Highly Selective Detection of Trace Copper(II) Using Bathocuproinesulfonate by Flow-Injection Electrospray Ionization Mass Spectrometry

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Trace Cu²⁺ was detected with high selectivity using specific complexation with bathocuproinesulfonate (BCS) through flow-injection electrospray ionization mass spectrometry (FI-ESI-MS), which separates Cu²⁺ from coexisting metal ions by forming a Cu-BCS complex with a high mass number. Here, only [Cu(BCS)₂]³⁻ was obtained with a high ion count. Its calibration curve was linear from 1.0×10^{-8} to 1.0×10^{-5} M. This method was applied to determine the Cu-complexing capacities of humic acid solution and river water samples by adding traces of Cu²⁺.

Keywords Electrospray ionization mass spectrometry, flow injection, copper, chelating agent, complexing capacity

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Introduction

Electrospray ionization mass spectrometry (ESI-MS) is an analytical method that ionizes, and then measures the chemical species in sample solutions under mild conditions. It is mainly applied to the determination of organic species, such as carboxylates or proteins. It has also often been used as an analytical method to measure the speciation of metal complexes, based on their nature.¹⁻⁴

Typically, a heavy metal ion in a water sample that is softly ionized by ESI forms various metal-hydration complexes.⁵ Therefore, the coordination of a target metal ion and a chelating agent can prevent this hydration, and can effectively separate the ion from coexisting-ionic species. For example, several researchers have reported²⁻⁴ the quantification and speciation of metal complexes with chelating agents, such as ethylenediaminetetraacetate (EDTA) or diethyldithiocarbamate (DDC).

In this study, we attempted to develop a selective and sensitive detection of Cu²⁺ by ESI-MS with a flow injection (FI-ESI-MS) using a carrier containing a chelating agent, and to use the method to determine the Cu-complexing capacity (CuCC) of ligands in water samples. CuCC refers to the maximum molar quantity of Cu²⁺ that can coordinate to the mass unit of unspecified ligands (fulvic acid, humic acid) that are dissolved in environmental water.⁶ The CuCC of an environmental water sample is determined from the inflection point of free Cu²⁺ concentration in the sample as a function of the total Cu²⁺ concentration in the sample. Typically, the concentration of ligands, estimated from the total organic carbon (TOC), is extremely low (< 1 ppm) in Japanese river water. Consequently, the trace levels of free Cu²⁺ (< 1.0×10^{-6} M) must be monitored in order to find an accurate inflection point for the determination of the CuCC. Itabashi *et al.* used the back-extraction method to

determine the CuCC of humic acid dissolved in water,⁷ but this method required the use of complicated procedures. This paper demonstrates that the CuCC of genuine river water samples could simply be determined by the proposed method using FI-ESI-MS.

The highly sensitive and selective detection of Cu²⁺ by FI-ESI-MS was developed in the following order: firstly, we selected an appropriate chelating agent for the selective detection of Cu²⁺. We used aromatic chelating agents, such as tetrakis(4-carboxyphenyl)porphyrin (TCPP) and tetraphenylporphyrin tetrasulfonate (TPPS), which are water-soluble porphyrins used for the fluorescence detection of metal ions,^{8,9} and bathocuproinesulfonate (BCS), which is a phenanthroline that selectively binds copper ions¹⁰ (Fig. S1, Supporting Information). We expected that the influence of the matrices in the ESI-MS analysis of Cu²⁺ should be minimized if Cu-complexes with larger mass numbers were obtained by using a chelating agent of larger molecular mass. Following this, we optimized the probe and curved desolvation line (CDL) voltages that were applied during the ESI-MS for more sensitive detection.

The optimized method was applied to the determination of the CuCC of a commercial humic acid dissolved in an aqueous solution and of ligands existing in a real river water sample (Watarase River in Gunma Prefecture, Japan).

Experimental

Reagents and chemicals

All reagents were of analytical grade and were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The water used for reagent dissolution and sample dilution was obtained from an ASK-2DS ultrapure water system (Iwaki Co., Ltd., Tokyo, Japan). Stock solutions of the metals (1.0×10^{-3} M) were prepared by dissolving copper(II) acetate monohydrate with an appropriate amount of water. Chelating agents, TCPP, TPPS, and BCS, were purchased from Dojindo Laboratories, Inc. (Kumamoto, Japan), and were diluted to 1.0×10^{-3} M in

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distilled-deionized water (water).

Apparatus

ESI-MS was performed with a Shimadzu high-performance liquid chromatograph mass spectrometer, LCMS-2010A, which comprises an ion-spray-type interface that promotes ionized gas. The injected sample volume of the sample solution containing Cu^{2+} was 30 μL . The length from injector to detector was 50 cm (0.13 μm i.d.). Using the flow-injection function in the apparatus, the injected sample was mixed with a carrier comprising 1.0×10^{-3} M ammonium acetate and 2.0×10^{-5} M chelating agent (pH 4) at a flow rate of 0.20 mL/min. The pH was selected with referring to a previous report.¹¹ A 1.0×10^{-3} M ascorbic acid used as a Cu^{2+} reductant was added into the carrier with BCS. The analyte was measured in the negative-ion mode. The other experimental conditions were as follows: nebulizer gas (flow rate), N_2 (1.5 L/min); drying gas (pressure), N_2 (0.04 MPa); probe voltage, -5.0 to 0.5 kV; CDL voltage, -30 to 0 V; and Q-array voltage, -50 V. TOCs in a humic acid-dissolved solution and a real river water sample solution were determined using a Shimadzu TOC-V (Shimadzu Co., Ltd.).

