

An Automated Batch-wise Flow System for Determination of Reactive Copper in Water Samples

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Abstract

A new type of flow system was proposed for the determination of reactive copper in water samples. The reactive copper was determined by spectrophotometric monitoring of the copper-catalyzed aerial oxidation of L-ascorbic acid to dehydroascorbic acid (DAA) and the formation of quinoxaline derivatives (QX) by the reaction of DAA with *o*-phenylenediamine. Absorbance of QX at 340 nm was measured after a reaction time of 5 min at 35°C. Considerable adsorption loss of copper was found for PTFE tube of a continuous flow FIA system. It was minimized by the proposed automated batch-wise flow system with individual injection lines for sample and reagent solutions and a cell for reaction and absorbance measurement. A linear calibration curve of copper up to 20 µg/l was obtained by the addition of Al (and F) ions as adsorption inhibitor. The usefulness of the proposed system was demonstrated in the determination of reactive copper in artificial water samples.

Keywords Flow injection, catalytic method, reactive copper, adsorption, water sample, humic substance

1. Introduction

Reactivity of certain elements changes their toxicity and bioavailability. Therefore, in speciation of elements, quantification of reactive species is important for environmental and biological studies. For the speciation, original physico-chemical property of an analyte must be kept in an analytical process or its change should be minimized [1]. For example, the acidification of sample for the storage is inappropriate with respect to dissociation of colloidal hydroxides and complexes. Automatic and rapid analysis based on FIA is attractive for the speciation in survey of aquatic systems. However, adsorption of copper and other elements on the inner wall of PTFE tube was reported for a neutral and basic solution, and accompanied the decrease in analytical sensitivity and maybe the discrimination of species [2,3]. Therefore, the adsorption leads to erroneous low analytical results.

In this study, a flow system with minimal adsorption loss of copper was developed for the determination of reactive copper based on the spectrophotometric monitoring of the copper-catalyzed aerial oxidation of L-ascorbic acid (AA) to dehydroascorbic acid (DAA) and the formation of quinoxaline derivatives (QX) by the reaction of DAA with *o*-phenylenediamine (OPDA) [4]. By this method, Cu^{2+} and $\text{Cu}^{\text{II}}\text{L}_i^{2-ni}$ ($i=1$ or 2 for a unidentate ligand and $i=1$ for a bidentate ligand (L^{n-})) are determined as reactive copper. Degrees of the adsorption for parts of flow system were evaluated by the normalized absorbance ratio [3]. The usefulness of the proposed flow system was demonstrated in the determination of reactive copper in artificial water samples.

2. Experimental

2.1. Reagents

High purity HCl and NaOH solutions (Kanto Chemical, Ultrapur) and water with ($\geq 18 \text{ M}\Omega\cdot\text{cm}$) were used throughout.

The other chemicals used were of analytical-reagent grade. A copper stock solution (1.00 g Cu/l) was prepared by dissolving $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$ with 1 mM HCl. Copper standard solutions were prepared by diluting the stock solution with the carrier solution (R_1 in Fig. 1) before use. AA solutions were prepared by dissolving L-ascorbic acid in HCl. OPDA solutions were prepared by dissolving the reagent with water. Humic substance (HS) ($< 0.45\mu\text{m}$) was prepared by dissolving humic acid (Wako Pure Chemical Industries) [5]. This reagent included fulvic-acid like substance showing no aggregation by acidification and with low molecular weights (MW) less than 1,000.

2.2. Apparatus

Figure 1 shows the flow system used in the determination of copper. PTFE tubes with 0.5 mm i.d. and Daiflon connectors were used for flow lines except a sample loop (1 mm i.d.). Four double-plunger pumps ($\text{P}_1 - \text{P}_4$) were used to propel carrier (R_1) and reagent solutions ($\text{R}_2 - \text{R}_4$). A valve-type injector (I) consisted of an automatically driven six-way valve and a PTFE sample loop. Sample or a copper standard solution (0.48 ml) was selected with another automatically driven valve (V) and introduced into I with a peristaltic pump (P_5). A Shimadzu UV-140-01 double-beam spectrophotometer equipped with a normal 10-mm fused silica cell and a strip-chart recorder was used for absorbance measurement. Four coils ($\text{C}_1 - \text{C}_4$) were submerged into a thermostated water bath ($35\pm 0.1^\circ\text{C}$) in order to control the temperature of the solution streams. The cell chamber was also controlled at the same temperature by circulating water from the water bath. Solution in the cell was pumped out with a peristaltic pump (P_6). The valves, pumps and the recorder were driven with a programmable controller.

