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Flow-injection determination of hydrogen peroxide based on fluorescence quenching of chromotropic acid catalyzed with Fe(II)

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ABSTRACT

Flow-injection analysis system (FIA system), which was based on Fe(II)-catalyzed oxidation of chromotropic acid with hydrogen peroxide, was developed for the determination of hydrogen peroxide. The chromotropic acid has a fluorescence measured at λ_{em} = 440 nm (emission wavelength) with λ_{ex} = 235 nm (excitation wavelength), and the fluorescence intensity at λ_{em} = 440 nm quietly decreased in the presence of hydrogen peroxide and Fe(II), which was caused by Fe(II)-catalyzed oxidation of chromotropic acid with hydrogen peroxide. By measuring the difference of fluorescence intensity, hydrogen peroxide (1.0×10^{-8}–1.0×10^{-3} mol L^{-1}) could be determined by the proposed FIA system, whose analytical throughput was 40 samples h^{-1}. The relative standard deviation (RSD) was 1.03% (n = 10) for 4.0×10^{-8} mol L^{-1} hydrogen peroxide. The proposed FIA technique could be applied to the determination of hydrogen peroxide in rain water samples.

1. Introduction

In oxidation process, hydrogen peroxide at trace level plays an important role. In the wide field of biochemistry [1], clinical chemistry [2,3], food chemistry [4], and environmental chemistry [5–7], hydrogen peroxide must be simply and sensitively determined in order to elucidate the behavior of hydrogen peroxide, such as oxidation processes and interaction mechanisms. Therefore, the development of the novel analytical method for hydrogen peroxide is one of the aims in the analytical science.

Hydrogen peroxide is stoichiometrically produced on the oxidation process in the presence of the oxidase enzyme in human body. In the field of food industry, hydrogen peroxide is added as an antibacterial agent to some foods. Hydrogen peroxide is correlated with redox reaction. Chemical interest in the field of environment study is the elucidation of hydrogen peroxide reactivity on the oxidation reaction in atmosphere. Nitrogen oxides (NO_x) and sulfur oxides (SO_x) are generated by hydrogen peroxide, and it also caused acidic rain.

For the determination of hydrogen peroxide, various kinds of analytical techniques have been reported, such as spectrophotometric [8–18], fluorimetry [19–26], chemiluminescence [27–31] and electrochemical [32–44]. Most of these techniques have some disadvantages, such as time consuming, tedious procedures, expensive reagents and low sensitivity. Considering their disadvantages, we aimed to develop the new analytical method for determining the hydrogen peroxide by simple and rapid technique using inexpensive chemicals and apparatuses.

Some detection techniques, such as spectrophotometric detection [45] and fluorescence quenching method [46], have been applied to the determination of hydrogen peroxide in rain water. Each technique can detect hydrogen peroxide at 7.0×10^{-7} mol L^{-1} and 5.0×10^{-7} mol L^{-1}, respectively. However, more sensitive analytical method is required for elucidating the behavior of hydrogen peroxide in rain water. In this study, the novel flow-injection analysis (FIA) system was developed for the determination of hydrogen peroxide (1.0×10^{-8}–1.0×10^{-3} mol L^{-1}). The hydrogen peroxide was detected on the florescence quenching of chromotropic acid,

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which was based on the oxidation reaction of chromotropic acid with hydrogen peroxide using Fe(II) as a catalyst. We described the development of the proposed FIA system and the elucidation of its analytical performance in this paper.

2. Experimental

2.1. Reagent

All reagents were of analytical-reagent grade. The standard solution (5.0×10^{-2} mol L^{-1} hydrogen peroxide) was prepared daily by diluting hydrogen peroxide solution (30%, 9.0 mol L^{-1}). The concentrations were determined by titration technique with permanganate. The working solutions were accurately diluted with ultrapure water. Reagent solution (RS) was prepared by dissolving chromotropic acid (0.0046 g) in 0.2 mol L^{-1} monochloroacetate buffer (100 mL, pH 3), where 0.25 g of $FeSO_4 \cdot 7H_2O$ was added. And the reagent solution (250 mL) was accurately prepared with volumetric flask. The final concentrations of chromotropic acid and Fe(II) in the RS were 5.0×10^{-5} mol L^{-1} and 3.5×10^{-3} mol L^{-1}, respectively. Ultrapure water (18.3 MΩ cm^{-1}), prepared by a Milli-Q System (Nihon Millipore, Tokyo, Japan), was used throughout.

