

Selected Paper

Discrimination of Copper(II) Ions and Humate Complexes by Successive Desorption from Thiacalix[4]arenetetrasulfonate-Loaded Sephadex A-25

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A facile method for the analytical fractionation of copper humates and free copper(II) species is described. A chelating agent, thiacalix[4]arenetetrasulfonate (TCAS, 20 μmol), was electrostatically immobilized onto a strongly basic anion-exchanger, QAE-Sephadex A-25 (50 mg). The resulting TCAS-loaded Sephadex A-25 was packed into a column (6 mm i.d. × 7 mm high) and a water sample (10–50 cm^3) was passed through the column to collect both copper humates and free copper(II) species. A 1.0-cm^3 volume of 0.01 mol dm^{-3} nitric acid was passed through the column to selectively desorb the copper complexed with humic substances. Subsequently, 1.0 cm^3 of 1 mol dm^{-3} nitric acid was passed through the same column to desorb the remaining copper, the fraction of free copper(II) species. The proposed two-step desorption method was successfully applied to the speciation analysis of copper in natural water samples by graphite-furnace atomic absorption spectrometry.

Humic and fulvic acids are the major components of dissolved organic substances in many aquatic systems, such as river, lake, and pond water.[1] These humic substances, formed via random reactions involved in chemical and microbial degradation of biological tissues, generally consist of highly complicated mixtures of polyelectrolytes. Therefore, they do not have a well-defined chemical structure, but can be described as acidic, hydrophilic, and aromatic polymers containing carboxylic and phenolic OH groups. Humic and fulvic acids are also known to interact with heavy metals to form negatively charged humate complexes,[2] whose biological and geochemical behavior may differ greatly from those of free metal species (e.g., simple hydrated ions and hydroxo-complexes). For example, free copper(II) species are reported to be highly toxic, whereas their humate complexes are relatively non-toxic.[3] The chemical speciation, therefore, has attracted much attention from researchers engaged in water analysis and environmental studies, and different approaches have been proposed and discussed in detail.[4–6]

In a previous study, the authors have developed a rapid column adsorption method for concentrating traces of heavy metals in water samples.[7] A chelating agent, thiacalix[4]arenetetrasulfonate (TCAS), was electrostatically immobilized onto a macroreticular cross-linked dextran-based anion-exchanger, QAE-Sephadex A-25. Owing to the high selectivity of TCAS toward heavy metals,[8] the column packed with the TCAS-loaded Sephadex A-25 offered a quantitative collection of heavy metals in seawater for the determination of their total-concentrations by graphite-furnace atomic absorption spectrometry (GFAAS).

The present paper describes the potential and utility of the TCAS-loaded Sephadex A-25 in chemical speciation, by taking the case of copper(II) in water as an example. The supporting material, QAE-Sephadex A-25, is a strongly basic anion-exchanger, and thus seems to collect negatively charged humate complexes. From this point, the authors conceived an idea of fractionating copper humates and free copper(II) species by two-step desorption after collecting them on the TCAS-loaded A-25 column. Negatively charged copper humates were collected by ion-exchange, while free copper(II) species were collected by complexation with the loaded TCAS. The fraction of copper humates was first recovered by desorbing with 0.01 M nitric acid, and the remaining fraction (i.e., free copper(II) species) was subsequently recovered by desorbing with 1 M nitric acid (1 M ≡ 1 mol dm^{-3}). The proposed two-step desorption method enables one to concentrate not only the humic (relatively non-toxic) fraction but also the free (toxic) fraction, at least 50-fold, by a simple procedure. The practical applicability of the proposed method was demonstrated by the speciation analysis of copper in natural water samples (e.g., river, pond, and ground water samples) by GFAAS.

Experimental

Apparatus. A Perkin-Elmer Model AAnalyst 600 graphite-furnace atomic absorption spectrometer equipped with a Model AS-800 autosampler and a Zeeman effect background corrector (Norwalk, CT, USA) was used for the determination of copper under the following furnace-operating conditions. The graphite tube was gradually warmed during 1 s to 110 °C and held for 30 s. The tube was further heated during 15 s to 130 °C and held for 30 s; it was then heated during 10 s to a pyrolysis temperature of 700 °C and held for 20 s. The tube was immediately heated to an atomization temperature of 2000 °C and held for 5 s. Clean-up was done at 2450 °C for 3 s to eliminate the memory effect. The wavelength and hollow-cathode lamp current were 324.8 nm and 15 mA, respectively.

