_2$ into humic acid was assumed, and then the partition coefficients were evaluated by measuring the water solubility of $Cu(OX)_2$ in the presence of humic acids. Moreover, the evaluation of the partition coefficients was performed to the various humic

acids extracted from the peat and the marine sediment. The dependences of pH, ionic strength, copper(II) complexing abilities and the amounts of acidic functional groups on the partition coefficients were investigated, and then the interaction between $Cu(OX)_2$ and humic acids was discussed according to these results.

8.2 Evaluation of partition coefficients

The $Cu(OX)_2$ can slightly dissolve in water, and then product the many species such as $CuOX^+$, Cu^{2+}, OX^-, HOX and H_2OX^+. In the presence of humic acid, the following solubility and partition equilibria were assumed,

$$Cu(OX)_2\,(s) \underset{S_w}{\rightleftharpoons} Cu(OX)_2\,(aq) \underset{K_{doc}}{\rightleftharpoons} Cu(OX)_2\,(HA) \qquad (8.1)$$

where S_w and K_{doc} represent the water solubility of $Cu(OX)_2$ and the $Cu(OX)_2$ partition coefficient, respectively. In the absence of humic acid, the water solubility of $Cu(OX)_2$, S_w, can be written as follows,

$$S_w = [Cu(OX)_2]_{aq} + [CuOX^+] + [Cu^{2+}] \qquad (8.2)$$

where the subscription, aq, means the species in aqueous phase. In the presence of humic acid, if the species, $Cu(OX)_2$, would be only distribute into humic acid according to the solubility equilibrium, the apparent water solubility of $Cu(OX)_2$, S_w^*, could be written as follows,

$$S_w^* = [Cu(OX)_2]_{aq} + [CuOX^+] + [Cu^{2+}] + [Cu(OX)_2]_{HA} \qquad (8.3)$$

where the subscript, HA, means the species distributed into humic acid. The partition coefficient, K_{doc}, can be defined as the ratio of the amounts of $Cu(OX)_2$ distributed into unit gram of carbon in humic acid to the $Cu(OX)_2$ in aqueous phase. Therefore, the partition coefficient, K_{doc}, can be written as,

$$K_{doc} = \frac{[Cu(OX)_2]_{HA}}{[DOC][Cu(OX)_2]_{aq}} \qquad (8.4)$$

where the [DOC] (mol l^{-1}) represents the concentration of dissolved organic carbon originated from humic acid. The following relationship was derived from Eqns. (8.2) - (8.4),

$$S_w^* = S_w + K_{doc}[Cu(OX)_2]_{aq}[DOC] \qquad (8.5).$$

The Eqn. (8.5) represents the linear relationship between S_w^* and [DOC], and then the K_{doc} can be evaluated by the slope and the $[Cu(OX)_2]_{aq}$. Therefore, if the $[Cu(OX)_2]_{aq}$ is constant according to the solubility equilibrium, the partition coefficients can be evaluated by measuring the water solubility of $Cu(OX)_2$ in various concentration of humic acid.

On the other hand, the $[Cu(OX)_2]_{aq}$ can be calculated by considering the dissociation equilibria of $Cu(OX)_2$ in the absence of humic acid. In the absence of humic acid, the dissociation equilibria of $Cu(OX)_2$ are as follows,

$$Cu(OX)_2 \rightleftharpoons CuOX^+ + OX^-$$

$$K_2 = \frac{[CuOX^+][OX^-]}{[Cu(OX)_2]} \qquad (8.6)$$

$$CuOX^+ \rightleftharpoons Cu^{2+} + OX^-$$

$$K_1 = \frac{[Cu^{2+}][OX^-]}{[CuOX^+]} \qquad (8.7).$$

The proton dissociation of oxine (HOX) and the acid dissociation constants, $K_{a1}$and K_{a2}, can be written as follows,

$$HOX \rightleftharpoons H^+ + OX^-$$

$$K_{a2} = \frac{[H^+][OX^-]}{[HOX]} \qquad (8.8)$$

$$H_2OX^+ \rightleftharpoons H^+ + HOX$$

$$K_{a1} = \frac{[H^+][HOX]}{[H_2OX^+]} \qquad (8.9).$$

From Eqns. (8.2) and (8.6) - (8.9), the following equation can be derived,

$$[Cu(OX)_2]_{aq} = \frac{[OX^-]^2 S_w}{[OX^-]^2 + K_2[OX^-] + K_1K_2} \qquad (8.10).$$

The total concentration of oxine, C_{OX}, can be written as,

$$C_{OX} = 2[Cu(OX)_2]_{aq} + [CuOX^+] + [OX^-] + [HOX] + [H_2OX^+] \qquad (8.11).$$

The following cubic equation can be derived from Eqns. (8.6) - (8.11),

$$[OX^-]^3 + K_2[OX^-]^2 + \frac{K_1K_2\alpha - S_wK_2}{\alpha}[OX^-] - \frac{2S_wK_1K_2}{\alpha} = 0 \qquad (8.12)$$

where α is defined as below,

$$\alpha = 1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}} \qquad (8.13).$$

The $[OX^-]$ can be estimated by solving in Eqn. (8.12). The $[Cu(OX)_2]_{aq}$ can be calculated by substituting the $[OX^-]$ value for Eqn. (8.10). The dissociation constants of $Cu(OX)_2$ and the acid dissociation constants of HOX were used for the reported values [26].

