FLOW INJECTION DETERMINATION OF ULTRATRACE MOLYBDENUM IN NATURAL FRESH AND TAP WATER SAMPLES BY CATALYTIC SPECTROPHOTOMETRY

Susumu Kawakubo[*], Ryo Fukasawa and Masaaki Iwatsuki

Department of Applied Chemistry and Biotechnology, Faculty of Engineering,

Yamanashi University, Takeda, Kofu 400, Japan

ABSTRACT

A sensitive flow injection method was developed for the determination of molybdenum using the molybdenum-catalyzed oxidation of L-ascorbic acid with hydrogen peroxide and the successive condensation of dehydroascorbic acid with o-phenylenediamine to produce quinoxaline derivatives. Absorbance was monitored at 345 nm under the conditions at pH 3.2 and 50°C. A calibration graph of molybdenum was linear up to at least 3 µg l^{-1} for 163 µl of sample. The detection limit was 0.06 µg l^{-1} (0.01 ng), and a typical sampling rate was 20 samples h^{-1}. The addition of trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid was effective to eliminate the interferences from vanadium, iron and copper ions. Without any preconcentration and separation, the proposed method was successfully applied to analyses of river, lake, rain and tap water samples.

Keywords: Catalytic method, molybdenum determination, FIA, L-ascorbic acid, o-phenylenediamine, natural fresh and tap water.

---

[*]To whom correspondence should be addressed.

The survey and monitoring of trace concentrations of molybdenum is required for the quality control of tap and drinking water[1]. The FIA method is useful for automatic and routine analysis of water samples. A detection limit (DL) of 0.4 μg Mo l^{-1} was reported for a flow injection method combined with inductively coupled plasma mass spectrometry[2]. The molybdenum-catalyzed oxidation of iodide by H_2O_2 and its potentiometric[3], amperometric[4] and spectrophotometric[5,6] detections was applied to FIA with DL in a range of 1 - 10 μg l^{-1}. However, these values[2-6] of DL were insufficient for analyses of natural fresh and tap water samples[7,8]. A sufficient DL of 0.02 μg l^{-1} was reported for a FIA method based on a chemiluminescence reaction of Mo(III) and lucigenin[9].

We recently proposed manual-batch methods for the highly sensitive determination of molybdenum based on the molybdenum-catalyzed oxidation of L-ascorbic acid (AA) to dehydroascorbic acid (DAA) and the spectrofluorometric[7] and spectrophotometric[8] detections of 3-(1,2-dihydroxyethyl)furo[3,4-b] quinoxalin-1-one (QX) formed by the successive condensation of DAA with o-phenylenediamine (OPDA). This paper describes a combination of spectrophotometric FIA with the above reaction system. The reaction variables and FIA system were optimized for the sensitive and rapid determination of molybdenum. The proposed method has successfully been applied to analyses of river, lake and tap water samples containing molybdenum at 0.1 - 1 μg l^{-1}.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade. De-ionized and distilled water was used throughout. AA solutions in 0.8 M HCl were prepared by dissolving L-ascorbic acid in chilled water and by adding 16 ml of 5 M HCl. OPDA solutions (16 - 48 mM) were prepared by dissolving o-phenylenediamine in water.

The AA and OPDA solutions were stored in the refrigerator at 5°C and used within about 6 h. Buffer solutions containing different concentrations of acetic acid, 0.4 M sodium acetate and 0.8 M NaOH were prepared for adjusting the pH of the reaction solution. A stock molybdenum solution (1.00 g Mo l^{-1}) was prepared by dissolving $Na_2MoO_4 \cdot 2H_2O$ (purity≥99%) in water. Working molybdenum standard solutions were prepared by diluting the stock solution with water just before use. The other reagent solutions were prepared by the same manners described previously[7,8].
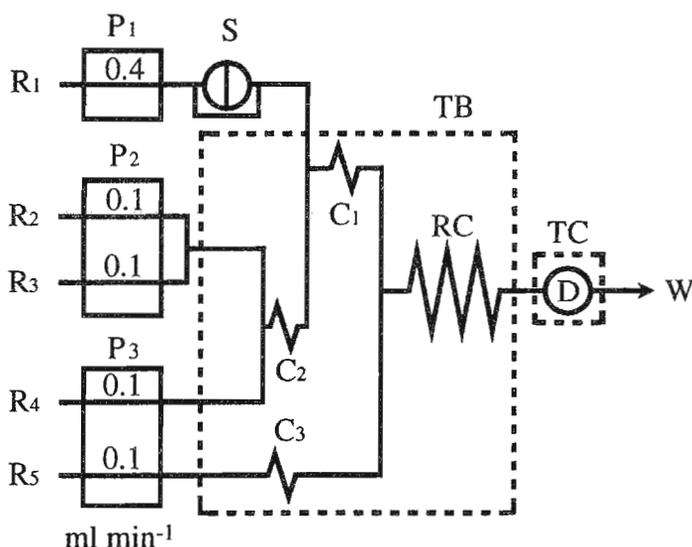
FIA System