2.2. Apparatus

A schematic diagram of the flow-injection analysis (FIA) system proposed is shown in Fig. 1. The system consisted of a spectrofluorometer (Shimadzu, Rx-10, λ_{ex} = 235 nm, λ_{em} = 440 nm) equipped with a flow cell (8 μL volume, 10 mm path length), a double plunger-type pump, and a recorder (TOA, FBR-252). PTFE tubing (0.5 mm i.d.) was used for all flow lines. The Corning Model 12 pH/mV meter was also used for pH measurements.

2.3. Procedure

Reagent solution (RS) containing chromotropic acid (5.0×10^{-5} mol L^{-1}) and $FeSO_4$ (3.5×10^{-3} mol L^{-1}) adjusted at pH 3.0 and carrier solution (CS, ultrapurewater) were propelled at flow rate of 0.8 mL min^{-1}. A sample solution (200 μL) was injected into the CS stream by a sample injector. The sample in the CS stream was mixed with RS, and it was flowed into the detector (spectrofluorometer) through the reaction coil (RC, 2 m). The chromotropic acid as a base line was detected at λ_{em} = 440 nm with λ_{ex} = 235 nm. The hydrogen peroxide was determined using the difference of the fluorescence intensity between the non-oxidized chromotropic acid and the oxidized chromotropic acid. The fluorescence intensity of chromotropic acid oxidized by hydrogen peroxide at λ_{em} = 440 nm decreased in the presence of Fe(II) as the catalyst. The optimized conditions of the proposed FIA system were summarized in Table 1.

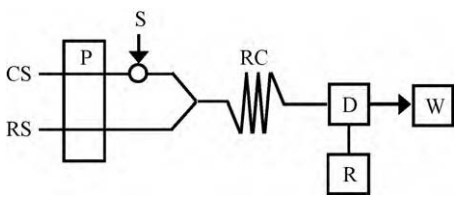

Fig. 1. Schematic diagram of the flow-injection analysis system proposed. CS (carrier solution), ultrapure water; RS (reagent solution), 5×10^{-5} mol L^{-1} chromotropic acid + 3.5×10^{-3} mol L^{-1} $FeSO_4$ (pH 3.0); S (sample), 200 μL of sample volume; RC (reaction coil), 2 m; P (pump), 0.8 mL/min of flow rate; D (detector), λ_{em} = 440 nm and λ_{ex} = 235 nm; R, recorder; W, waste.

Table 1
Operating conditions for the determination of hydrogen peroxide by the proposed FIA system.

Pump	PFA-300 FIA Instrument
Carrier solution (CS)	Ultrapure water
Reagent solution (RS)	5×10^{-5} mol L^{-1} chromotropic acid + 3.5×10^{-3} mol L^{-1} $FeSO_4$ + monochloroacetic acid buffer (pH 3.0)
Flow rate	0.8 mL min^{-1}
Sample volume	200 (L
Reaction coil length	2 m
Detector	Shimadzu, RF-10AX_L
Wavelength	λ_{ex} = 235 nm (excitation) λ_{em} = 440 nm (emission)
Recorder	TOA, FBR-252

3. Results and discussion

3.1. Optimization of chemical conditions

The proposed flow-injection analysis (FIA) system was based on the oxidation reaction of chromotropic acid with hydrogen peroxide catalyzed with Fe(II). Some factors, such as the detected fluorescence, catalyst, reaction pH and concentration of chromotropic acid, were significant for the determination of hydrogen peroxide. Their chemical conditions were examined as follows.