A Jasco Model V-550 UV–vis double-beam spectrophotometer (Tokyo, Japan) was used with a 1-cm quartz cell for the determination of humic and fulvic acids at 400 nm. A Horiba Model M-12 pH meter equipped with a Model 6366-10D elec-

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trode (Kyoto, Japan) was used for the pH measurement. Separation procedures were carried out in a Hitachi Model ECV-843 BY clean bench (Hitachi, Japan).

Reagents. The solid support, QAE-Sephadex A-25 (0.05–0.1 mm particles, macroreticular cross-linked dextran gel with diethyl(2-hydroxypropyl)aminoethyl groups, chloride-form), was purchased from Pharmacia (Uppsala, Sweden). The chelating agent, tetrasodium thiacalix[4]arenetetrasulfonate (TCAS), was synthesized as described in the literature.[9] The TCAS-loaded A-25 column was prepared as follows. A 10-cm^3 volume of a 2.0 mM TCAS solution was added to 50 mg of QAE-Sephadex A-25 gel. The suspension was stirred for 15 min to load TCAS on the gel particles. After the supernatant solution was discarded, the resulting TCAS-loaded A-25 gel was packed into a polypropylene column (6 mm i.d. × 7 mm high). The column was washed with 10 cm^3 of water before use.

A standard copper(II) solution (10 $\mu g\,cm^{-3}$ in 0.01 M nitric acid) was prepared from a commercial standard solution (1.00 $mg\,cm^{-3}$, Nacalai Tesque, Kyoto, Japan). A humic acid (HA) solution (0.30 $mg\,cm^{-3}$) was prepared by dissolving HA powder (extracted from peat soil, Nacalai Tesque) in a 0.1 M potassium hydroxide solution and passing through a 0.4-μm pore-size Nuclepore polycarbonate membrane filter. A fulvic acid (FA) solution (0.50 $mg\,cm^{-3}$) was prepared by dissolving FA powder (extracted from brown forest soil based on the method of International Humic Substances Society[10]) in water.

All reagents used were of guaranteed reagent grade (Wako Pure Chemicals, Osaka, Japan), unless otherwise stated. Water was purified by ion-exchange and distillation, and then passed through a Milli-Q SP reagent water system (Millipore, Bedford, MA, USA).

Procedure. An outline of the procedure is schematically represented in Fig. 1. A water sample (10–50 cm^3, containing traces of copper(II) and HA and/or FA) was passed through the TCAS-loaded A-25 column at a flow rate of 5–10 $cm^3\,min^{-1}$. A 1.0-cm^3 volume of 0.01 M nitric acid was introduced onto the column to desorb the copper complexed with humic substances, and the eluate was reserved for the GFAAS determination. Subsequently, 1.0 cm^3 of 1 M nitric acid was introduced onto the same column to desorb the remaining copper (the fraction of free copper(II) species), and the eluate was reserved. A 20-mm^3 aliquot of each eluate (after dilution, if necessary) was individually injected into the graphite furnace and analyzed by GFAAS. The measurement was repeated three times and the atomic absorption signals were averaged. Calibration graphs were constructed using 0.01 or 1 M nitric acid solutions containing copper at $ng\,cm^{-3}$ levels.

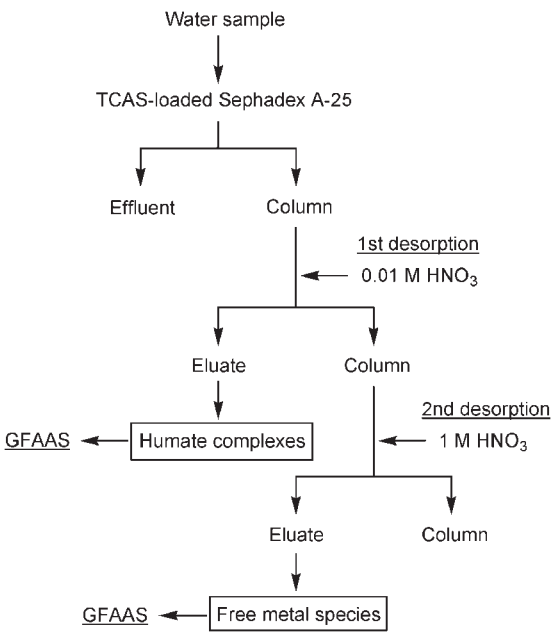

Fig. 1. Flow chart of the procedure.

Results and Discussion

Collection of Copper Humates and Free Copper(II) Species. The TCAS-loaded Sephadex A-25 was prepared by a batch adsorption method. By simply mixing 50 mg of QAE-Sephadex A-25 with 10 cm^3 of 2.0 mM TCAS solution, TCAS was quantitatively loaded on the gel particles. The resulting TCAS-loaded A-25 gel was packed into a column for subsequent studies.