Figure 1 shows the FIA system used in this study. PTFE tubing (0.5 mm i.d.) and Daiflon connectors were used throughout. Three double plunger pumps (Nihon Seimitsu Kagaku NP-FX-1U, Sanuki Kogyo SSP DM2M-1024 and a pumping part in Hitachi 655A-11 liquid chromatograph) were used for propelling a carrier (R_1) and reagent solutions (R_2 - R_5). A valve-type sample injector[10] (S) comprised a six-way valve, a sample loop (0.5 mm i.d.) and a bypass coil (0.25 mm i.d., 0.2 m). The sample volume injected was measured photometrically[11]. An Atto SJ-1220 peristaltic pump was used to introduce a sample solution into the sample injector. A Shimadzu UV-140-01 double-beam spectrophotometer with a silica glass flow cell (optical path, 10 mm long and 18 µl) and a strip-chart recorder was used for absorbance measurements. The temperature of the solution streams was controlled by submerging a reaction coil (RC) and the other coils (C_1 - C_3) in a thermostated water bath (50±0.1°C, unless otherwise stated). The cell chamber (TC) of the spectrophotometer was also kept at a constant temperature by circulating water from the water bath.

Procedure

The water carrier (R_1 in Fig. 1) and the reagent solutions (R_2 -R_5) were pumped at the constant flow rates indicated in Fig. 1. A sample solution (163 µl) was

introduced into the sample injector (S) by a peristaltic pump, and then injected into the carrier flow. The absorbance of QX was monitored at 345 nm which gave the maximum absorbance. For constructing a calibration graph, the height of the resulting peak signal was plotted against the concentration of molybdenum injected.

Fig. 1 FIA system. P_1 - P_3, pumps; R_1, water carrier; R_2, 0.64 M AA and 0.8 M HCl solution (5℃); R_3, 5.4 M acetic acid, 0.4 M sodium acetate and 0.8 M NaOH solution; R_4, 40 mM H_2O_2 solution; R_5, 40 mM OPDA solution (5℃); S, sample injector (sample volume, 163 μl); D, flow cell; RC, reaction coil (0.5mm i.d., 6 m); C_1-C_3, coils (0.5mm i.d., 1 m) for temperature-control of the solution streams; TB and TC, temperature-controlled water bath and cell chamber (50℃), respectively; W, waste.

RESULTS AND DISCUSSION

Combination of FIA with catalytic reaction

As described in the previous paper[8], the production process of QX shown by reactions (1) and (2) gives a relationship of eq. (3) between absorbance (*A*) of QX and reaction time (*t*), when the concentrations of AA, H_2O_2 and OPDA are high

enough to be approximately constant in the rate equations of the reactions.

$$
\begin{aligned}
&\text{Mo catalyst}\\
&\text{AA} + \text{H}_2\text{O}_2 \quad \xrightarrow{\quad} \quad \text{DAA} \quad\quad\quad\quad\quad (1)\\[4pt]
&\text{DAA} + \text{OPDA} \;\xrightarrow{\quad}\; \text{QX} \quad\quad\quad\quad\quad\quad (2)
\end{aligned}
$$

$$
A = \varepsilon\,[\text{QX}] = \varepsilon\,(k_c[\text{Mo}]+k_b)[t-\{1-\exp(-k_1 t)\}/k_1]
$$
$$
+ \varepsilon\,[\text{DAA}]_0\{1-\exp(-k_1 t)\} \quad\quad\quad\quad (3)
$$

where ε is the molar absorption coefficient of QX, $[\text{DAA}]_0$ is the initial concentration of DAA introduced from the oxidation of the AA solution in storage, k_c and k_b are apparent rate constants of catalyzed and uncatalyzed parts in reaction (1), respectively, and k_1 is the apparent rate constant of reaction (2). From eq. (3), absorbance ($A = A_f$) at a fixed time ($t = t_f$) is represented by eq. (4).