The chromotropic acid has a fluorescence, which can be measured at λ_{em} = 440 nm (emission wavelength) with λ_{ex} = 235 nm (excitation wavelength), whereas the fluorescence intensity of chromotropic acid itself at λ_{em} = 440 nm is quenched by its oxidation with hydrogen peroxide. Fig. 2 shows the emission spectra of (a) chromotropic acid (blank solution) and (b) chromotropic acid in the presence of 5×10^{-6} mol L^{-1} hydrogen peroxide (sample solution), which were measured at λ_{em} = 440 nm with λ_{ex} = 235 nm. The fluorescence intensity at λ_{em} = 440 nm of (b) chromotropic acid in the presence of 5×10^{-6} mol L^{-1} hydrogen peroxide (sample solution) was lower than that of (a) chromotropic acid (blank solution). The fluorescence intensity of chromotropic acid itself decreased by the presence of hydrogen peroxide. It was caused by the oxidation of chromotropic acid by the hydrogen peroxide. The difference of the fluorescence intensity between (a) chromotropic acid (blank solution) as a base line and (b) chromotropic acid in the presence of 5×10^{-6} mol L^{-1} hydrogen peroxide (sample solution) as a signals provided the negative peaks, which was used as its peak height for the detection of hydrogen peroxide by the proposed FIA system.

Some metal ions as a catalyst, such as Fe(II), Cu(II), Mn(II), Zn(II), Ni(II) and Fe(III), were examined for the effect on the oxidation efficiency of chromotropic acid with hydrogen peroxide. Fig. 3 shows the peak height by the proposed FIA system using 10^{-3} mol L^{-1} Fe(II), Fe(III), Cu(II), Mn(II), Zn(II) and Ni(II) as catalysts for the oxi-

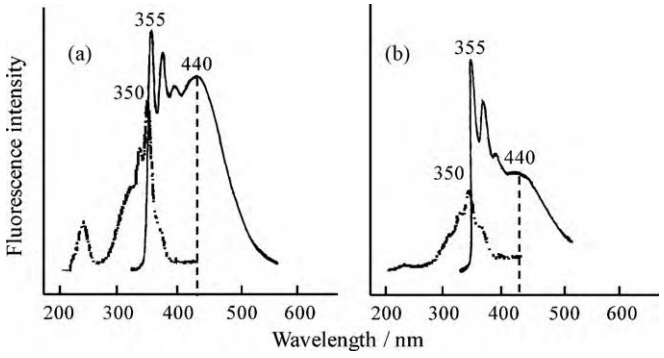

Fig. 2. Effect of hydrogen peroxide on the fluorescence intensity of chromotropic acid at λ_{em} = 440 nm. (a) Chromotropic acid (blank solution) and (b) chromotropic acid in the presence of 5×10^{-6} mol L^{-1} hydrogen peroxide (sample solution).

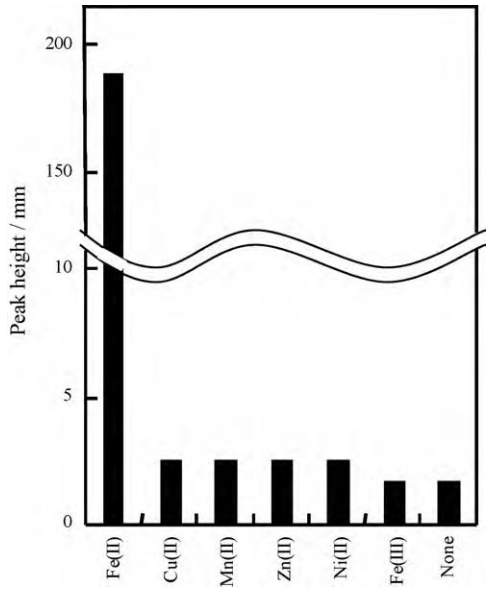

Fig. 3. Effect of various catalysts on the peak height for the determination of hydrogen peroxide with chromotropic acid by the proposed FIA system. RS: 1×10^{-4} mol L^{-1} chromotropic acid + 1×10^{-3} mol L^{-1} catalyst (pH 3.0); S: 5×10^{-5} mol L^{-1} hydrogen peroxide. The other experimental conditions were the same as in Fig. 1.

