# Stopped-Flow Method for Evaluating Copper(II) Complexing Ability of Humic Acid

Hideyuki ITABASHI*, Hisayo MIYAMAE, and Hiroshi KAWAMOTO

Department of Applied Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan

## Abstract

A simple method for evaluating the copper(II) complexing ability of humic acid was developed based on spectrophotometric determination of copper(II). The method, using a stopped-flow system, enabled us to determine not only copper(II) complexing capacity but also the lability of copper(II)-humic acid complex. Feasibility of the proposed system was confirmed by using some aminopolycarboxylic acids instead of humic ones. The established method was applied to humic acid sample solutions, and the results revealed that the humic acids form a considerably stable complex with copper(II).

**Keywords** Complexing capacity, lability, copper(II), humic acid, stopped-flow system

## 1. Introduction

In recent studies of analytical chemistry, speciation of heavy metals in natural waters is one of the most important tasks, because bioavailability of the metal ions to aquatic organisms depends heavily on their chemical forms [1,2]. For toxicity of copper(II) to aquatic organisms such as algae, the effect significantly varies by the lability of copper(II) species: generally, the toxicity of labile species is much higher than inert ones [3]. In the speciation of copper(II), therefore, evaluation of the lability is also important as well as discrimination of their chemical forms.

In our previous studies, to estimate the chemical form of copper(II) in natural waters, methods for measuring copper(II) complexing capacity (CuCC) were developed based on the solvent extraction technique, and applied to speciation of copper(II) in river- and pond-water samples [4-6]. The results indicated that most of the copper(II) in the samples exists as complexes binding with naturally occurring ligands; the concentration of free copper(II) was estimated to be $10^{-12}$ - $10^{-13}$ mol dm$^{-3}$. Further, our recent studies, in which stability constants of copper(II) complex formed with naturally occurring ligands were measured, revealed that the copper(II) species in natural waters are likely to be humic acid complexes [7,8]. Therefore, the evaluation of the complexing ability of humic acid is extremely important estimating the toxicity of copper(II) in natural water.

This paper describes a novel method for evaluating the complexing ability of humic acid. The method provides a simple and effective means of measuring the lability of copper(II)-humic acid complex as well as CuCC of humic acid. By using a stopped flow system, both values, CuCC and lability, are obtained only for one measurement. In this paper, feasibility of the proposed method was confirmed by using some chelating agents instead of humic acid. The established method was applied to sample solutions containing commercial humic acids.

* Corresponding author
E-mail: itabashi@chem.gunma-u.ac.jp

## 2. Experimental

### 2.1 Reagents

A stock solution of copper(II) was prepared by dissolving copper(II) sulfate pentahydrate of analytical grade in $5 \times 10^{-2}$ mol dm$^{-3}$ sulfuric acid. Stock solutions of bathocuproinedisulfonic acid (BCS), ethylenediaminetetraacetic acid (EDTA), diaminopropanoltetraacetic acid (DPTA-OH) and Etylenediaminedipropionic acid (EDDP) were prepared by dissolving 2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid disodium salts (Dojindo Lab.), ethylenediamine-$N,N,N',N'$-tetraacetic acid disodium salt dehydrate (Dojindo Lab.), 1,3-diamino-2-hydroxypropane-$N,N,N',N'$-tetraacetic acid (Dojindo Lab.) and ethylemediamine-$N,N'$-dipropionic acid dihydrochloride (Dojindo Lab.) in water, respectively. Stock solutions of humic acid, Wako HA, Aldrich HA and Cheetah HA, were prepared by dissolving commercial humic acids (Wako Pure Chemical Co., Aldrich Chemical Co. and Cheetah Chemical Co.) in 0.2 mol dm$^{-3}$ 3-morpholinopropanesulfonic acid (MOPS) buffer (pH 7.0) solution and filtered through $\phi$0.45 μm membrane filter. Concentrations of the humic acid solutions were determined by measuring the absorbance of the solutions at 410 nm [9].
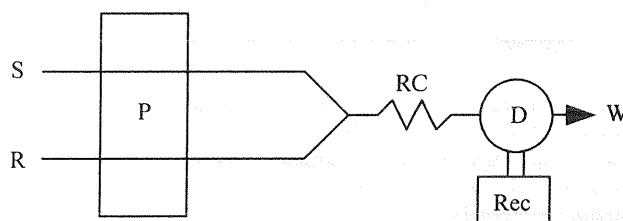