8.3 Experimental Section

Humic acids

The humic acids used in this chapter were SHHA, BHA and SAHA and FBHA. The humic acids extracted by sodium hydroxide, subsequently precipitated by hydrochloric acid and then purified according to Chapter 2. The concentration of the dissolved organic carbon originated from humic acid, [DOC], was calculated by the following equation,

$$[\text{DOC (mM)}] = \frac{[\text{humic acid (mg l}^{-1})]\text{x}(\%\text{C}/100)}{12.011\ (\text{mg mmol}^{-1})} \qquad (8.14)$$

Reagents

A copper(II) oxinate dihydrate was prepared by mixing the aqueous solution of copper(II) acetate with the ethanol solution of oxine [27]. The stock solution of copper(II) oxinate was prepared as the saturated solution of copper(II) oxinate in ethanol. The concentration of copper(II) oxinate in the stock solution was determined by measuring copper(II) by means of an atomic absorption spectrophotometry (AAS). The concentration of the stock solution measured was 300 mM. Sodium acetate (pH<5.5), 2-(Morpholino)ethanesulfonic acid (MES, pH 6) and *N*-(2-Hydroxyethyl)piperidine-*N'*-2-ethanesulfonic acid (HEPES, pH 7, 8) were used as the buffering agents (Dojindo Laboratories).

Measurement

An 1-ml aliquot of the stock solution of copper(II) oxinate and the buffer were put into the 25 ml of volumetric flask and was shaken with humic acid for 4 hours at 20 °C. The solution was filtered through by a membrane filter (0.45 mm). The copper(II) species in the filtrate were determined by an AAS. The total concentrations of copper(II) species in the filtrate represent the

apparent water solubility of copper(II) oxinate, S_w^*. The procedure for the measurement of the water solubility of HOX was also performed by the same method as that in the case of copper(II) oxinate. The absorbance of oxine at 310 nm was determined in this case.

Evaluation of the copper(II) complexing capacity or the amounts of the acidic functional groups was done by the cation-exchange method or the conductimetry according to the previous works [28,29]. Moreover, in order to determine copper(II) species bound with humic acid, the method using a diethylaminoethyl Sephadex A-25 (an A-25 method) was adopted [30]. In this case, the filtrate was passed through the A-25 column.

Apparatus

A Hitachi 170-50 type atomic absorption spectrometer was used to determine the copper(II) in the filtrate. A CG-201 PL type conductimetric cell and a CM-5b type conductimeter (TOA Electronics Ltd.) were used to measure the conductivity. A 6366 type glass electrode and a M-13 type pH meter (Horiba Ltd.) were used to measure the pH. The infrared spectra were measured by using a 1720-X type FTIR spectrometer (Perkin Elmer). The measurements were done with a KBr disk method. Furthermore, the UV-vis spectra were measured by using an Ubest-30 type spectrophotometer (Japan Spectroscopic Co., Ltd.).

8.4 Dependence of the water solubility of $Cu(OX)_2$ on [DOC].

The relationships between S_w^* and [DOC] were shown in Fig. 8.1. As was expected in Eqn. (8.5), the values of S_w^* linearly increased with a increase of [DOC]. The Y-intercepts of these lines (Fig. 8.1) represents the water solubility of $Cu(OX)_2$, S_w, in the absence of humic acid. Therefore, the K_{doc} values of humic acids could be evaluated by the slopes of the lines and $[Cu(OX)_2]_{aq}$. These values were summarized in Table 8.1.

On the other hand, the concentration of the species dissociated from $Cu(OX)_2$ were calculated by the S_W values in the absence of humic acid (Fig. 8.2). The $Cu(OX)_2$ was the predominant species at pH 7 or 8, and then the concentration of other species were negligible. Therefore, the adoption of Eqn. (8.5) could be appropriate at these pHs. However, the $[CuOX^+]$, $[Cu^{2+}]$, $[OX^-]$, [HOX] and $[H_2OX^+]$ values could not be negligible for the $[Cu(OX)_2]_{aq}$ value below pH 6. Especially, the $[CuOX^+]$ and $[Cu^{2+}]$ may be bound with humic acid. Therefore, the partition of oxine and the complexation with humic acid have to be considered.