Different synthetic samples containing 10 $ng\,cm^{-3}$ of copper(II) and 3.0 $\mu g\,cm^{-3}$ of HA or 10 $\mu g\,cm^{-3}$ of FA were prepared. A 10-cm^3 aliquot of the solution was passed through the TCAS-loaded A-25 column and the effluent was analyzed by GFAAS to determine the amount of copper passing through the column. From the determined value, the amount of copper collected on the column was calculated. For a comparison, a second 10-cm^3 aliquot was passed through a column packed with 50 mg of untreated (without TCAS) QAE-Sephadex A-25, and the amount of copper collected on the column was calculated in the same manner. A third 10-cm^3 aliquot was used for the determination of copper complexed with humic substances in the samples by the indium-treated XAD-2 method,[11] where humate complexes were selectively collected on the indium-treated Amberlite XAD-2 resin by low-energy physical sorption.

Table 1 gives the amounts of copper collected on the untreated or TCAS-loaded A-25 column. When passing a sample containing no humic substance, the untreated A-25 column did not retain copper. On the other hand, in the presence of humic substances, approximately 40–90% of copper was collected on the untreated A-25 column. This fraction coincided with the amount of copper complexed with humic substances in the samples, indicating that QAE-Sephadex A-25 is inherently capable of sorbing copper humates. QAE-Sephadex A-25 is a macroreticular strongly basic anion-exchanger, and copper humates are negatively charged species, as described in Introduction. Therefore, this selective retention is most probably caused by ion-exchange; in fact, a weakly basic anion-exchanger, DEAE-Sephadex A-25, is often used for the selective collection of humate complexes.[6,12]

As shown in Table 1, loading TCAS on QAE-Sephadex A-25 offered the quantitative collection of copper over a pH range of 4.5–7.5, which is a practical pH range for environmental water monitoring. This quantitative collection indicates that the copper species being not collectable by ion-exchange (i.e., free copper(II) species) can also be collected by complexation with the loaded TCAS.

Desorption of Copper Humates from the Untreated A-25 Column. As discussed above, the TCAS-loaded A-25 column retained both copper humates and free copper(II) species, hence their chemical speciation can be achieved by desorbing them individually. Along this line, the desorption behavior of

Table 1. Collection of Copper on QAE-Sephadex A-25

Sample solution, 10 cm^3	Cu collected/μg	
	Untreated A-25	TCAS-loaded A-25
pH 4.5		
Cu 0.10 μg	0.002	0.10
Cu 0.10 μg; HA 30 μg	0.042 (0.045)[a)]	0.10
Cu 0.10 μg; FA 100 μg	0.063 (0.064)	0.10
pH 6.0		
Cu 0.10 μg	0.004	0.10
Cu 0.10 μg; HA 30 μg	0.064 (0.061)	0.10
Cu 0.10 μg; FA 100 μg	0.083 (0.078)	0.10
pH 7.5		
Cu 0.10 μg	0.005	0.10
Cu 0.10 μg; HA 30 μg	0.070 (0.073)	0.10
Cu 0.10 μg; FA 100 μg	0.089 (0.090)	0.10

a) The values in parentheses indicate the amounts of Cu complexed with humic substances, which were determined by the indium-treated XAD-2 method.[11]

copper complexed with humic substances was first investigated with the untreated A-25 column because it retained copper humates selectively.

In a previous study, copper humates were found to be stable down to pH 4, but rapidly decompose at lower pH's.[13] Therefore, the authors attempted to desorb copper complexed with humic substances by decomposition of the humate complexes under an acidic condition. Synthetic samples containing 10 ng cm^{-3} of copper(II) and 3.0 μg cm^{-3} of HA or 10 μg cm^{-3} of FA were prepared. The copper complexed with humic substances was determined by the DEAE-Sephadex A-25 method.[12] A 10-cm^3 aliquot of the sample solution was passed through the untreated A-25 column to collect humate complexes. A 1.0-cm^3 volume of dilute nitric acid (pH 0–3) or 0.01 M formic acid-sodium hydroxide buffer (pH 3–4.5) was introduced onto the column and the eluate was analyzed by GFAAS. As shown in Fig. 2a, the copper complexed with HA or FA was quantitatively recovered by desorbing with 0.01–1 M nitric acid.