Fig. 1 Schematic diagram of the flow system.
S: sample solution, R: reagent solution ($4 \times 10^{-4}$ mol dm$^{-3}$ BCS, $2 \times 10^{-3}$ mol dm$^{-3}$ hydroxylamine hydrochloride), P: pump (flow rate, 1.2 cm$^3$ min$^{-1}$), RC: reaction coil (20 cm × 1 mm i.d.), D: spectrophotometric detector, Rec: recorder, W: waste.

## 2.2 Apparatus

A schematic flow diagram for the estimation of the complexing ability of humic acid is shown in Fig. 1. A double plunger pump (Sanuki Kogyo, DMX-2000) was used for propelling a sample solution and a reagent solution. The flow lines were made of PTFE tubing (1mm i.d.) and connectors. The absorbance was measured at 485 nm with a Soma Kogaku S-3250 spectrophotometer with a 10-mm micro flow cell (8 mm$^3$) and was recorded on a Hitachi Model 056 recorder.
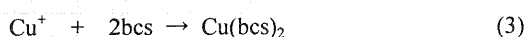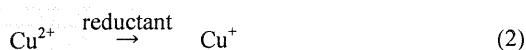
## 2.3 Procedure

A humic acid (or chelating agent) sample solution containing $4 \times 10^{-5}$ mol dm$^{-3}$ copper(II) and 0.2 mol dm$^{-3}$ MOPS buffer (pH 7.0) and a $4 \times 10^{-4}$ mol dm$^{-3}$ BCS solution containing $2 \times 10^{-3}$ mol dm$^{-3}$ hydroxylamine hydrochloride were pumped at a flow rate of 1.2 cm$^3$ min$^{-1}$. After the flow of the solutions had been stopped by turning off the pump, the absorbance was continuously measured at 485 nm. The copper(II) concentrations which reacted with BCS were calculated from the absorbance and the molar absorption coefficient ($\varepsilon = 1.38 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$ at 485 nm ) of copper(I)-BCS complex.

# 3. Results and Discussion

## 3.1 Principles

In the case in which a complexing site, L, of humic acid forms a complex CuL by adding an excess amount of copper(II) to the sample solution, the reaction between CuL and BCS in the presence of the reductant (hydroxylamine hydrochloride) proceeds as follows:

$$CuL \rightarrow Cu^{2+} + L \qquad (1)$$

$$Cu^{2+} \xrightarrow{\text{reductant}} Cu^{+} \qquad (2)$$

$$Cu^{+} + 2bcs \rightarrow Cu(bcs)_2 \qquad (3)$$

where Cu(bcs)$_2$ represents the copper(I)-BCS complex having $\varepsilon = 1.38 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$ at 485 nm. Assuming that the rate determining step of the above reaction is the dissociation of CuL complex shown in reaction (1), the rate of the reaction is defined as:

$$\text{rate} = - \frac{d[CuL]}{dt} = k_d[CuL] \qquad (4)$$

where $k_d$ represents the rate constant for reaction (1). Integration results in

$$-\ln[CuL]_t = -\ln[CuL]_{t=0} + k_d t \qquad (5)$$

According to Eq. (5), a plot of $-\ln[CuL]_t$ against $t$ should give a straight line having a slope of $k_d$ and an intercept of $-\ln[CuL]_{t=0}$. Here, $k_d$ and $[CuL]_{t=0}$ were defined as lability of CuL and copper(II) complexing capacity (CuCC) of a humic acid solution, respectively.