$$
A_f = k_c{}'[\text{Mo}] + k_b{}' \quad\quad\quad\quad\quad\quad (4)
$$

where $k_c{}' = \varepsilon\,k_c[t_f-\{1-\exp(-k_1 t_f)\}/k_1]$ and $k_b{}' = \varepsilon\,k_b[t_f-\{1-\exp(-k_1 t_f)\}/k_1] + \varepsilon\,[\text{DAA}]_0$ $\{1-\exp(-k_1 t_f)\}$. On signal outputs of FIA, $k_c{}'[\text{Mo}]$ and $k_b{}'$ in eq. (4) are corresponding to the peak height and base-line absorbance, respectively. From eq. (4), an increase of $[\text{DAA}]_0$ originated from the oxidation of AA in storage does not affect the peak height, but it increases the base-line absorbance. Therefore, the AA solution was stored at 5°C[8]. The oxidation product of OPDA in storage did not seriously interfere with the measurement of QX at 345 nm. However, the OPDA solution was also stored at 5°C to keep at a constant concentration. When the reaction proceeded to $A_f = 1$ under the initial concentrations of 80 mM of AA and each 5 mM of H_2O_2 and OPDA, the decreases of the concentrations were less than 3 %[8], which did not affect the reaction kinetics as shown by Figs. 2(B) to (D).

Optimization of reaction variables

In FIA, sample solution injected is diluted in a flow system by the dispersion,
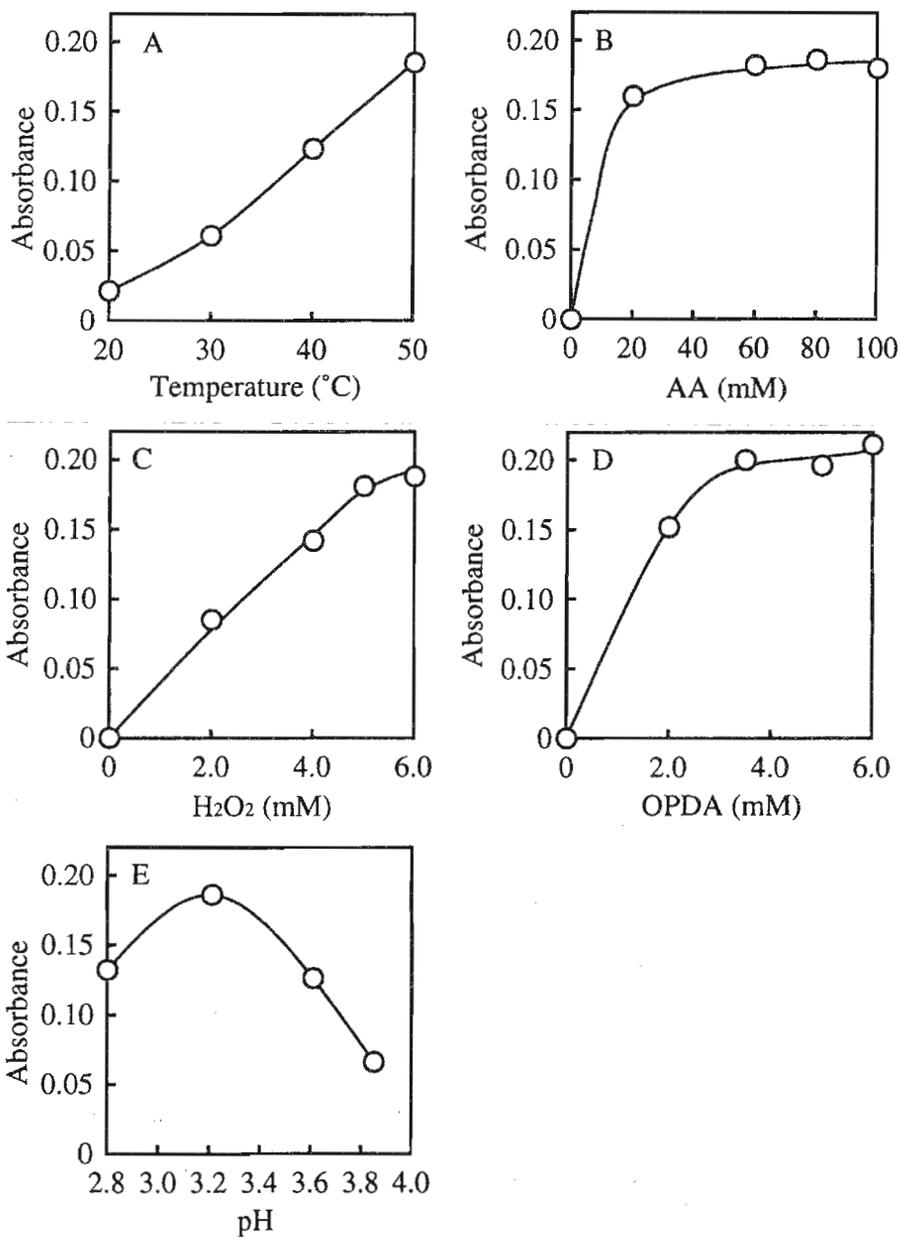
Fig. 2 Effects of reaction temperature (A), AA (B), H_2O_2 (C) and OPDA (D) concentrations and pH (E) on absorbance of peak height for 2 μg Mo I^{-1} under the conditions as shown in Fig. 1.