dation of chromotropic acid with hydrogen peroxide. Each metal ion was added into the RS (reagent solution). Each peak height of chromotropic acid with hydrogen peroxide in the presence of Fe(III), Cu(II), Mn(II), Zn(II) and Ni(II) as a catalyst was almost the same with each other, whereas the peak height in the presence of Fe(II) was quite higher than that of the other metal ions. The catalytic activity of Fe(II) for accelerating the oxidation of chromotropic acid with hydrogen peroxide was considered to be higher than that of the other metal ions, such as Fe(III), Cu(II), Mn(II), Zn(II) and Ni(II). Then, Fe(II) was selected as a catalyst for the following experiments. The catalyst, Fe(II), could accelerate the oxidation of chromotropic acid with hydrogen peroxide, and the concentration of Fe(II) might effect on the oxidation efficiency. The concentration of Fe(II) was examined by varying it in the RS (reagent solution). The results were shown in Fig. 4. The peak height of chromotropic acid increased up to 3.0×10^{-3} mol L^{-1} Fe(II), whereas it decreased over 5.0×10^{-3} mol L^{-1} Fe(II). Higher concentration of Fe(II) might form some complexes with hydroxide or chromotropic acid. Considering the sensitivity, the concentration of Fe(II) was determined to be 3.5×10^{-3} mol L^{-1}.

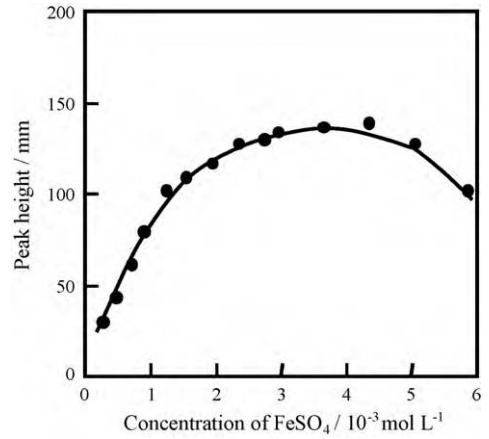

Fig. 4. Effect of $FeSO_4$ concentrations on the peak height by the proposed FIA system. RS: 1×10^{-4} mol L^{-1} chromotropic acid + $FeSO_4$ (pH 3.0); S: 5×10^{-5} mol L^{-1} hydrogen peroxide. The other experimental conditions were the same as in Fig. 1.

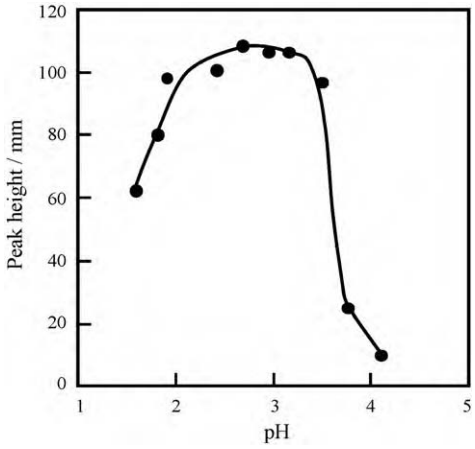

Fig. 5. Effect of pH of reagent solution (RS) on the peak height by the proposed FIA system. RS: 1×10^{-4} mol L^{-1} chromotropic acid + 3.5×10^{-3} mol L^{-1} $FeSO_4$; S: 5×10^{-5} mol L^{-1} hydrogen peroxide. The other experimental conditions were the same as in Fig. 1.

Fig. 5 shows the examination of the pH dependence on the oxidation reaction of chromotropic acid with hydrogen peroxide using Fe(II) as a catalyst. The peak height was almost constant at pH regions from 2.5 to 3.5. At higher and lower pH regions, the fluorescence intensity was quenched as shown in Fig. 5. The hydroxyl groups in the chromotropic acid are protonated at lower pH and deprotonated at higher pH. Especially, the chromotropic acid deprotonated is known to be chemically unstable. Further, Fe(II) is easy to form the precipitate at higher pH regions. Therefore, the fluorescence intensity might be wrong at higher and lower pH regions. Considering the sensitivity, pH 3.0 was selected.