## 3.2 Estimation of complexing ability of chelating agents

To confirm the feasibility of the proposed method, several chelating agents, EDTA, DTPA-OH and EDDP, were used. A plot of $-\ln[CuL]_t$ against $t$ in which DTPA-OH as a chelating agents is shown in Fig. 2, where three straight lines show the validity of Eq. (5). When EDTA and EDDP were used, the plots also fall on straight lines.
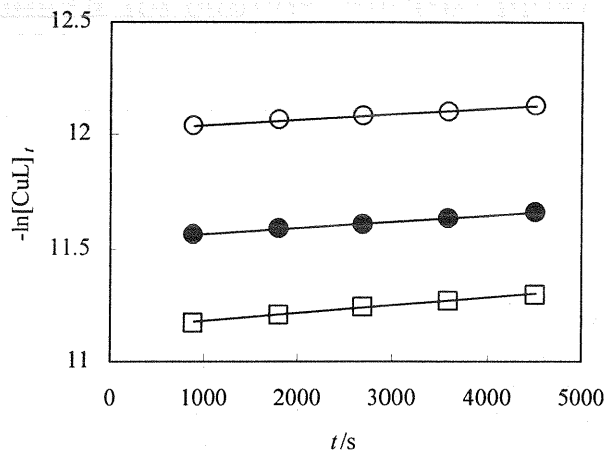


Fig. 2　Plot of $-\ln[CuL]_t$ against reaction time ($t$) in DPTA-OH.
Concentration of DPTA-OH ($\times 10^{-5}$ mol dm$^{-3}$): $\bigcirc$, 0.50; ●, 1.00; □, 1.50.

The $k_d$ and CuCC values for the chelating agents obtained by the proposed method are summarized in Table 1. The CuCC values were approximately equal to the concentration of ligand added, indicating the usefulness of the method for the estimation of complexing capacity. The $k_d$ values are independent of the ligand concentration, but depend only on the kind of ligand. The order of the $k_d$ values shows the lability of copper(II) complex decreases in the following order: Cu(II)-EDDP > Cu(II)-DTPA-OH > Cu(II)-EDTA. Since an inert copper(II) complex with small $k_d$ value is expected to have strong bonding between copper(II) and the ligand, the complexing ability of the ligand would be in inverse order of the $k_d$ value. Therefore, the copper(II) complexing ability of these ligands increases in the following order: EDDP < DPTA-OH < EDTA, which corresponds to the order of stability constants ($\beta$) of the copper(II) complexes: $\beta_{\text{Cu(II)-EDDA}} = 10^{15.1} < \beta_{\text{Cu(II)-DPTA-OH}} = 10^{17.2} < \beta_{\text{Cu(II)-EDTA}} = 10^{18.8}$ [10]. The results suggest that the $k_d$ value reflects the stability of the formed copper(II) complex.

Table 1　Copper(II) complexing capacity (CuCC) and lability ($k_d$) for chelating agents.

| Ligand | [L]$_{added}$[a] | CuCC[a] | $k_d$[b] |
|---|---|---|---|
| EDTA | 1.50 | 1.46 | $8.4 \times 10^{-6}$ |
| | 1.00 | 1.03 | $1.0 \times 10^{-5}$ |
| | 0.50 | 0.65 | $8.3 \times 10^{-6}$ |
| DPTA-OH | 1.50 | 1.44 | $3.9 \times 10^{-5}$ |
| | 1.00 | 0.99 | $3.6 \times 10^{-5}$ |
| | 0.50 | 0.59 | $2.9 \times 10^{-5}$ |
| EDDP | 1.50 | 1.37 | $5.7 \times 10^{-5}$ |
| | 1.00 | 0.97 | $6.0 \times 10^{-5}$ |
| | 0.50 | 0.56 | $4.9 \times 10^{-5}$ |

a. $10^{-5}$ mol dm$^{-3}$,　b. s$^{-1}$.