which decreases the sensitivity. Therefore, the acceleration of the catalyzed reaction was studied for the sensitive determination of molybdenum by FIA. Figure 2 shows the effects of the reaction temperature, reagent concentrations and pH on the peak height for 2 µg l^{-1} of molybdenum. In Fig. 2, the reagent concentrations indicate the concentrations at the mixing point where all the reagent solutions are mixed. Figure 2(A) shows the effect of the reaction temperature. An increase in the temperature increased the peak height. Therefore, the maximum temperature of 50°C was selected as a recommended temperature. Figures 2 (B) to (D) show the effects of the AA, H_2O_2 and OPDA concentrations, respectively. An increase in the concentrations of these reactants increased the peak height and was not effective beyond the concentrations of 60 mM of AA, 5 mM of H_2O_2 and 3.5 mM of OPDA, respectively. Therefore, 80 mM of AA and each 5 mM of H_2O_2 and OPDA were adopted as the optimized concentrations. From Fig. 2 (E), a pH value of 3.2 was adopted to obtain the maximum peak height. Consequently, a peak height about five times higher than that under the previous conditions[8] was obtained by altering the reaction temperature from 25°C[8] to 50°C.

An increase of base-line absorbance (BA) often increases the variation of BA caused by the fluctuation of pulsating flows from the pumps[12] and therefore increases the DL. The variation of BA was comparable to an instrumental noise (about ±0.002) under the conditions shown in Fig. 2, although the effects of the above reaction variables on BA were similar to those on the peak height and changed BA from 0.01 to 0.5. This result indicates that the flow rates of pumps used were probably controlled well.

Optimization of FIA system

Figure 3 shows the effects of the flow rate, length of reaction coil and sample volume on the peak height for 2 µg l^{-1} of molybdenum and the sampling rate (samples h^{-1}). A decrease in the total flow rate and an increase in the length of the reaction coil and in the sample volume increased the peak height. The system