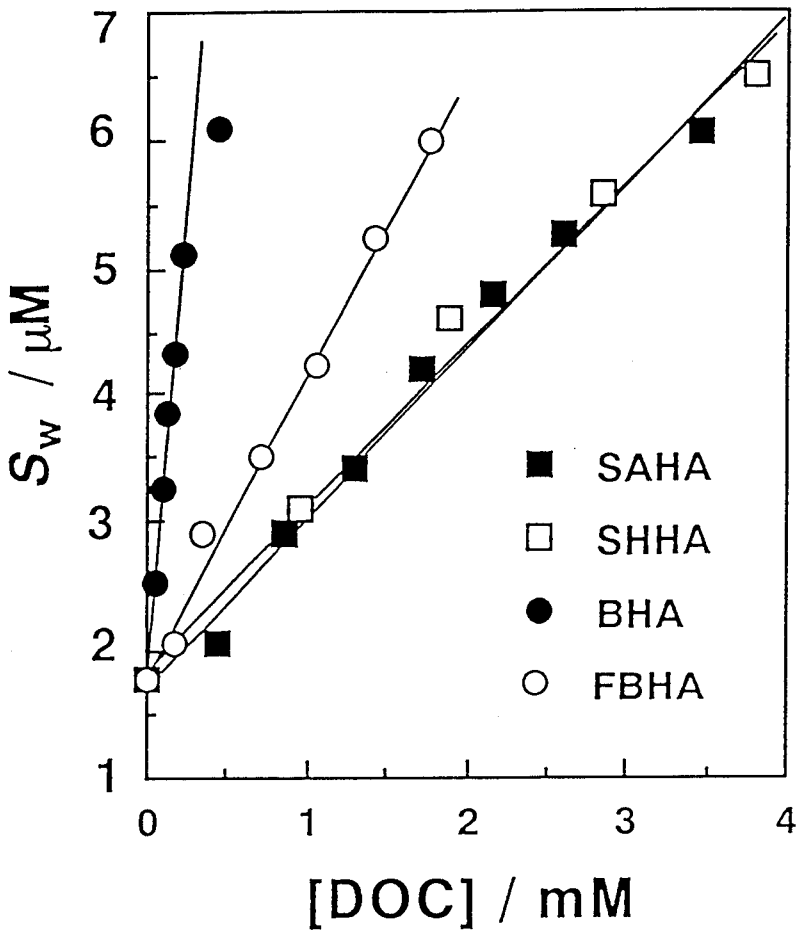

Fig.8.1 Typical S_w^* *vs* [DOC] plot of various humic acids.

0.01 M MES/NaOH buffer (pH 6)

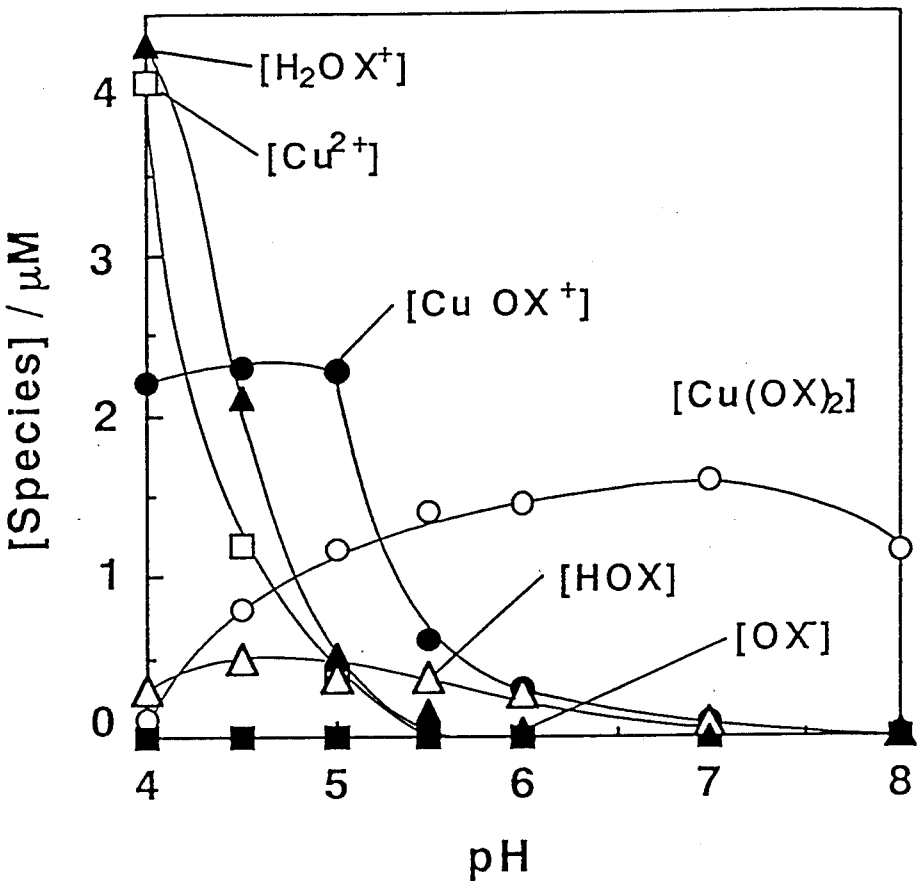

Fig.8.2 Concentration of species derived from $Cu(OX)_2$ in the absence of humic acids.

Buffer concentration 0.01M, pH 4 - 5.5: HOAc/NaOAc buffer, pH 6: MES/NaOH buffer, pH 7 - 8: HEPES/NaOH buffer.

Table 8.1 Partition coefficients and amounts of functional groups of various humic acids.

Humic acids	SHHA	SAHA	FBHA	BHA
$\log K_{doc}$	2.82	2.89	3.18	3.91
$N^{a)}$	421	410	339	230
$A^{b)}$	82.1	64.8	56.3	43.5
(O+N)/C	0.885	0.776	0.795	0.668

a) copper(II) complexing capacities measured at pH 6: μmol Cu^{2+} per gram of carbon in humic acid.

b) amounts of acidic functional groups: mmol H^+ per gram of carbon in humic acid.

