Measurement of Copper Complexing Ability of Humic Acids by Using Diethylaminoethyl Sephadex A-25 Column

Mitsuhiko TAGA, Shunitz TANAKA and Masami FUKUSHIMA

Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, Japan

A method for the measurement of copper complexing ability of humic acids by using diethylaminoethyl Sephadex A-25 is reported. Since the A-25 resin can adsorb copper-humic acid complexes, but not free copper ions, the free copper ions were fractionated and then determined by graphite-furnace atomic absorption spectrometry. Conditional stability constants and complexing capacities of the humic acids were estimated by Scatchard plot adapting to two-site model. The method could be applied to the humic acids from peat soil, and the copper complexing ability of the humic acids could be estimated sensitively and simply.

Keywords: Humic acid, copper, complexing ability, diethylaminoethyl Sephadex A-25, Scatchard plot

Humic substances widely distribute in natural waters. Information about the interaction between humic substances and heavy metals (e.g. Cu, Cd, Ni, Co, etc.) is important for environmental chemistry. Recently, methods for measurement of the metal complexing ability of humic substances have been developed with ion selective electrodes, dialysis membrane and gel chromatography. However, these methods are not sensitive and simple enough to be applied directly to natural waters.

A macroreticular 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 nucleotides, peptides 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.

Using this property of A-25, Hiraide et al. evaluated the copper complexing capacities of humic acids in river waters after concentration by coprecipitation and floatation.

In this paper, the copper complexing abilities of humic acids were investigated more quantitatively using an A-25 resin column. A Scatchard plot was constructed to investigate the interaction between metal ions and organic ligands as humic substances in aqueous environment, and the complexing capacity was estimated by using the two-site model for these organic ligands which have many binding sites. In this paper, a Scatchard plot adapted to the two-site model was also used to estimate the conditional stability constants and the copper complexing capacities for humic acids after fractionation with A-25 resin.

Experimental

Reagents and materials

The humic acids were obtained from Fluka Chemie AG (HA) and peat soil (PHA). The peat soil was leached in 300 ml of 1 M potassium hydroxide solution for a week, and the supernatant (humic substances) and residue (humin) were separated by centrifugation. A 50 ml of concentrated hydrochloric acid was added to the supernatant and the mixture was kept for 2 d. The precipitate (humic acid) and the supernatant (fulvic acid) were fractionated by centrifugation. The humic acid obtained here was washed 5-6 times with hydrochloric acid, then with water, and dried at 30°C. The stock solutions of humic acids were prepared by dissolving 0.1 g of the humic acid powder in 0.1 M 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 0.1 M hydrochloric acid solution, washed with water and then swollen in the 0.05 M potassium chloride solution after adjusting pH to 6-7 by potassium hydroxide solution. The standard copper solution (1 g/l) was prepared by dissolving electric copper (purity 99.999%) in concentrated nitric acid and diluting to 0.01 M nitric acid solution. The ethylenediaminetetraacetic acid (EDTA) solution was prepared by dissolving EDTA-4H (Wako Pure Chemical Industries, Ltd.) in 0.1 M potassium hydroxide solution. The citric acid was of analytical reagent grade.
**Apparatus**

A polypropylene column (Bio-Rad) was used. 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 D2 lamp.

**Procedure**

After the pH of the sample solution containing copper and humic acid was adjusted, 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. Then the A-25 column was washed with water. By this procedure, copper-humic acid complex was adsorbed on A-25 resin, and only free copper ion was eluted down. The column system is shown in Fig. 1. The effluent was enriched by heating it on the hotplate and was then 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 GFAAS. The copper adsorbed on A-25 resin was determined as follows. The A-25 resin with copper-humic acid complex was transferred to a centrifuge tube and 8 ml of 4 M nitric acid solution was added. The copper 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 supernatant 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 the mixture to 25 ml. The 20 µl aliquot was injected into the graphite-furnace.

**Scatchard plot to estimate the copper complexing ability of humic acid**

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

\[
Cu^{2+} + L_e \rightleftharpoons CuL_e^{(e-2)}
\]

where \(L_e\) 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 complex formation, which are independent of each other, in the sample solution, the mass balance can be described by Eqs. (1)–(3):

\[
[M_e] = [M] + \sum [ML]
\]

\[
N_e = [L_e] + [ML_e]
\]

\[
K'_e = [ML] / [M][L_e]
\]

where \([M_e]\) is the total copper concentration in the sample solution, \([M]\) is the concentration of free copper ions, and \(K'_e\) and \(N_e\) show the conditional stability constants and copper complexing capacities of each site.