The base line for the flow signals derives from the fluorescence of the chromotropic acid itself. Its concentration was examined by varying it in reagent solution (RS), whose results were shown in Fig. 6. The peak height increased up to around 5.0×10^{-5} mol L^{-1} of it, and it decreased over 5.0×10^{-5} mol L^{-1}. At higher concentration ranges of chromotropic acid in RS, the mixing efficiency might be decreased between chromotropic acid and hydrogen peroxide. Therefore, the concentration of the chromotropic acid in RS

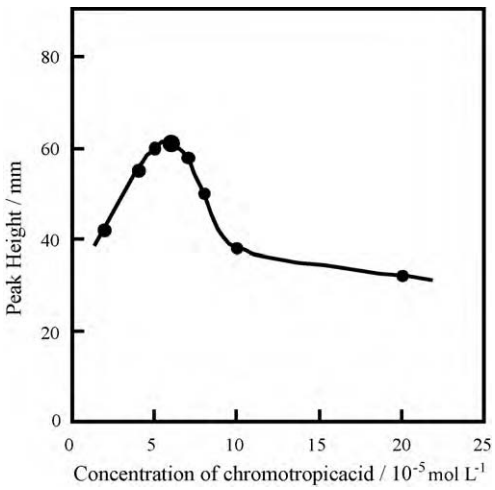

Fig. 6. Effect of concentrations of chromotropic acid on the peak height by the proposed FIA system. RS: chromotropic acid + 3.5×10^{-3} mol L^{-1} $FeSO_4$ (pH 3.0); S: 5×10^{-6} mol L^{-1} hydrogen peroxide. The other experimental conditions were the same as in Fig. 1.

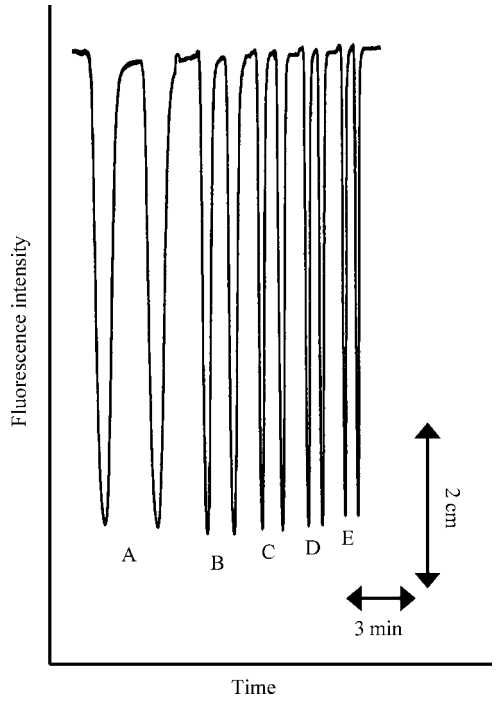

Fig. 7. Effect of flow rate on the peak height by the proposed FIA system. The flow rates of CS and RS were varied from 0.2 ml min^{-1} to 1.0 ml min^{-1} as follows: A, 0.2 ml min^{-1}; B, 0.4 ml min^{-1}; C, 0.6 ml min^{-1}; D, 0.8 ml min^{-1}; E, 1.0 ml min^{-1}. The other experimental conditions were the same as in Fig. 1.