2.3. Determination procedure of reactive copper

The determination of reactive copper was carried out by the automated operation (Table 1). In step 4 to 7, 1 ml of the carrier (R_1) with the sample or copper standard solution, 0.5 ml of phosphate buffer solution (R_2), 0.5ml of OPDA solution (R_3) and 0.5ml of AA solution (R_4) were introduced in turn into the cell. In step 8, absorbance at 340 nm was recorded for 310 s on the

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strip chart. Absorbance at 300 s after the addition of R₄ was measured for the determination of copper. Then, the solution in

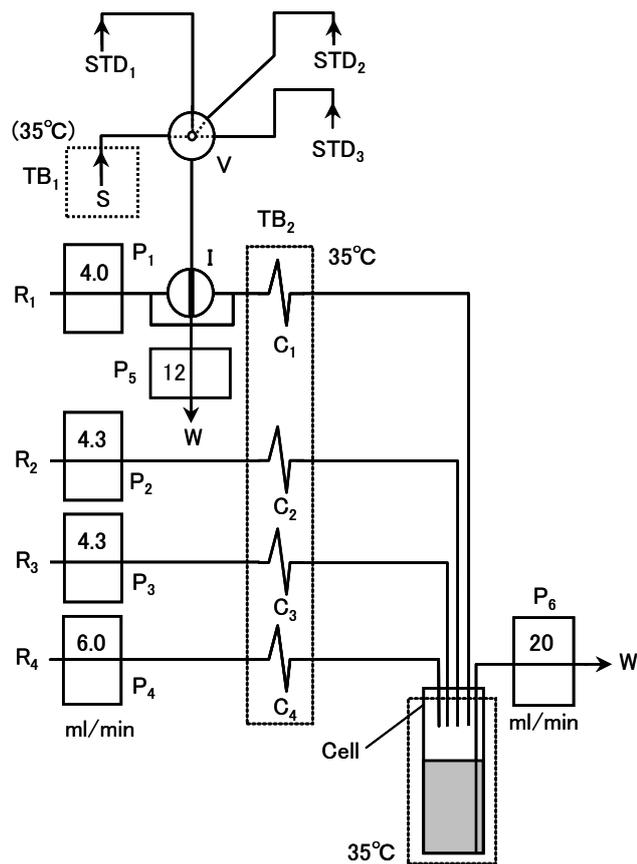


Fig. 1 Automated flow system for determination of reactive copper. R₁:carrier, R₂:phosphate buffer (17.5 mM Na₂HPO₄-17.5 mM NaH₂PO₄-65.7 mM NaOH), R₃:25 mM OPDA, R₄:25 mM AA-50 mM HCl, P₁– P₄:double-plunger pumps, P₅ and P₆:peristaltic pumps, S:sample, STD₁–STD₃:copper standard solutions, V:selector, I:injector (0.48 ml), C₁– C₄:coils (0.5 mm i.d., 2 m), W:waste.

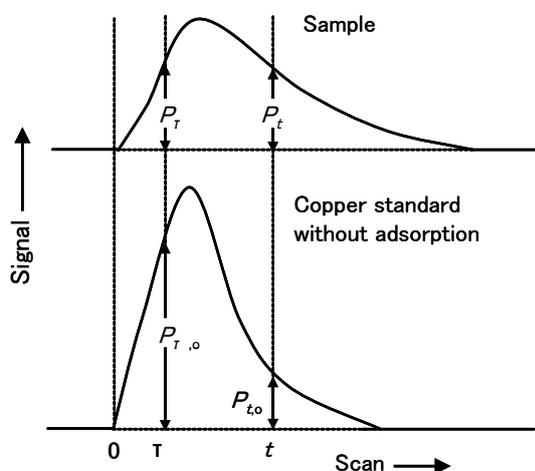


Fig. 2 Measurement of absorbance of peak profiles for evaluation of adsorption of copper. t and τ : scanning times from the start point of the profile, P_t and $P_{t,0}$: values of absorbance from the baseline for sample and copper standard solution, respectively, at a scanning time of t , P_τ and $P_{\tau,0}$: values of absorbance from the baseline for sample and copper standard solution, respectively, at a scanning time of τ .