Substitution and rearrangement of these equations give

\[
[M_e] - [M] = \Sigma(K'_e[M]N_e) / (1 + K'_e[M]).
\]

In case of one-site, from Eq. (4)

\[
([M_e] - [M]) / [M] = -K([M_e] - [M]) + K'N_e.
\]

If \((([M_e] - [M]) / [M])\) is plotted against \(([M_e] - [M])\), Scatchard plot becomes linear and the conditional stability constant \(K'\) and copper complexing capacity \(N_e\) 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 have from Eq. (4)

\[
[M_e] - [M] = \frac{K'_eN_1}{1 + K'_e[M]} + \frac{K'_eN_2}{1 + K'_e[M]}.
\]

On the other hand, in the case of a two-site model assuming two binding sites independent of each other, we have from Eq. (4)

\[
\frac{[M_e] - [M]}{[M]} = \frac{K'_eN_1}{1 + K'_e[M]} + \frac{K'_eN_2}{1 + K'_e[M]}
\]

where \(K'_1\) and \(K'_2\) are the conditional stability constants of the two sites, and \(N_1\) and \(N_2\) are the copper complexing capacities of each site.
In this case, Scatchard plot is the lower projected curve, as shown in Fig. 2. 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 \( (N_1, N_2) \).

For the division into two lines, the regression analysis was used. We use the name “strong site” for the higher stability site \( (K'_1, N_1) \) and “weak site” for the lower stability site \( (K'_2, N_2) \).

Results and Discussion

**Adsorption of copper-humic acids complexes on A-25 resin**

Figure 3 shows the adsorption behavior of copper-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 \( pK_{a1}=3.08 \), \( pK_{a2}=4.74 \) and \( pK_{a3}=5.40 \), the stable anionic complex was formed at the pH region beyond \( pK_{a3} \), and approximately quantitative adsorption was achieved.

The amount of copper-humic acids (HA or PHA) 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.

**[M] vs. [M] plots**

Figure 4 shows the \([M]\) vs. \([M]\) plots, where free copper concentration \([M]\) is plotted against the total copper concentration in sample solution \([M]\).

When sample solution without the ligands was passed through an A-25 resin column, the \([M]\) vs. \([M]\) plot was linear and the slope of this line (Fig. 4 (a)) was 1. Therefore, it was confirmed that free copper 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 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-EDTA complex was quantitatively adsorbed on A-25 resin (Fig. 4 (d)). The \([M]\) vs. \([M]\) plots of HA or PHA as the ligands were the lower projected curves (Fig. 4 (b), (c)) because of complexing formation with the humic acids was weaker than that of EDTA.

**Copper complexing ability of citric acid**

In order to ascertain how suitable it was to estimate the copper complexing ability by using A-25 resin column, the measurement for 3 µM of citric acid as a standard ligand was performed. The Scatchard plot at pH 7 was linear, as shown in Fig. 5. \( K' \) and \( N \) were calculated from the slope and intercept of this line. The values estimated were \( \log K'=5.7 \) and \( N=2.2 \) µM. The value for \( \log K' \) was in good agreement with the

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Fig. 3 Effect of pH on the adsorption of copper complexes on A-25. The sample solution contained 60 nmol of copper and the ligands: 0.25 µmol of citric acid (a), 1 mg of HA (b) and 0.25 mg of PHA (c).

Fig. 4 [M] vs. [M] plot. A 25 ml of sample solution (pH 6.0) containing 0 - 3.8 µM copper was used. (a) without ligands, (b) 10 mg/l of PHA, (c) 40 mg/l of HA and (d) 2 µM of EDTA.

Fig. 5 Scatchard plot for citric acid. A 25 ml of sample solution (pH 7.0) was used. Citric acid, 3 µM.
These results suggest that the method could be applied to the measurement of the complexing ability of humic acids.

Copper complexing ability of humic acids

Figure 6 shows a Scatchard plot for PHA 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 copper complexing capacities \((N_1', N_2')\) 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 1.

The obtained conditional stability constants between copper and humic acids were within the range of that reported by Bresnahan et al., Montoura et al. (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, 40 mg/l) than for other methods (over 1 g/l). The proposed method would be a sensitive and simple one for the measurement of copper complexing ability of humic acids in natural waters.

Table 1  Conditional stability constants \((K_1', K_2')\) and copper complexing capacities \((N_1', N_2')\) for the humic acids at pH 6

<table>
<thead>
<tr>
<th>Humic acids</th>
<th>Strong site</th>
<th>Weak site</th>
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<tbody>
<tr>
<td>HA (40 mg/l)</td>
<td>(\log K_1') 7.6 (N_1'/\mu M) 2.0 (\log K_2') 5.6 (N_2'/\mu M) 0.8</td>
<td></td>
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<tr>
<td>PHA (10 mg/l)</td>
<td>(\log K_1') 6.8 (N_1'/\mu M) 1.5 (\log K_2') 5.1 (N_2'/\mu M) 1.7</td>
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References


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