8.5 Effect of partition of free oxine

If the interaction between oxine (HOX) and humic acid would be remarkable, the dissociation equilibria of $Cu(OX)_2$ and HOX would change in the presence of humic acid. The HOX partition coefficient (K_{doc}^{HOX}) can be written as described in the case of $Cu(OX)_2$,

$$K_{doc}^{HOX} = \frac{[HOX]_{HA}}{[HOX]_{aq}[DOC]} \qquad (8.15).$$

And then, by combining Eqns. (8.4), (8.11) with (8.15), the following equation can be derived,

$$2(S_w^* - S_w) = 2K_{doc}[Cu(OX)_2][DOC] + K_{doc}^{HOX}[HOX][DOC] \quad (8.16).$$

The $Cu(OX)_2$ slightly dissolved in pure water, and then this water solubility was 8.0×10^{-7} M (20 °C). However, the water solubility of HOX was 4.5×10^{-3}M (20 °C). Therefore, HOX seems to be more hydrophilic character than $Cu(OX)_2$. Hence, it can be predicted that HOX is more slightly distributed into humic acid than $Cu(OX)_2$. If the interaction between HOX and humic acid could be negligible, the term of the $S_w^* - S_w$ would be larger than the term of $K_{doc}^{HOX}[HOX][DOC]$ in Eqn. (8.16).

In order to ascertain this, the HOX partition coefficients, K_{doc}^{HOX}, was evaluated. The relationship between the [DOC] and the water solubility of HOX, S_W^{HOX} and S_W^{HOX*}, can be represented as described in the case of $Cu(OX)_2$,

$$S_W^{HOX*} = S_w^{HOX} + K_{doc}^{HOX}[HOX]_{aq}[DOC] \qquad (8.17).$$

According to Eqn. (8.17), the K_{doc}^{HOX} could be evaluated by measuring the water solubility of HOX. In the absence of humic acid, the water solubility of HOX, S_w^{HOX}, can be written as follows,

$$S_W^{HOX} = [OX^-](1 + \frac{[H^+]}{K_{a2}} + \frac{[H^+]^2}{K_{a1}K_{a2}})$$

$$= \alpha[OX^-] \qquad (8.18)$$

The $[HOX]_{aq}$ can be calculated by the Eqn. (8.18). The partition coefficients

measured at pH 4.5 were summarized in Table 8.2. And then, the $S_w^*-S_w$ term and the K_{doc}^{HOX}[HOX][DOC] term was calculated by measuring S_W^* under [DOC] = 0.23 mM (Table 8.2). From these results, the following approximation can be constrained: $S_w^*-S_w >> K_{doc}^{HOX}$[HOX][DOC]. Therefore, following equation can be written as,

$$S_w^*-S_w \approx K_{doc}[Cu(OX)_2]_{aq}[DOC] \qquad (8.19).$$

Eqn. (8.19) was the same as Eqn. (8.5). Therefore, the HOX partition was negligible.

Table 8.2. Calculation of S_w^*-S_w and K_{doc}^{HOX}[HOX][DOC].

pH	logK_{doc}^{HOX}	S_w^*-S_w /M	K_{doc}^{HOX}[HOX][DOC] /M
BHA; [DOC]: 0.23 mM			
4.5	1.07	1.21×10^{-6}	1.31×10^{-9}
5.0	1.59	2.38×10^{-6}	3.41×10^{-9}
5.5	1.70	3.49×10^{-6}	4.39×10^{-9}
6.0	1.92	3.34×10^{-6}	5.49×10^{-9}
SHHA; [DOC]: 0.95 mM			
4.5	1.07	7.50×10^{-7}	5.41×10^{-9}
5.0	1.59	1.06×10^{-6}	1.41×10^{-8}
5.5	1.70	3.15×10^{-6}	1.81×10^{-8}
6.0	1.92	1.34×10^{-6}	2.27×10^{-8}

8.6 Effect of copper(II) complexation with humic acid

It found in Fig. 8.2 that the positive charged species such as Cu^{2+} and $CuOX^{+}$ increased below pH 6. If these positive species would form the complex with humic acid, amounts of these species in the absence of humic acid would be different from those in the presence of humic acid. The complexation between copper(II) ions and humic acid was well-known [32-34]. In Fig. 8.2, the dissociation of Cu^{2+} species was remarkable below pH 4.5. It was examined by the method using an A-25 [31] how the species, $CuOX^{+}$ or Cu^{2+}, bound with humic acid. The A-25 is a macroreticular, weak-base anion exchanger, and then the A-25 can retain the anionic complexed species such as Cu(II)-humic acid and CuOX-humic acid complexes but not the other species [31,35,36]. Hence, the anionic complexed species of copper(II) in the filtrate was retained by the A-25 method. If the complexation with humic acid would be remarkable, it could be predicted that the concentration of the copper(II) species by the A-25 method was larger than the value of the S_w^*-S_w. The relationship between the values by the A-25 method and the values of the S_w^*-S_w were shown in Fig. 8.3. These points existed on the line which was the slope 1 above pH 4.5. Therefore, the complexation between humic acid and Cu^{2+} or $CuOX^{+}$ could be negligible above pH 4.5. However, the values measured below pH 4.5 (data in the circle of Fig.8.3) were far away from the line of slope 1. From these results, the complexation with humic acid could not be negligible below pH 4.5.