conditions indicated in Fig. 1 were selected to obtain the higher peak height for the sensitive determination of molybdenum without an extreme decrease of the sampling rate.
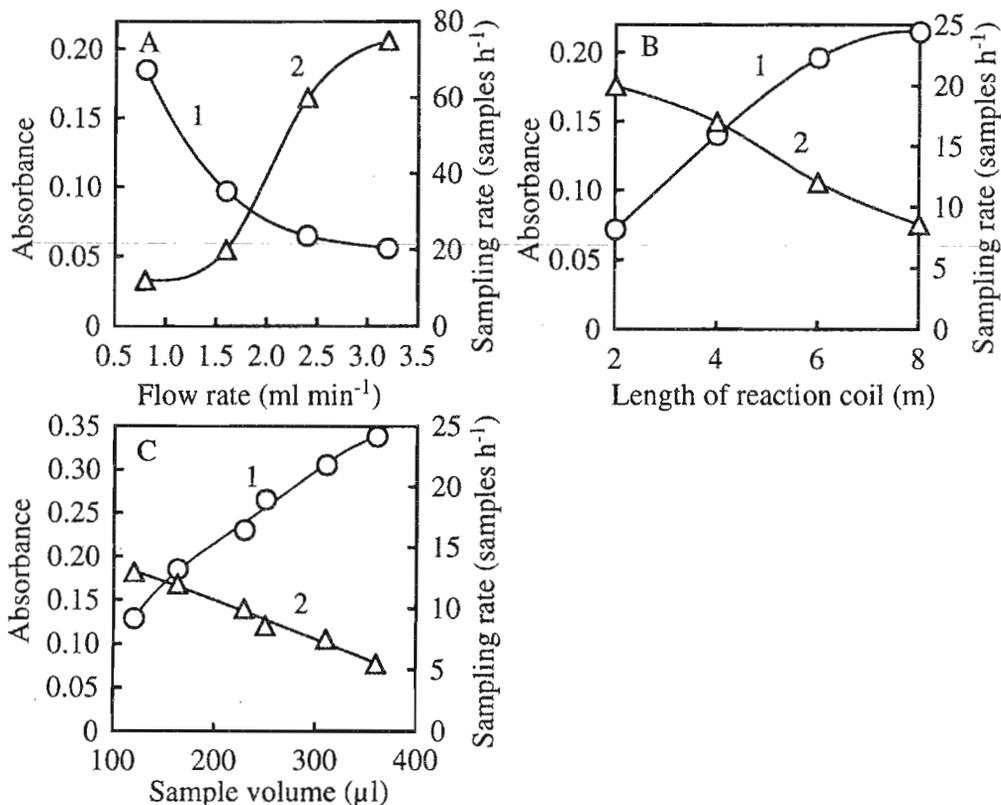

Fig. 3 Effects of flow rate (A), length of reaction coil (B) and sample volume (C) on absorbance of peak height (1) for 2 μg Mo l^{-1} and sampling rate (2) under the conditions as shown in Fig. 1.

The variation of *BA* at 2 m of reaction coil or 3.2 ml min^{-1} of flow rate became twice larger than that under the other system conditions. These results indicate that the increase of variation is caused by an extreme increase in the flow rate and an extreme decrease in the length of reaction coil[13]. Therefore, a certain extent of dispersion is required to decrease the variation of *BA* caused by the fluctuation of pulsating flows from the pumps.

Determination characteristics

Under the reaction conditions optimized above, typical peak signals were recorded for different concentrations of molybdenum (Fig. 4). The variation (s, ±absorbance) of BA was used to estimate the value of DL of molybdenum corresponding to $3s$. As shown in Table 1, the relative value of DL obtained by the FIA method was 0.06 µg l^{-1}, comparable to that by the manual-batch fluorometric[7] and photometric[8] methods. The relative standard deviation was 3 - 5 % in the determination of 0.5 - 3 µg l^{-1} of molybdenum by the FIA method. An absolute value (0.01 ng) of DL by the FIA method was 15-times lower than that by the manual-batch method[8]. The calibration graph by the FIA method was linear up to at least 3 µg l^{-1} of molybdenum. The linear range of the calibration graph was comparable to that obtained by the manual-batch methods[7,8]. The sampling rate in the determination of 0.5 µg l^{-1} of molybdenum was about three times higher than that by the manual-batch method, although it was changed by depending on the concentration of molybdenum as shown in Fig. 4.
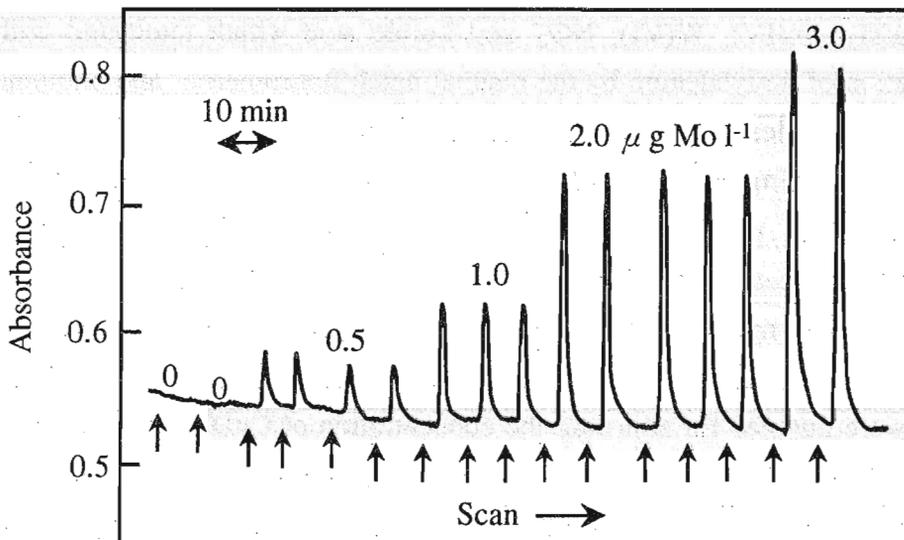
Fig. 4 Typical peak profiles for different concentrations of molybdenum under the conditions as shown in Fig. 1. Arrows indicate injection points.