the cell was discarded (step 9). For washing, 2.8 ml of the carrier was introduced into the cell (step 10) and then discarded (step 11). The cell was washed twice.

Table 1 Operation of the flow system (Fig. 1)

Step No.	Time /sec	Part	Flow rate /ml min ⁻¹	Function
1	—	V	—	Selection of solution loaded
2	20	P ₅	12	Introduction of the above solution into I
3	—	I	—	Change of the position of flow way to cell
4	15	P ₁	4.0	Introduction of carrier (R ₁ , 1 ml) with the injected solution into the cell
5	7	P ₂	4.3	Introduction of phosphate buffer solution (R ₂ , 0.5 ml) into the cell
6	7	P ₃	4.3	Introduction of OPDA solution (R ₃ , 0.5 ml) into the cell
7	5	P ₄	6.0	Introduction of AA solution (R ₄ , 0.5 ml) into the cell
8	310	Recorder	—	Measurement of absorbance
9	20	P ₆	20	Discard of the solution in the cell
10	42	P ₁	4.0	Introduction of R ₁ (2.8 ml) into the cell
11	20	P ₆	20	Discard of the solution in the cell
12	—	—	—	Repetition of step 10 and 11
13	—	I	—	Change of the position to solution injection

Symbols are indicated in Fig.1 and the text.

2.4. Evaluation of adsorbed copper

In this study, adsorption of copper on inner wall of PTFE tube, valve and cell was evaluated by the normalized absorbance ratio (NAR) [3], *i.e.*, $(P_t/P_{t,0})/(P_\tau/P_{\tau,0})$, where symbols are indicated in Fig. 2. The adsorption is indicated by NAR value; NAR>1 by the adsorption, NAR<1 by the dissociation of adsorbed copper and NAR=1 in no adsorption.

3. Results and discussion

3.1. Adsorption of copper

For the evaluation of the adsorption of copper, a single-line FIA system consisted of a double-plunger pump, the injection (I in Fig. 1) and an atomic absorption spectrophotometer AAS for the detection of total copper. A carrier of water, reagent solution (R₁, R₂ or R₃) or the mixed solution *i.e.*, the reacting solution, was pumped into a PTFE tube or a fused silica flow cell at 1.2 ml/min. The carrier containing copper was injected as sample. Acidic carrier (0.1 M HCl) can eliminate adsorbed copper from a PTFE tube [2]. Therefore, the peak profile without the adsorption was obtained by using the copper standard and the carrier with 0.1 M HCl. Figure 3 shows resulting NAR profiles ($\tau=15$ s). Significant adsorption was observed for water and the phosphate buffer solution (a and b in Fig. 3A) in the case of the PTFE tube, whereas it was found for the buffer solution (b in Fig. 3B) alone in the case of the flow cell. Increase of NAR with increasing the scanning time (Fig. 3A) suggests the adsorption was caused by the contact of sample zone with the inner wall of

PTFE tube [3].

The peak maximum of absorption profiles appeared at around 20 s for the PTFE tube. Ratio ($P_{max}/P_{max,o}$) between the peak