### 3.3 Estimation of complexing ability of humic acid

The present method was applied to the humic acid sample solutions. A plot of $-\ln[CuL]_t$ against $t$ is shown in Fig. 3. The straight lines show that the Eq. (5) can be used for the estimation of the copper(II) complexing ability of humic acid. The CuCC and $k_d$ values obtained from the plot were summarized in Table 2. The copper(II) binding site (CuBS) in unit mass of the humic acids, which were obtained by dividing CuCC (mol $dm^{-3}$) by humic acid concentration (mg $dm^{-3}$), is $10^{-7}$ mol $mg^{-1}$ order of magnitude. The results approximately corresponded with the values obtained from the previous study using a solvent extraction method: CuBS for Wako HA and Aldrich HA were $3.8 \times 10^{-7}$ and $1.6 \times 10^{-7}$ mol $mg^{-1}$, respectively [6]. The consistency shows that the CuCC of humic acid can easily be obtained by the proposed method.

From the comparison of the $k_d$ values between humic acids and the above chelating agents, it was found that the humic acids form the most inert copper(II)-complex among them. This result indicates that the copper(II)-humic acid complex is significantly stable, and such inertness of the complex presumably plays an important role in reducing the toxicity of copper(II) in natural water.
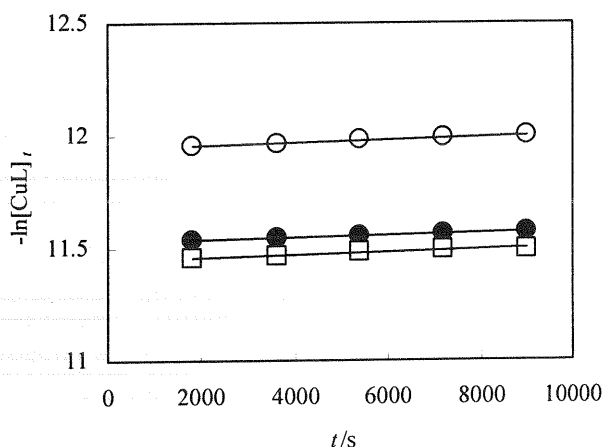
Table 2    Copper(II) complexing capacity (CuCC), copper(II) binding site (CuBS) and lability ($k_d$) for humic acids.

| Sample solution | CuCC[a] | CuBS[b] | $k_d$[c] |
|---|---|---|---|
| Wako HA (21 mg $dm^{-3}$)) | 0.65 | 3.1 | $6.4 \times 10^{-6}$ |
| Aldrich HA (41 mg $dm^{-3}$) | 0.98 | 2.4 | $4.2 \times 10^{-6}$ |
| Cheetah HA (47 mg $dm^{-3}$) | 1.07 | 2.3 | $5.8 \times 10^{-6}$ |

a. $10^{-5}$ mol $dm^{-3}$,   b. $10^{-7}$ mol $mg^{-1}$,   c. $s^{-1}$.

## 4. Conclusion

A stopped-flow system, which can evaluate the copper(II) complexing ability of humic acid, was developed based on a spectrophotometric analysis of copper(II). This method made it possible to determine not only the copper(II) complexing capacity of humic acid but also the lability of copper(II)-humic acid complex. The proposed method, in which the amount of waste fluid and consumption of reagents are extremely low, is expected to be a useful system providing fundamental data concerning complexation property of humic acid.

Fig. 3   Plot of $-\ln[CuL]_t$ against reaction time ($t$) in humic acids.
O: Wako HA (21 mg $dm^{-3}$), ●: Aldrich HA (41 mg $dm^{-3}$), □: Cheetah HA (47 mg $dm^{-3}$).

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