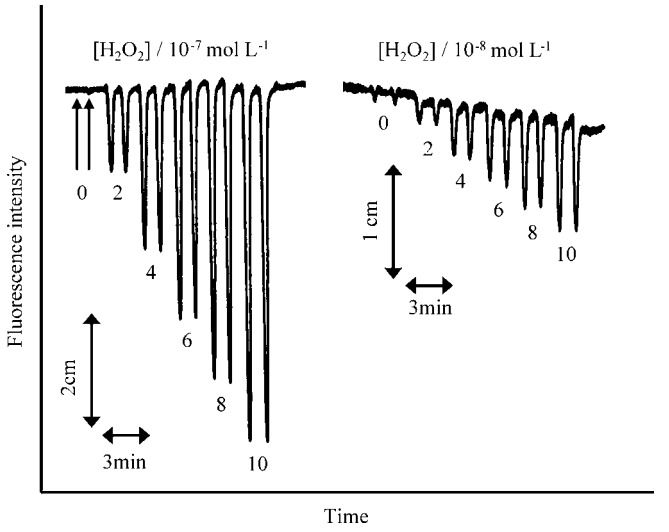

Fig. 8. Flow signals for hydrogen peroxide by the proposed FIA technique. (a) $0-10\times10^{-7}$ mol L^{-1} hydrogen peroxide and (b) $0-10\times10^{-8}$ mol L^{-1} hydrogen peroxide were used as standard samples. The other experimental conditions were the same as in Fig. 1.

was decided to be 5.0×10^{-5} mol L^{-1} for the sensitive and rapid measurement.

3.2. Optimization of flow conditions

The proposed FIA system consisted of a spectrofluorometer, a double plunger-type pump and a recorder. PTFE tubing (0.5 mm i.d.) was used for all flow lines. The flow conditions, such as sample volume, flow rate and reaction coil length, were examined as follows.

The effect of the sample volume on peak profiles was examined by varying it from 100 μL to 400 μL. With an increase in the sample volume, the peak height also increased up to 200 μL, and then the peak height with sample volume more than 200 μL was almost the same with that with 200 μL of sample volume. Therefore, the sample volume was decided to be 200 μL.

The flow rate of carrier solution (CS) and reagent solution (RS) on the peak profiles was examined by varying it from 0.2 mL min^{-1} to 1.0 mL min^{-1}, whose results are shown in Fig. 7. The peak height was almost the same with an increase in the flow rate of CS and RS. Considering the analytical throughput, each flow rate was fixed at 0.8 mL min^{-1}.

The effect of the reaction coil (RC) length was tested by varying it from 1 m to 5 m. The peak height increased along with an increase in the RC length which is due to reaction efficiency, whereas the peak height gradually decreased in the RC length over 2 m. The 2 m RC satisfied the analytical performance, which was used throughout this experiment.

3.3. Calibration graphs and analytical performance

The optimized conditions for the determination of hydrogen peroxide by the proposed FIA system were summarized in Table 1. The calibration graph showed good linearity over a range from 1.0×10^{-8} to 1.0×10^{-3} mol L^{-1} of hydrogen peroxide. Typical flow signals at the concentration range of 10^{-7} mol L^{-1} and 10^{-8} mol L^{-1} levels are shown in Fig. 8. The relative standard deviation (RSD) of 10 measurements of 4.0×10^{-8} mol L^{-1} hydrogen peroxide was 1.0%, and the limit of detection (LOD) was 5.0×10^{-9} mol L^{-1}. The analytical throughput was 40 samples h^{-1}. Some detection techniques, such as spectrophotometric detection [45] and fluorescence quenching method [46], for hydrogen peroxide determination in rain water have been reported, whose LODs are 7.0×10^{-7} mol L^{-1} and 5.0×10^{-7} mol L^{-1}, respectively. The LOD of the proposed FIA techniques in this study is 5.0×10^{-9} mol L^{-1}, which is better than the previous research.

When the proposed FIA system is applied to the determination of hydrogen peroxide in real samples, some species coexisting in the samples might be interfered to it. Table 2 shows the effect of

Table 2
Effect of coexisting ions on the detection of hydrogen peroxide by the propose FIA system.