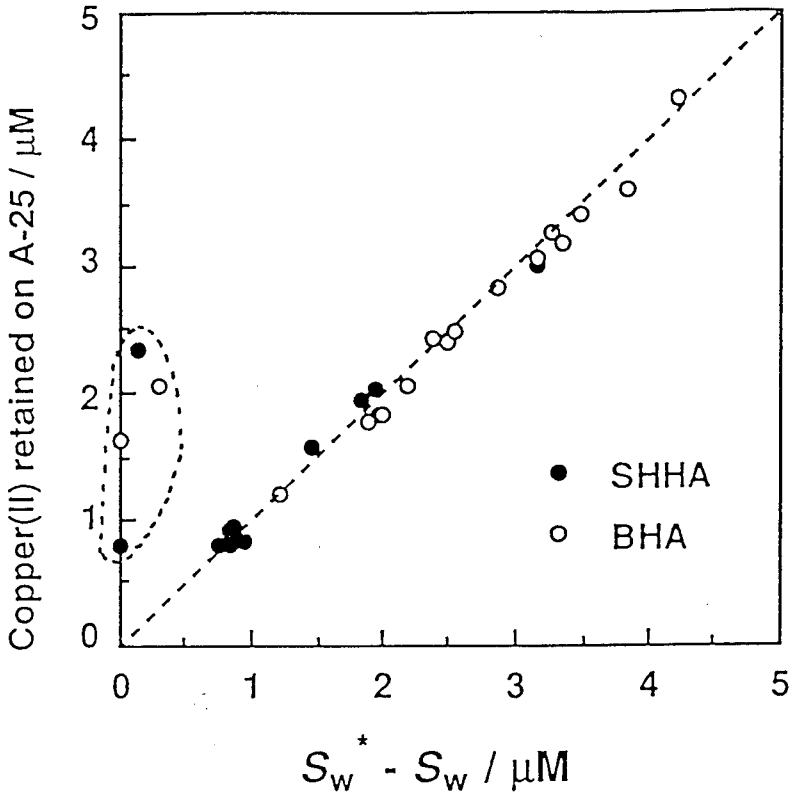

Fig. 8.3 Comparison of values of adsorbed Cu(II) species on humic acid derived from two methods.

[DOC]: 0.89 - 2.85 mM.

8.7 Effect of pH and ionic strength on partition coefficients

Dependence of pH on the K_{doc} of BHA and SHHA was shown in Fig. 8.4. The maximum of K_{doc} was at pH 5.5 for both BHA and SHHA. When the protonation of functional groups in polyelectrolyte would occur, the polarity of polyelectrolyte would decrease [7]. Therefore, the K_{doc} seems to increase with a decrease of pH from 8 to 5.5. However, the K_{doc} decreased below pH 5.5. This is due to decrease the amounts of $Cu(OX)_2$ with the dissociation. Furthermore, the decrease of the K_{doc} shows that the interaction between the Cu^{2+} or $CuOX^{+}$ and humic acid does not seem to be strong.

On the other hand, dependence of ionic strength on the K_{doc} values was shown in Fig. 8.5. The value of K_{doc} increased with an increase of ionic strength. When the ionic strength increase, the negative charges of humic acid shielded by the counter cation (in this case, Na^{+}). Moreover, it was reported that the aggregation point of humic acid decreased by adding inorganic salt like the CMCs of surface active agents [7]. Therefore, the increase of the K_{doc} values is attribute to decreasing of polarity of humic acid.

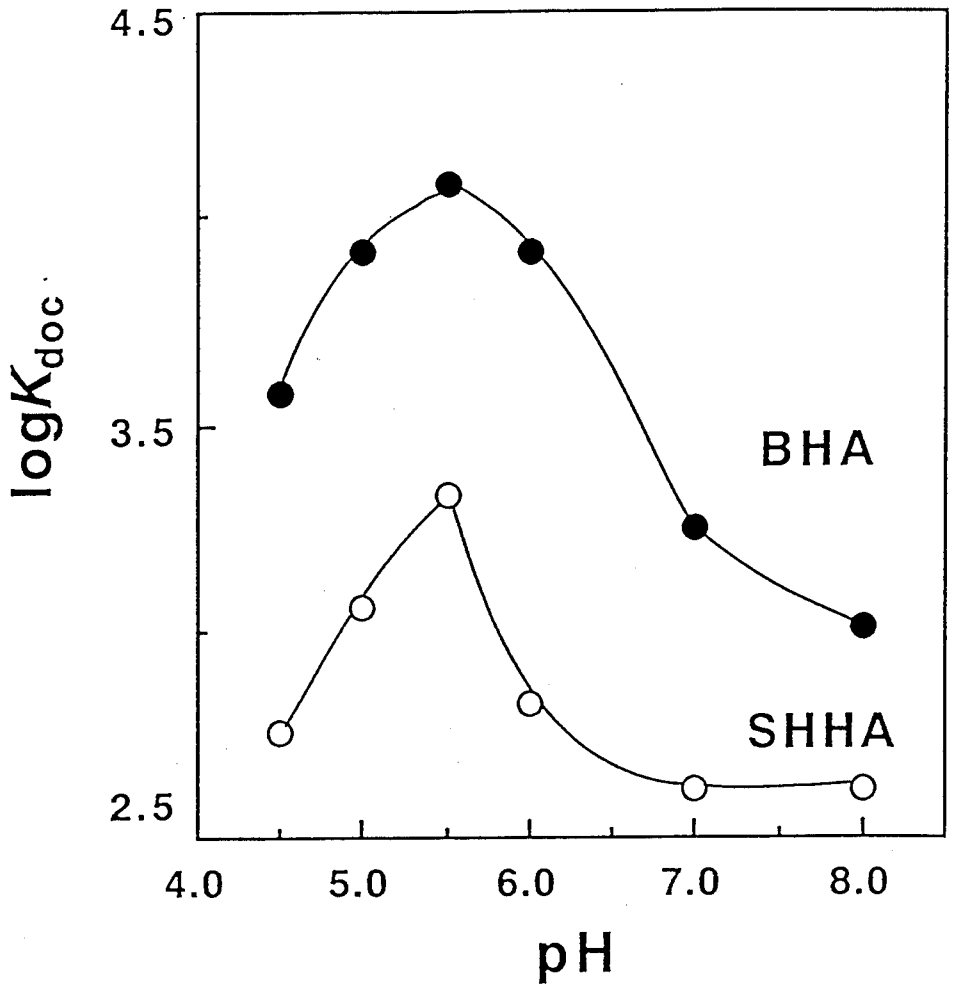

Fig.8.4 Effect of pH on K_{doc}.