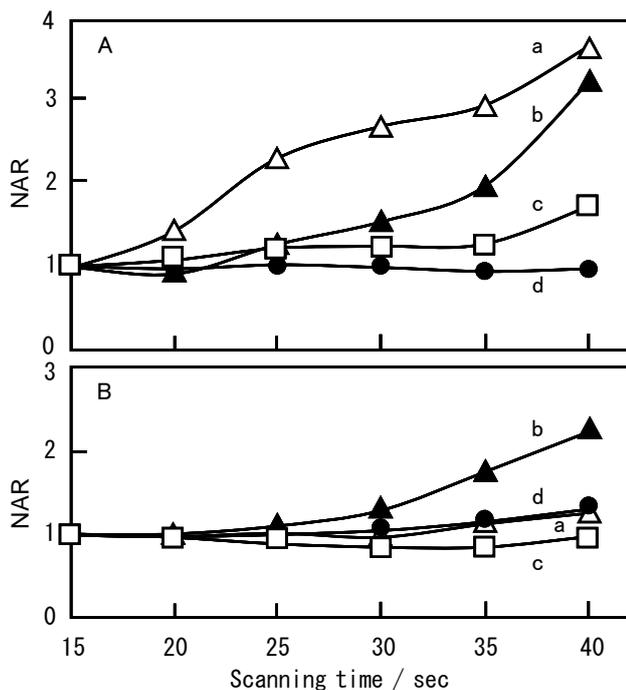


Fig. 3 Normalized absorbance ratio (NAR) for a PTFE tube (0.5 mm i.d., 1.5 m) (A) and a fused silica flow cell (10 mm, 1.5 mm i.d.) (B). carrier: water (a), phosphate buffer (b), all reagent (c) and 0.1 M HCl (d). sample: 0.4 $\mu\text{g ml}^{-1}$ of copper solution (0.2 ml) containing reagents the same as the carrier.

height of the carrier tested and that of 0.1 M HCl also shows the degree of adsorption. The absorption profile of copper was measured by AAS in order to obtain peak heights. The difference in sensitivity among media was corrected by the direct AAS measurement without the FIA system. For the PTFE tube, carrier solutions except OPDA-buffer solution gave 30 or 40% decrease of peak height, supporting that the adsorption of copper was significant in neutral and basic solution [2]. The adsorption is presumed to be the association of cations with the anion sites on the surface of PTFE [6]. From such adsorption properties, the injected solution was directly introduced into the fused silica cell, as shown in Fig. 1. A sampling loop (PTFE tube) with a larger diameter was connected with the injector (I) in order to reduce the adsorption by minimizing the ratio of contacting surface area to volume of injected sample.

Table 2 Comparison of ratio of peak height ($P_{max}/P_{max,o}$) for different carrier solutions

Carrier solution	$P_{max}/P_{max,o}$	Adsorption
PTFE tube		
Water	0.65	Strong
Phosphate buffer	0.57	Strong
OPDA + phosphate buffer	0.90	Weak
All reagents	0.69	Strong
Fused silica flow cell		
Water, phosphate buffer, or all reagents	0.90 - 0.93	Weak
OPDA + phosphate buffer	0.99	Negligible

$P_{max}/P_{max,o}$ is defined in the text.

3.2. Determination of copper

Figure 4 shows calibration curves for copper. Poor linearity of the calibration curve (Fig. 4, b) indicates the influence of the adsorption. For the speciation, the carrier with higher concentration of acid is not recommended to keep the original condition of sample [4]. Magnesium ions were reported as the inhibitor of adsorption by their occupation of the anionic adsorption sites on the surface of PTFE tube [2,6]. In this study, magnesium or aluminum ions were added to the carrier (R_1 in Fig. 1) and the sample (S). Aluminum ions were more effective than magnesium and gave the linear calibration curve (Fig. 4, a). Therefore, aluminum ions were adopted as the inhibitor. Copper was determined with an error of about 1 ng/ml. Six samples can be analyzed in 1 hour.

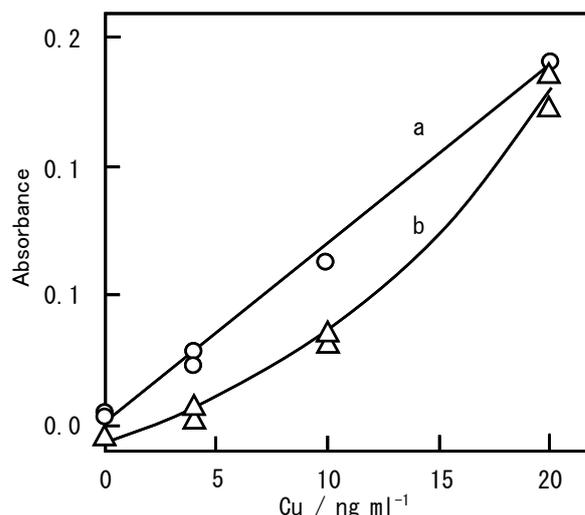


Fig. 4 Calibration graph for copper under the conditions of 5 mM AA and 5mM OPDDA at pH 6.9 and 35°C using a carrier of (a) 10 $\mu\text{g ml}^{-1}$ of Al(III) and (b) water.