Table 1 Comparison of determination characteristics between the manual-batch and FIA methods for the determination of molybdenum

	FIA method	Manual-batch method[Ref.8]
Detection limit[a]		
Relative value ($\mu g\ l^{-1}$)	0.06	0.03
Absolute value (ng)	0.01	0.15
Linear range of calibration graph[a]		
Relative value ($\mu g\ l^{-1}$)	0 - 3	0 - 4
Absolute value (ng)	0 - 0.5	0 - 20
Sampling rate (samples h^{-1})	20 ($0.5\ \mu g\ Mo\ l^{-1}$)	6

[a]Concentration and amounts indicate those in the sample solution injected for FIA and in the reaction solution (5 ml) for the manual-batch method.

Interferences

The interference from foreign ions was investigated for Fe(II), Fe(III), V(IV), V(V), Cu(II), Sn(IV), W(VI), NO_2^- and humic acid which interfered with the determination of molybdenum by the manual-batch fluorometric[7] and photometric[8] methods. The tolerable limit was defined as the maximum concentration of a foreign ion causing about a $\pm 5\%$ error in the determination of 2 $\mu g\ l^{-1}$ of molybdenum, as in the previous papers[7,8]. *trans*-1,2-Cyclohexanediamine-*N*,*N*,*N'*,*N'*-tetraacetic acid (CyDTA) effectively masked Fe(II), Fe(III), V(IV), V(V), Cu(II) and humic acid[7,8]. Therefore, CyDTA was used for the practical analysis. The buffer (R_3) and H_2O_2 (R_4) solutions containing each 0.8 mM of CyDTA were pumped for adjusting the concentration of CyDTA to 0.2 mM in the reaction. The addition of CyDTA did not significantly affect the peak height of molybdenum. Table 2 shows the tolerable limits of the interfering ions in the FIA determination. Tolerable limits of Fe(II), Fe(III) and W(IV) in the FIA method were lower than those in the manual-batch method[8]. A higher concentration of CyDTA was not used to increase the tolerable limits, because many fresh water

samples probably contain these ions at tolerable concentrations, *e.g.*, the reported concentrations[14] in river water samples are at a 0.01 mg l^{-1} level for iron and at 0.01 to 0.1 μg l^{-1} levels for tungsten.

Table 2 Tolerable limits of interfering ions in the FIA and manual-batch methods for the determination of molybdenum

Interfering ions	Tolerable limits[a,b] (mg l^{-1})	
	FIA method	Manual-batch method[Ref.8]
Fe(II)	0.1	0.5
Fe(III)	0.1	0.5
V(IV)	0.05	0.1^c
V(V)	0.05	0.05^c
Cu(II)	0.05	0.05^c
Sn(IV)	0.1	-
W(VI)	0.01	0.05^c
NO$_2^-$	0.2^c	0.1^c
Humic acid	2^c	2^c

^{a}Concentration indicates that in the sample solution injected for FIA and in the reaction solution (5 ml) for the manual-batch method.
^{b}In the determination of 2 μg Mo l^{-1} in the presence of 0.2 mM CyDTA.
^{c}Maximum concentrations tested.