Influence of coexisting metals

We added 1000-fold concentrations of Zn^{2+} and Cd^{2+} than that of Cu^{2+} to the tested solution, in order to observe the influence of excess quantities of metals on the formation of the Cu-BCS complex. The sample used for evaluating the influence of coexisting metals for the detection of Cu^{2+} (as a Cu-BCS complex) was obtained by mixing 1.0×10^{-5} M copper(II) acetate monohydrate with 1.0×10^{-2} M cadmium(II) acetate dihydrate, or 1.0×10^{-2} M zinc(II) acetate dihydrate. The carrier was a mixture of 1.0×10^{-3} M ammonium acetate, 2.0×10^{-5} M BCS, and 1.0×10^{-3} M ascorbic acid. The other conditions were as described above.

Determination of CuCC

Each mixture of various concentrations of copper(II) acetate monohydrate (1.0×10^{-7} – 2.0×10^{-6}) was injected with humic acid (TOC: 6.4 mg/L) into the FI-ESI-MS. The carrier, consisting of 1.0×10^{-3} M ammonium monohydrate, 6.0×10^{-6} M BCS, and 2.0×10^{-6} M ascorbic acid, was mixed with the sample by FI.

For determining the CuCC of ligands in river water, various concentrations of copper(II) acetate monohydrate (1.0×10^{-8} to 2.0×10^{-7} M) were added to a river water sample filtered by a membrane filter (0.45 μm ϕ). The concentrations of the ligands in the river water were estimated from the TOC. The CuCC values was estimated by referring to a report by Itabashi *et al.*⁷

Results and Discussion

Influence of the chelating agent

The ion counts of the main peaks of each FI-ESI-MS spectrum of Cu^{2+} (1.0×10^{-5} M) coordinated with three different chelating agents (2.0×10^{-5} M each) in the negative-ion mode were compared.

Firstly, anionic porphyrins, such as TCPP and TPPS, were employed as Cu^{2+} chelating agents. The use of TCPP provided the isolated peak of $[\text{Cu}(\text{H}_2\text{TCPP})]^{2-}$ at $m/z = 425$, and there were no peaks corresponding to free TCPP or other Cu-TCPP complexes (Fig. 1a). This result provided an isolated peak to allow easy identification of the analyte Cu^{2+} , but it had a small ion count. Because a weakly acidic compound TPCC is partially neutralized at pH 4–5, it might conduct the lower ionization

Fig. 1 Mass spectra of Cu-chelating complexes. Injected sample, $[\text{Cu}^{2+}] = 1.0 \times 10^{-5}$ M; carrier solution, 2.0×10^{-5} M chelating agent in 1.0×10^{-3} M $\text{CH}_3\text{COONH}_4$. 1.0×10^{-4} M ascorbic acid was added into the carrier with only BCS as a chelating agent.

efficiencies in ESI part, though the detail mechanism could not be cleared at this stage.

The use of TPPS provided a high ionization efficiency of $[\text{Cu}(\text{TPPS})]^{4-}$ at $m/z = 248$, accompanied by trivalent and divalent species at $m/z = 331$ and 497 , respectively (Fig. 1b). This would be possible to determine how many functional groups were coordinated to copper from the spectra. However, the peaks of the Cu-TPPS complexes detected at $m/z = 248$ and 331 overlapped with those from the spectrum of Zn-TPPS (Fig. S2, Supporting Information). In that of Zn-BCS, the peaks between $m/z = 300$ and 400 appeared, but they were not detected with the peak at $m/z = 367$ that originated from $[\text{Cu}^{\text{I}}(\text{bcs})_2]^{3-}$.

The use of BCS afforded a trivalent anionic species only; that is, $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$ (Fig. 1c). Ideally, if monovalent and divalent anionic species could be simultaneously detected, this method could be used for the speciation of Cu-BCS complexes. However, this method, using BCS, is very selective for Cu^+ , and the complexation of BCS to other tested metal ions (Fe^{3+} , Al^{3+} , Zn^{2+} , Cd^{2+}) did not occur (data not shown). Additionally, the sensitivity for $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$ was much higher than that of the Cu-TCPP and Cu-TPPS complexes.

Optimization of the probe and CDL voltages

Next, we optimized the probe and CDL voltages to enhance the sensitivity for $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$.

The probe voltage is the voltage applied to the capillary tip of the ESI interface, and it is the ionization source for the conversion of the sample into charged droplets when passing through the capillary. Figure 2a shows changes in the ion count

Fig. 2 Ion count of $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$ ($m/z = 367$) as a function of the probe and CDL voltages.