3.3. Applicability of the proposed flow system

HS, Fe(III) and Si (silicate) are important components controlling physicochemical properties of humic iron aggregates and trace elements in/on the aggregates [5, 7, 8]. Humic acid is also aggregated by the addition of aluminum ions [9]. Therefore, the influence of aluminum was investigated in the determination of reactive copper in artificial river water (Fig. 5). The monitoring of concentrations was started by the addition of aluminum. Artificial river water samples with typical concentrations of HS, Fe(III) and Si (pH 6) were prepared and equilibrated for 24 h at 25°C in polystyrene bottles before analysis [5]. The sample bottle was placed in the water bath (BT_1 in Fig. 1) and automatically injected into the flow system. The addition of aluminum ions (10 $\mu\text{g/ml}$) decreased pH value to 4.5. For the speciation of copper, the samples before and after the addition were fractionated by filtration and ultrafiltration technique [5, 8]. The concentrations of HS, Fe and Si in the fractions were determined by spectrofluorometry for HS and inductively coupled plasma emission spectrometry for Fe and Si [5] (Table 3). The concentration of HS was monitored fluorometrically with a flow system with another injector in-lined between I and V in Fig. 1. The concentrations of HS, Fe, Si and reactive copper after the equilibration did not agree with the initial concentrations at the addition, because of the adsorption on the inner wall of the bottle.

For Sample 1 in Table 3, higher concentrations of HS and iron in the particle fraction ($>0.45\mu\text{m}$) indicate the formation of humic iron aggregates by the addition of aluminum ions. In Fig.

5, a decrease in concentration of HS was caused by a decrease of fluorescent humic acid (HA) molecules, indicating the aggregation occurred (fulvic acid does not contribute to the aggregation [5]). Thus, a decrease in concentration of reactive copper also indicates the aggregation of copper with humic iron.

Table 3 Analytical results of humic substance (HS), Fe and Si in artificial river water samples before and after addition of 10 $\mu\text{g Al ml}^{-1}$ (and 2 mg F ml^{-1})

Addition	HS / $\mu\text{g ml}^{-1}$			Fe / ng ml^{-1}			Si / $\mu\text{g ml}^{-1}$		
	A	B	C	A	B	C	A	B	C
Aluminum for Sample 1									
Before	-	0	0.10	-	35	32	-	4.1	4.7
After	0.05	0	0.03	63	0	0	0.1	0.5	8.2
Aluminum and fluoride for Sample 2									
Before	-	0	0.08	-	28	26	-	3.3	3.8
After	-	-	-	-	0	52	0.1	1.7	7.2

Sample 1 : 20 ng Cu ml^{-1} - 0.2 $\mu\text{g HS ml}^{-1}$ - 100 ng Fe ml^{-1} - 10 $\mu\text{g Si ml}^{-1}$. Sample 2 : 16 ng Cu ml^{-1} - 0.16 $\mu\text{g HS ml}^{-1}$ - 80 ng Fe ml^{-1} - 8 $\mu\text{g Si ml}^{-1}$. Fractions : $>0.45\mu\text{m}$ (A), $\text{MW}1000-0.45\mu\text{m}$ (B) and $\text{MW}<1000$ (C)