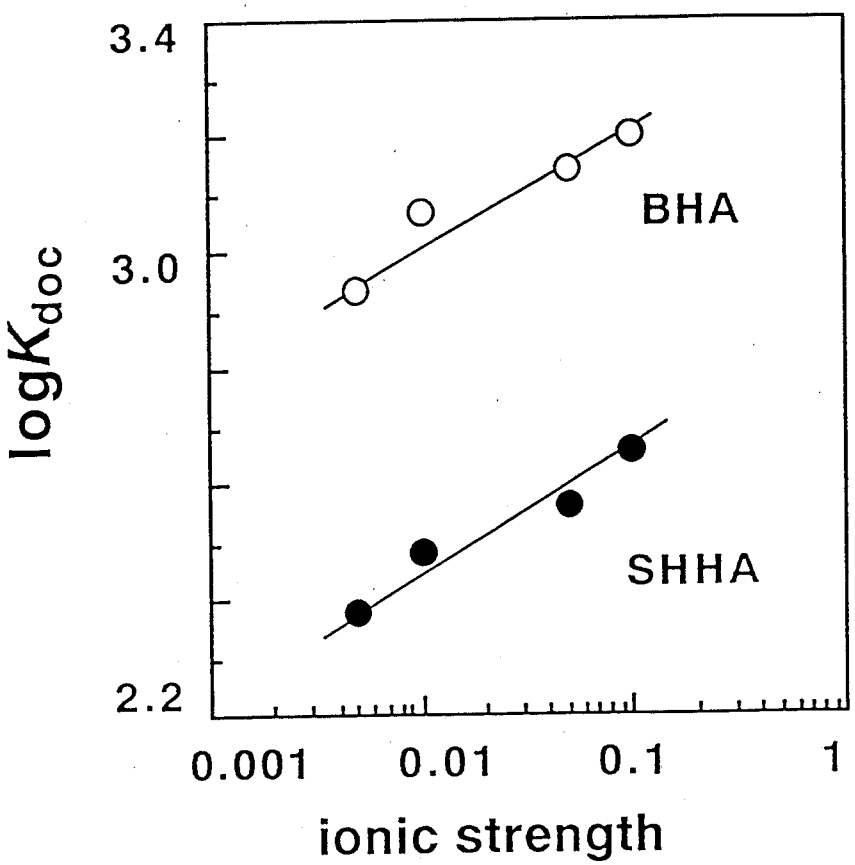

Fig.8.5 Effect of ionic strength on K_{doc}.

5 mM HEPES/NaOH buffer (pH 7.0).

8.8 Evaluation of the K_{doc} values of various humic acids

It was predicted by the results of pH and ionic strength dependence of the K_{doc} values that the polarity of humic acid might concern with the partition of $Cu(OX)_2$ into humic acid. One factor of polarity of humic acid was the copper(II) complexing capacity (N) and the amounts of acidic functional groups (A). In the previous work, the evaluations of the N and the A values were performed by the conductimetry and the cation exchange method [30]. The N and the A values were shown in Table 8.1. The order of the K_{doc} value was as follows: BHA>FBHA>SAHA≥SHHA. However, the orders of the N and the A values were SHHA>SAHA>FBHA>BHA. These were contrary to the order of the K_{doc} value. The orders of the N and the A values correspond to the orders of polarity of humic acids. Therefore, the smaller amounts of acidic functional groups and copper(II) binding site corresponds to the higher K_{doc} values.

On the other hand, Chiou et al. reported that the ratio of polar element such as O and N to nonpolar element such as C corresponded to the polarity of humic acids and then this fact concerned with the partition coefficients [18]. The (O+N)/C value of humic acids used in the present work are summarized in Table 8.1. The orders of these values are as follows: SHHA≥FBHA>SAHA>BHA. This tendency is different from those in the A or the N values. Especially, the humic acid from the marine sediment, FBHA, is the higher value. This reason will be discussed below.

The absorbance parameter, e_{400}/e_{600}, are summarized in Table 8.3. This corresponds to amounts of chromophore or auxochrome in humic acid [36]. The order of this value is as follow: SHHA> SAHA≥BHA>FBHA. This shows that the absorbance of FBHA in the ultraviolet region is the lowest. Since it has been known that the chromophore of humic acid is mainly the aromatic compounds [37,38], the lower absorbance in ultraviolet region suggests that