Desorption of Free Copper(II) Species from the TCAS-Loaded A-25 Column. The desorption behavior of free copper(II) species was investigated with the TCAS-loaded A-25 column. A 10-cm^3 aliquot of a synthetic sample (Cu 10 ng cm^{-3}) was passed through the TCAS-loaded A-25 column and the desorption was carried out in the same manner as described above. The results are shown in Fig. 2b. In contrast to the case of desorbing copper complexed with humic substances, no copper was desorbed down to pH 2. By using 1 M nitric acid, copper was quantitatively desorbed from the TCAS-loaded A-25 column.

Fractionation of Copper Humates and Free Copper(II) Species. Based on the above-mentioned findings, an attempt was made to fractionate copper humates and free copper(II) species by two-step desorption after collecting them on the TCAS-loaded A-25 column. The first desorption was carried out with 1.0 cm^3 of 0.01 M nitric acid (pH 2) to selectively recover the humic fraction of copper. Subsequently, the second desorption was carried out with 1.0 cm^3 of 1 M nitric acid to recover the remaining fraction (i.e. free copper(II) species).

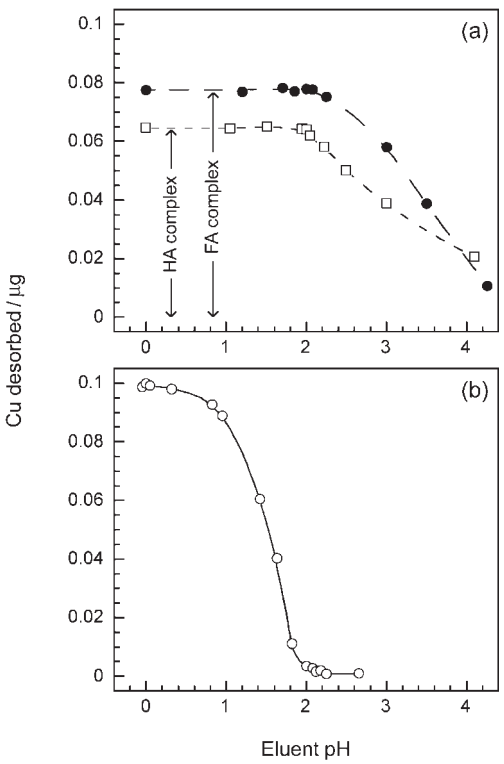

Fig. 2. Desorption of copper humates (a) and free copper species (b). Cu–HA (□): A 10-cm^3 sample (Cu 0.10 μg, HA 30 μg, pH 6.7, Cu–HA 65%) was passed through the untreated A-25 and the desorption was carried out. Cu–FA (●): A 10-cm^3 sample (Cu 0.10 μg, FA 100 μg, pH 6.5, Cu–FA 78%) was passed through the untreated A-25 and the desorption was carried out. Free Cu species (○): A 10-cm^3 sample (Cu 0.10 μg, pH 6.0) was passed through the TCAS-loaded A-25 and the desorption was carried out.

Table 2 gives the results obtained with different synthetic samples containing traces of copper(II) and HA and/or FA. The amounts of copper recovered in the first desorption step were

Table 2. Fractionation of Free Copper Species and Humate Complexes in Synthetic Water Samples

Sample solution					Cu recovered/μg		
Cu/μg	HA/μg	FA/μg	Volume/cm^3	pH	1st desorption[a)]	2nd desorption[b)]	Total
0.10	5.0	0	10	6.4	0.031 (0.032)[c)]	0.066	0.097
0.10	10	0	10	6.0	0.049 (0.051)	0.047	0.096
0.10	30	0	10	6.5	0.068 (0.067)	0.033	0.10
0.10	0	20	10	6.8	0.048 (0.042)	0.054	0.10
0.10	0	50	10	6.9	0.072 (0.079)	0.032	0.10
0.10	0	100	10	6.3	0.077 (0.078)	0.022	0.099
0.20	10	40	20	6.3	0.12 (0.14)	0.074	0.19
0.20	20	40	20	6.7	0.13 (0.14)	0.070	0.20
0.20	20	100	20	6.7	0.16 (0.17)	0.034	0.19
0.25	15	25	50	6.0	0.10 (0.10)	0.15	0.25
0.25	25	100	50	6.5	0.18 (0.19)	0.065	0.25
0.25	50	250	50	6.7	0.21 (0.21)	0.045	0.26

a) Performed with 1.0 cm^3 of 0.01 M nitric acid. b) Performed with 1.0 cm^3 of 1 M nitric acid. c) The values in parentheses indicate the amounts of Cu complexed with humic substances, which were determined by the DEAE-Sephadex A-25 method.[12]

in agreement with those of copper complexed with humic substances in the samples. In addition, the total amounts of copper recovered in the first and second desorption steps well coincided with the amounts of copper added to the sample solutions. These results indicate that the two-step desorption method offers the fractionation of copper complexed with humic substances and free species, where the former is recovered in the first desorption step and the latter is recovered in the second desorption step. Furthermore, both fractions can be concentrated by a simple, one-column procedure, at least 50-fold, by loading 50 cm^3 of a water sample and desorbing with 1.0 cm^3 of nitric acid, which should improve the accuracy and precision of the analytical results for both fractions.