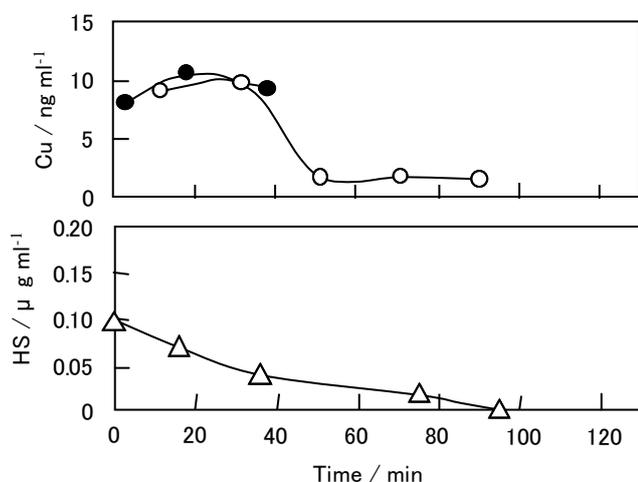


Fig. 5 Effect of addition of aluminum ($10\mu\text{g ml}^{-1}$) on concentrations of reactive copper (\circ, \bullet) and humic substance (HS) (\triangle) in an artificial river water sample (20 ng Cu ml^{-1} - $0.2\mu\text{g HS ml}^{-1}$ - 100 ng Fe ml^{-1} - $10\mu\text{g Si ml}^{-1}$).

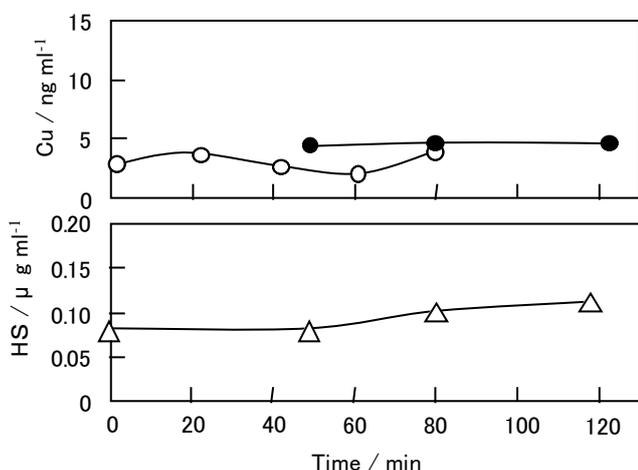


Fig. 6 Effect of addition of aluminum ($10\mu\text{g ml}^{-1}$) and fluoride (2 mg ml^{-1}) on concentrations of reactive copper (\circ, \bullet) and humic substance (HS) (\triangle) in an artificial river water sample (16 ng Cu ml^{-1} - $0.16\mu\text{g HS ml}^{-1}$ - 80 ng Fe ml^{-1} - $8\mu\text{g Si ml}^{-1}$).

An increase of concentration of reactive copper within 20 min was probably caused by the dissociation of colloidal $\text{Fe}(\text{OH})_3$ including copper as non reactive form, because pH value decreased to 4.5 by the addition of aluminum. In use of aluminum as the inhibitor, immediate measurement after the addition of aluminum was required to determine reactive copper in the original sample.

For Sample 2 in Table 3, fluoride ions (2 mg/ml) were added with aluminum ions to prevent the aggregation of humic iron by changing $\text{Al}(\text{OH})_3$ and/or hydroxo- $\text{Al}(\text{III})$ complex to fluoro- $\text{Al}(\text{III})$ complex. The concentration of fluoride ions was as low as not to interfere with the determination. The calibration curve of copper was similar to that of Fig. 4, a. The value of pH was 6.5 in the presence of aluminum and fluoride. After the addition, most of iron existed in the low molecular fraction ($\text{MW}<10^3$) (Table 3), indicating the dissociation of the original humic iron aggregates. On the other hand, the concentration of reactive copper was not affected by the addition of aluminum and fluoride (Fig. 6). Therefore, the addition of aluminum and fluoride is recommended to the speciation of copper.

4. Conclusion

An automated batch-wise flow system was proposed for the determination of reactive copper in water samples. The reactive copper was determined by spectrophotometric monitoring of the copper-catalyzed oxidation of L-ascorbic acid in the presence of *o*-phenylenediamine. Adsorption of copper on the inner wall of PTFE tube was evaluated and minimized in the proposed flow system and by the addition of Al (and F) ions as the adsorption inhibitor. The usefulness of the proposed system was demonstrated in the determination of reactive copper in artificial water samples.

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