Plots: (upper) probe voltage; and (bottom) CDL voltage. Injected sample, $[\text{Cu}^{2+}] = 1.0 \times 10^{-5}$ M; carrier solution, 2.0×10^{-5} M BCS, 1.0×10^{-4} M ascorbic acid, and 1.0×10^{-3} M $\text{CH}_3\text{COONH}_4$.

of $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$ as a function of the probe voltage. In this case, the ion count increased as the probe voltage rose to the negative range. The maximum ion count was obtained by applying -4.5 kV. The ion count then decreased upon the application of -5 kV, possibly due to the fragmentation of BCS, or the dissociation of copper from the Cu-BCS complex. The optimal probe voltage was thus concluded to be -4.5 kV.

The CDL is the section that removes the solvent contained in the fine charged particles that are generated by ESI. Applying a voltage to the CDL can also prevent the formation of larger droplets, which cause noise, bending the CDL. That is, the strength of the CDL voltage correlates to the sensitivity of an analyte peak. As shown in Fig. 2b, the ion count increased as the CDL voltage was increased to the negative range, and the

maximum ion count was obtained at -25 kV. The ion count decreased when applying -30 kV because a portion of the analyte Cu-BCS complex might be removed with large droplets. Accordingly, the optimal CDL voltage was -25 kV.

Analytical performances

Using the optimized conditions described above, the calibration curve of Cu^{2+} was created from variations in the ion count of $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$ ($m/z = 367$) as a function of the concentration of Cu^{2+} injected. The calibration curve was linear in the range of 1.0×10^{-8} to 1.0×10^{-5} M with a good correlation coefficient ($r^2 = 0.9985$) (Fig. S3, Supporting Information).

The detection limit (DL) of Cu^{2+} as $[\text{Cu}^{\text{I}}(\text{BCS})_2]^{3-}$ at a signal-to-noise ratio of 3 was 3.5×10^{-9} M. This value is approximately 1000-fold lower than that of bare Cu^{2+} (3.2×10^{-6} M) detected by the FI-ESI-MS without any chelating agents. In addition, when this DL was compared to several previous reports on the ESI-MS of Cu^{2+} , it was found to be almost equal to or lower than the reported values. For example, the DL is 9.4×10^{-9} M for Cu-DDC $[\text{Cu}(\text{DDCH})^+]$,⁴ and 6.0×10^{-9} M for Cu-EDTA $[\text{Cu}(\text{HEDTA})^-]$.²

Next, the effect of coexisting heavy metal ions, Zn^{2+} and Cd^{2+} , was examined. A solution containing a 1000-times higher concentration of coexisting metal than that of Cu^{2+} was injected into the FI-ESI-MS. Only the Cu-BCS complex was detected, with no coexisting metal-BCS complexes evident.

CuCC of humic acid and ligands in river water

To demonstrate the application of this study, we attempted to determine the CuCC of a humic acid solution and a Watarase River water.

As shown in Fig. 3, the ion counts hardly increased when injecting the humic acid solution with Cu^{2+} solutions ranging from 1.0×10^{-7} M to 7.0×10^{-7} M, but rapidly increased when higher concentrations than 7.0×10^{-7} M were injected. The inflection point, estimated from the intersection of the two approximations in Fig. 3, was 6.8×10^{-7} M. Consequently, the CuCC of the humic acid solution obtained from the inflection point with a varying ion count was 1.1×10^{-4} mol/g. This value means that a mass unit of the humic acid can coordinate Cu^{2+} up to 1.1×10^{-4} mol, and almost equal to the values (1.0×10^{-4} to 3.0×10^{-4} mol/g) obtained by the back-extraction method.⁷ In addition, the CuCC of the river water sample (TOC: 0.54 mg/L), determined by the procedures described above, was 1.5×10^{-4} mol/g, which is almost equal to that of commercial humic acid (Fig. 3).

Conclusions

The FI-ESI-MS method developed in this study allowed the successful detection of Cu^{2+} with high selectivity and sensitivity, by complexation with BCS in the carrier. Our study demonstrates that this is useful as a simple method for the identification of Cu^{2+} .

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Fig. 3 Ion count of $[\text{Cu}(\text{BCS})_2]^{3-}$ ($m/z = 367$) as a function of the Cu^{2+} concentration added in the humic acid and river water sample solutions.

Injected sample, (humic acid) a mixture of 1.0×10^{-7} to 2.0×10^{-6} M Cu^{2+} and humic acid (6.4 mg/L TOC); and (river water) a mixture of 1.0×10^{-8} to 2.0×10^{-7} M Cu^{2+} and river water (0.54 mg/L TOC). Carrier solution, 6.0×10^{-6} M BCS and 2.0×10^{-5} M ascorbic acid in 1.0×10^{-3} M $\text{CH}_3\text{COONH}_4$.

Supporting Information

Chemical structures of the chelating agents used in this study are shown in Fig. S1. Mass spectra of Zn-chelating complexes are shown in Fig. S2 for comparison with Fig. 1. A calibration curve of the ion count of $[\text{Cu}(\text{BCS})_2]^{3-}$ as a function of the copper(II) concentration is shown in Fig. S3. This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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