the lower amounts of aromatic compounds in the FBHA are lower than those in the others. On the other hand, the FTIR spectra of humic acids are shown in Fig. 8.6. All humic acids have the peaks at about 3400 and 1620 cm^{-1}. These peaks represent the O-H stretching of the hydroxyl groups and the C=O stretching of the carboxylic acids, respectively. The absorption maximum at about 1200 cm^{-1} in SHHA, SAHA and BHA is shifted to about 1100 cm^{-1} in FBHA. It was generally known that the C-O stretching of aromatic and aliphatic ether appears at about 1200 and 1100 cm^{-1}, respectively. Therefore, the humic acid from the marine sediment, FBHA, contains smaller amounts of aromatic compounds than the humic acids from peat, SHHA, SAHA and BHA. The ratios of the peak area at about 1100 cm^{-1} (A_{1100}) to that at about 3400 cm^{-1} (A_{3400}) or that at about 1620 cm^{-1} (A_{1600}) are summarized in Table 8.3. The ranges of the integration are also shown in Table 8.3. From these results, it found that the A_{1100}/A_{1600} and the A_{1100}/A_{3400} of FBHA were the largest value of the all. Therefore, alkyl chain may be largely contained in the FBHA. That is, the alcoholic hydroxyl groups or ether seems to be largely contained in this humic acid. Although the FBHA contained in the larger amounts of polar elements than the other humic acids from peat, the polarity of FBHA decreased. Therefore, the K_{doc} values of FBHA was larger.

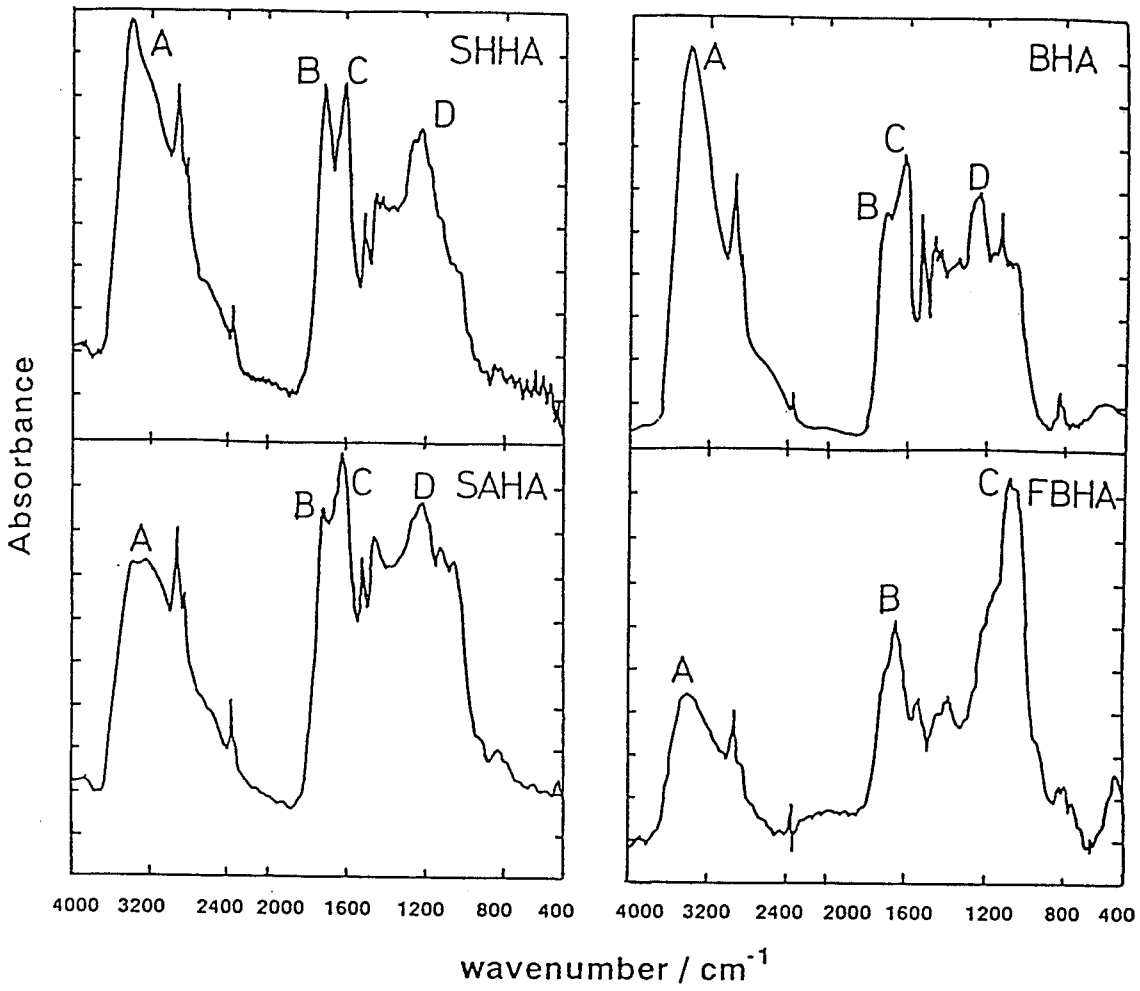

Fig. 8.6 FT-IR spectra of humic acids.

SHHA: A;3407, B;1718, C;1617, D;1224,

SAHA: A;3386, B;1708, C;1615, D;1216,

BHA; A;3387, B;1708, C;1615, D;1224,

FBHA; A;3399, B;1660, C;1085 (cm^{-1}),

KBr disk method.

TABLE 8.3. Photometric absorption parameter and data from FT-IR.

Humic acids	e_{400}/e_{600} a)	A_{1100}/A_{1600} b)	A_{1100}/A_{3400} c)
SHHA	9.00	1.131	0.826
SAHA	7.47	1.408	1.514
BHA	7.44	1.407	0.781
FBHA	6.65	2.714	2.427

a) ratio of absorptivity at 400 nm to 600 nm.

b) ratio of peak area at about 3400 cm^{-1} to 1600 cm^{-1}.

c) ratio of peak area at about 1100 cm^{-1} to 1600 cm^{-1}.