In this speciation procedure, an error can occur if the free copper(II) species react with humic substances sorbed on a Sephadex gel. According to the literature describing the DEAE-Sephadex A-25 method,[12] this error is negligible. On the other hand, the ligand-exchange reaction of copper humates with the loaded TCAS may also cause an error. To confirm that this error is negligible, solvent extraction was carried out with the parent, hydrophobic compound *p-tert*-butylthiacalix[4]arene (TCA). A 2.0-cm^3 volume of chloroform containing 20 μmol of TCA was vigorously shaking with 10 cm^3 of water containing 0.10 μg of copper at pH 6.5 for 3 min. Copper was completely extracted into the chloroform. On the other hand, in the presence of 10 μg of HA or 20 μg of FA, 42 or 38% of copper remained in the aqueous phase, respectively. The amounts of the remaining copper agreed with those of copper complexed with humic substances. These results suggest that the ligand-exchange reaction occurred negligibly during the separation procedure. The extracted copper was not back-extracted with water containing humic substances at pH 6.5, suggesting that the ligand-exchange reaction of copper–TCA complex with humic substances was also negligible.

Application to Natural Water Samples. The proposed method was applied to the chemical speciation of copper in river, pond, and ground water samples (sampled in Nagoya City on August 2004). After passing a sample through 0.4-μm pore-size Nuclepore polycarbonate membrane filters, a 25-cm^3 aliquot of the filtrate was subjected to the separation protocol, followed by GFAAS determination, as described in Procedure section. To validate the analytical results obtained by the proposed method, the DEAE-Sephadex A-25 method was employed as a reference method. Copper complexed with humic substances in a 50-cm^3 aliquot of the filtrate was selectively collected on the DEAE-Sephadex A-25 column, and then desorbed with 5.0 cm^3 of 1 M nitric acid for the GFAAS determination. The effluent was also analyzed by GFAAS for the determination of free copper species. However, a 10-fold preconcentration of the effluent by evaporation was required to obtain a reliable value for free copper species, which was in striking contrast to the case of the analysis by the proposed method. The amounts of humic substances were also determined by evaporating the filtrate 50-fold (for river and pond waters) or 200-fold (for ground water) and measuring the absorbance of 400 nm at pH 13. The FA solution (described in Reagents section) was used as a standard solution (absorptivity at pH 13: 3.8 cm^2 mg^{-1}) because FA was found to be a predominant species of aquatic humic substances.[14]

Table 3 shows the analytical results. The blank value through the whole procedure was less than the detection limit (0.3 ng). Approximately 30–50% of the total dissolved copper in the river and pond water samples was present as free species. On the other hand, the ratio of the free species to the total dissolved copper was 80% in the ground water sample. The higher percentage of free copper species in the ground water sample than those in the other samples is probably due to the lower concentration of humic substances. The analytical results obtained by the proposed method were in agreement with those obtained by the reference method, which clearly demonstrates the reliability of the proposed method.

In conclusion, a facile method for the analytical fractionation of copper humates and free copper(II) species has been developed. Chemical speciation provides useful information for a better understanding of the biological and geochemical behavior of trace metals. Hence, the simple speciation method proposed here will be helpful in various fields of environmental studies.

Table 3. Analytical Results for Natural-Water Samples

Filtered sample	Cu determined[a]/ng cm^{-3}			Humic substances/ μg cm^{-3}	pH
	Free	Humate complexes	Total dissolved species		
River water I	0.64 ± 0.06 (0.61)[b]	0.76 ± 0.08 (0.83)	1.4 ± 0.1 (1.4)	0.9	7.2
River water II	0.28 ± 0.05 (0.24)	0.63 ± 0.05 (0.69)	0.91 ± 0.03 (0.93)	3.1	7.1
Pond water	0.43 ± 0.06 (0.38)	0.67 ± 0.07 (0.60)	1.1 ± 0.1 (0.98)	1.4	7.4
Ground water	$3.5 \pm 0.0_2$ (3.9)	0.93 ± 0.07 (0.98)	4.4 ± 0.1 (4.9)	0.08	7.2

a) Average of five parallel runs with different columns. b) The values in parentheses were obtained by the DEAE-Sephadex A-25 method.[12]

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