Ion	Concentration (mol L^{-1}) (relative peak height)		Ion	Concentration (mol L^{-1}) (relative peak height)	
None	10^{-6} (1.00)	–	–	–	–
Na (I)	10^{-2} (0.98)	10^{-1} (0.89)	Sr (II)	10^{-4} (0.97)	10^{-3} (0.44)
B (III)	10^{-3} (1.03)	10^{-2} (1.11)	Cd (III)	10^{-4} (0.97)	10^{-3} (0.56)
Mg (II)	10^{-3} (0.97)	10^{-2} (0.89)	Ba (II)	10^{-4} (0.97)	10^{-3} (0.64)
K (I)	10^{-3} (0.97)	10^{-2} (0.27)	Mn (II)	10^{-5} (0.98)	10^{-4} (0.80)
Ca (II)	10^{-3} (0.98)	10^{-2} (0.78)	Ag (I)	10^{-5} (0.98)	10^{-4} (0.62)
Ni (II)	10^{-3} (0.98)	10^{-2} (0.84)	Fe (II)	10^{-6} (0.95)	10^{-5} (0.89)
Zn (II)	10^{-3} (0.98)	10^{-2} (0.73)	Fe (III)	10^{-6} (1.05)	10^{-5} (1.84)
Cr (III)	10^{-4} (0.96)	10^{-3} (0.33)	W (VI)	10^{-6} (0.99)	10^{-5} (0.73)
Co (II)	10^{-4} (1.02)	10^{-3} (1.16)	V (V)	10^{-7} (1.03)	10^{-6} (1.11)
Cu (II)	10^{-4} (1.02)	10^{-3} (1.40)	Mo (VI)	10^{-7} (0.93)	10^{-6} (0.67)

Table 3
Analytical results and recovery values of hydrogen peroxide in rain water calculated from standard addition method.

H_2O_2 added (10^{-7} mol L^{-1})	Peak height (mm)	H_2O_2 (10^{-7} mol L^{-1})		Recovery (%)
		Measured	Found	
0.00 (rain water[a])	9.1 ± 0.5	1.79 ± 0.11	1.79	–
0.40	10.0 ± 0.3	2.10 ± 0.07	1.70	95.0
0.80	12.4 ± 0.2	2.51 ± 0.04	1.71	95.5
1.00	13.5 ± 0.2	2.75 ± 0.04	1.75	97.8
2.00	18.2 ± 0.3	3.78 ± 0.07	1.78	99.4

[a] Rain water was sampled at 29th December, 2003.

Table 4
Determination of hydrogen peroxide in rain water by the proposed FIA.

Sample No.[a]	Concentration of H_2O_2 (10^{-7} mol L^{-1})	
	This method	Batchwise method[b]
1	9.0 ± 0.1	8.4 ± 0.1
2	0.15 ± 0.01	ND[c]
3	5.9 ± 0.1	6.2 ± 0.1
4	1.8 ± 0.1	ND[c]

[a] Samples 1–4 were sampled at 22nd October, 21st November, 28th December, 29th December 2003, respectively.
[b] Batchwise method with V(V)–H_2O_2–(5-Br-PADAP) reported in [47].
[c] Not detected.

coexisting ions on the determination of 1.0×10^{-6} mol L^{-1} hydrogen peroxide. Alkali metals and alkali earth metals even at high concentration did not show the significant interference, whereas some transition metal ions affected the determination of hydrogen peroxide. These metal ions might interfere the oxidation of chromotropic acid with hydrogen peroxide catalyzed with Fe(II), whereas it could be applied to the determination of hydrogen peroxide in rain water samples.

Then, the proposed FIA technique was applied to the determination of hydrogen peroxide in rain water samples. After sampling the rain water, it was filtered on the membrane filter with 0.45 μm of pore size. Table 3 shows the analytical results, $[H_2O_2]/10^{-7}$ mol $L^{-1} = 1.79 \pm 0.11$. Also, a trace amount of hydrogen peroxide at a similar concentration to the hydrogen peroxide present in rain water sample was added to it before the measurement, based on the standard addition method. The recovery values are also shown in Table 3, which are a sufficient recovery (95.0–99.4%). Table 4 also shows the analytical results of hydrogen peroxide in the rain water sample, which sampled on the different days. And the analytical values by the proposed FIA technique were compared with those by the batchwise technique by spectrophotometry using V(V)–H_2O_2–(5-Br-PADAP). These values are good agreement with each other. The propose FIA technique, which was the fluorescence detection based on the oxidation of chromotropic acid with hydrogen peroxide catalyzed with Fe(II), could be applied to the rain water samples.

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