Analysis of natural fresh and tap water samples

River, lake, rain and tap water samples were collected in Kofu, Japan. In order to eliminate particulate matter, each 100 ml of the sample solutions was centrifuged at about 1000*g* for 15 min and supernatant solutions were taken for analysis[7,8]. For a comparison, the molybdenum contents were also determined by the manual-batch photometric method using the reaction at 25°C[8]. Table 3 shows the results of the determination of molybdenum. For river and lake water samples, the recoveries of molybdenum (1 μg l^{-1}) added to the samples were 96 -103%, which were acceptable regarding the analytical precision. Except No.2 sample, the results

obtained by the proposed FIA method agreed with those obtained by the manual-batch photometric method.

Table 3 Determination of molybdenum (μg l^{-1}) in river, lake, rain and tap water samples by the present FIA and manual-batch methods

Sample No. and sampling data	Pretreatment[a]	FIA method[b]		Manual-batch method	
1 Ai river pH 7.8, 2/5/1997	Centrifugation	0.42±0.01 (n=4)		0.40±0.05 (n=3)	
2 Nigori river pH 7.3, 3/10/1997	Centrifugation Filtration	6.0 ±0.1 3.7 ±0.2	(n=3) (n=3)	4.7±0.3 3.8, 3.8	(n=3)
3 Lake Chiyoda pH 7.5, 2/5/1997	Centrifugation	0.31±0.04 (n=4)		0.28±0.02 (n=3)	
4 Rain water 1/24/1997	Centrifugation	0.25±0.02 (n=5)		-	
5 Tap water pH 7.0, 2/7/1997	Centrifugation	0.99±0.01 (n=3)		1.2, 1.0	

[a]For removal of solid particles in samples.
[b]No.2 sample was diluted with the same or twice volume of water.

For No.2 sample, by warming the supernatant solution at 50°C for 10 min, the manual-batch method gave higher analytical results (6.5±0.1 μg l^{-1}, n=3) comparable to those obtained by the FIA method. The warming at 50°C as in the FIA system probably accelerated the dissolution or dissociation of molybdenum from the inorganic substances[14] in the supernatant solution. The filtration through a membrane filter with a pore size of 0.3 μm gave the same result for the FIA and manual-batch methods, as shown in Table 3. The filtration may be required to remove the solid particles for the water samples as No.2 sample which was turbid

and contained a higher concentration of molybdenum compared to the other samples analyzed.

REFERENCES

1. M. Ando : *Bunseki Kagaku*, **1994**, 696.

2. J.M. Craig and D. Beauchemin : *Analyst* [London], **119**, 1677 (1994).

3. H. Müller and V. Müller : *Anal. Chim. Acta*, **180**, 30 (1986).

4. M. Trojanowicz, A. Hulanicki, W. Matuszewski, M. Palys, A. Fuksiewicz, T. Hulanicka-Michalak, S. Raszewski, J. Szyller and W. Augustyniak : *Anal. Chim. Acta*, **188**, 165 (1986).

5. Z. Fang and S. Xu : *Anal. Chim. Acta*, **145**, 143 (1983).

6. J.C. de Andrade, R.E. Bruns and S. de Paula Eiras : *Analyst* [London], **118**, 213 (1993).

7. S. Kawakubo, H. Suzuki and M. Iwatsuki : *Anal. Sci.*, **12**, 767 (1996).

8. S. Kawakubo, K. Ogihara and M. Iwatsuki : *Bunseki Kagaku*, in press.

9. J.R. Lu and Z.J. Zhu : *Fenxi Huaxue*, **22**, 909 (1994); [Anal. Abstr., **57**, 3D132 (1995)].

10. J. Ruzicka and E.H. Hansen : *"Flow Injection Analysis"*, p.108, Wiley-Interscience, New York, 1981.

11. S. Kawakubo, M. Iwatsuki and T. Fukasawa : *Anal. Chim. Acta*, **282**, 389 (1993).

12. S. Kawakubo, K. Kajihara and M. Iwatsuki : *Anal. Sci.*, **12**, 237 (1996).

13. S. Kawakubo, T. Katsumata, M. Iwatsuki, Tsug. Fukasawa and T. Fukasawa : *Analyst* [London], **113**, 1827 (1988).

14. Y. Tanizaki, T. Shimokawa and M. Nakamura, *Environ. Sci. Technol.*, **26**, 1433 (1992).