A_{3400}: 3600 - 3000 cm^{-1}, A_{1600}: 1800 ~ 1550 cm^{-1},

A_{1200}: 1300 - 950 cm^{-1}.

8.9 Conclusions

The partition coefficients of $Cu(OX)_2$ into humic acids could be evaluated by the water solubility of $Cu(OX)_2$ in the presence of humic acid. It found the polarity of humic acid depended on the partition of $Cu(OX)_2$ into the humic acid. Therefore, the interaction between $Cu(OX)_2$ and humic acid may occur mainly in the hydrophobic domain of the humic molecule.

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CHAPTER 9

SUMMARY

Recently, the detailed informations about the interactions between humic acids and heavy metal ions such as copper(II) or hydrophobic organic pollutants such as copper(II) oxinate have been desired. In order to obtain such the informations, the following investigation must be needed; (1) the development of the simple and sensitive methods for the speciation analysis of free metal forms and complexed forms, (2) characterization of the functional groups and hydrophobic domains in humic molecules, (3) the interpretation modes of the binding abilities which reflect the binding-sites in humic molecules. However, because of the poorer sensitivity of the usual methods, the discussions about complexing abilities were difficult at the trace metal level. Moreover, there are many data about the composition of elements and functional groups in humic substances but not the knowledges about the relation between the binding-sites and pollutants. Furthermore, the binding constants between the pollutants and humic substances were only evaluated by the mathematically attribution of experimental data without considering the form of binding-sites.

In the present work, the sensitive and simple methods for the speciation analysis of copper(II) in the presence of humic acid were developed by using the dextran ion-exchangers and anodic stripping voltammetry, and then these methods could be applied

to the evaluation of copper(II) complexing abilities (conditional stability constants and copper(II) complexing capacities). The copper(II) binding-site of humic acids were supposed by the comparison of the acid-base titration curves of humic acids with model compounds which resembled humic acids. The thermodynamic stability constant could be calculated by investigating the ionic strength dependence of copper(II) complexing abilities. The continuous stability function model were proposed in which this model reflected to the distribution of stability of binding-site in humic molecules. Finally, the interactions between copper(II) oxinate and humic acids were quantitatively discussed. The knowledges obtained in the present work are described as follows. In chapter 1, this was general introduction of the present work, and then the aids were described.

In chapter 2, the preparations of humic acids and the fundamental data such as the elemental analysis and UV-vis spectra were described.

In chapter 3, the sensitive method for the speciation analysis of copper(II) in the presence of humic acid was developed by using an anion-exchanger diethylaminoethyl Sephadex A-25 (A-25) column. The copper(II)-humic acid complex was separated and the free copper(II) species in effluent were determined by an AAS. Moreover, the method for speciation analysis of copper(II) was developed by using a cation-exchanger sulfopropyl Sephadex C-25 (C-25). This was performed by a batch operation. The

concentration of copper(II)-humic acid complex could be determined rapidly and simply, and then this method is useful for the treatment of water samples in the sampling spot. Still more, these methods described above were applied to the evaluation of copper(II) complexing abilities of humic acids extracted from peat in Hokkaido. The copper(II) complexing capacities and conditioning stability constants were evaluated by the Scatchard plot adopting to a two site model.

In chapter 4, the determination of uncomplexed labile copper(II) species were performed in the presence of humic acid by an anodic stripping voltammetry (ASV). If humic acid was present in the solution, the measurement of labile copper(II) was interfered because of the adsorption of humic acid on the electrode. To eliminate this interference, the addition of various surfactants were investigated. The addition of sodium dodecyl-sulfate (SDS) was found to be advantageous in measuring labile copper(II) species. This method was sensitive and could measure copper(II) at 10^{-7} M level.

In chapter 5, the relationship between copper(II) ions and copper(II) binding sites of humic acid was investigated by the acid-base titration, elemental analysis and FTIR. From these investigation, the carboxyl groups which had low pK_a values would concern with the strong binding site, and then the phenolic hydroxyl or amino groups which had high pK_a values would concern with the weak binding site in humic acid.

In chapter 6, effect of ionic strength on copper(II) complexing ability of humic acid was investigated, and then the thermodynamic stability constant was calculated.

In chapter 7, humic acid was separated by a size exclusion chromatography using a Sephadex G-50 column, and then the copper(II) complexing abilities were evaluated with respect to each fractions. In order to consider the distribution of binding sites in humic molecules, the continuous stability distribution function was proposed. The average stability constants between copper(II) ions and binding sites could be evaluated by this interpretation mode. It found that the copper(II) complexing ability of the mixture of each fractions was lower than that of each fractions. This suggested that the copper(II) binding sites were shield the binding site by the interaction between each fractions.

In chapter 8, interactions between the fungicide, copper(II) oxinate, and humic acids were investigated. In spite of water insoluble character of copper(II) oxinate, the water solubility of copper(II) oxinate dramatically increased in the presence of humic acids. This mechanism was attribute to the partition of copper(II) oxinate into the hydrophobic domain in humic acid, and then the partition coefficients were evaluated.

In chapter 9, the summary of the present work were described.

In the present work, the sensitive measurement of copper(II) can be performed in the presence of humic acids. Moreover, the

interaction between copper(II) ions or copper(II) oxinate and humic acids can be discussed quantitatively. The developed methods and the knowledges obtained in the present work will not give useful informations to only analytical chemistry but also environmental and soil chemistry.

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