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Notes

Indirect Detection of Halide Ions *via* Fluorescence Quenching of Quinine Sulfate in Microcolumn Ion Chromatography

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Halide ions could be visualized *via* fluorescence quenching in microcolumn ion chromatography. The fluorescence of quinine sulfate, which was contained in an acidic eluent, was quenched by halide ions. The observed fluorescence quenching values increased in this order: iodide, bromide, and chloride. The present detection system was relatively sensitive to halide ions except for fluoride: other anions gave smaller signals than halide ions. The present detection system provided quantitative information, so it could be applied to the determination of chloride in water samples.

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Introduction

Quenching of fluorescence is a well-known physicochemical phenomenon that has been utilized for the selective determination of quenching species.[1–11] The quenching of fluorescence was typically used for metal cations, but the ability of halide ions to quench fluorescence has also been reported to involve quinine sulfate[1,2] and other dyes.[3–7]

Fluorescence quenching has been utilized for the detection of non-fluorescent species in various separation methods.[8–11] Amankwa and Kuhr[8] accomplished indirect fluorimetric detection using quinine sulfate as the visualization agent in micellar electrokinetic chromatography. They found that the detection mechanism involved a combination of displacement of the fluorophore from the micelle by the analytes and net reduction of the quantum efficiency of the fluorophore in the sample zone. Smith and El Rassi[9] also employed *N*-phenyl-naphthylamine (PNA) as a background fluorophore for indirect fluorimetric detection in micellar electrokinetic capillary chromatography. The negative signal was primarily the result of quenching of the PNA by the analyte in the micelle's core. Reijenga *et al.*[10] presented an apparatus for simultaneous fluorescence and UV absorption detection in isotachophoresis. They demonstrated fluorescence quenching as a method of identification for non-fluorescent compounds, using quinine as a fluorescent counter ion. Stalikas *et al.*[11] reported an ion chromatographic method for the simultaneous determination of nitrite and nitrate by postcolumn indirect fluorescence detection with tryptophan as the fluorescent agent.

Conductimetric and indirect UV absorption detection have been commonly utilized in ion chromatography. However, these detection methods are relatively universal and sometimes encounter a difficulty in identification of analytes, especially for complex mixtures. Selective detection is therefore preferred in the determination of ions of interest in a complex matrix. On the other hand, microcolumn ion chromatography has attracted a great deal of attention because it provides advantages over conventional ion chromatography in terms of mass sensitivity and decreased consumption of eluents.[12] Conductivity detectors for microcolumn ion chromatography are not commercially available, and direct UV and indirect UV absorption detection have been therefore utilized in microcolumn ion chromatography.

Fluorimetric detection has been widely utilized in liquid chromatography because it provides sensitive and selective detection. Since most inorganic ions are not fluorescent, indirect fluorimetric detection is a good way for the determination of inorganic ions when fluorimetric detectors are used. However, there are few fluorescent ions available for the ion chromatography eluent, involving cerium(III) and salicylate. Indirect fluorimetric detection based on quenching is another option for the determination of ions in ion chromatography since there are a number of candidates. Indirect fluorimetric detection based on quenching is promising in microcolumn ion chromatography, because there are a few detection methods commercially available.

The present paper describes indirect fluorimetric detection of halide ions in microcolumn IC (μIC), based on the fluorescence quenching of quinine sulfate due to the halide ions. Quinine sulfate is a good candidate for the present purpose because it is soluble in water.

Experimental

Apparatus

A μIC system was assembled from Model MF2 Microfeeder (Azumadenki Kogyo, Tokyo, Japan) equipped with a 0.5-mL MS-GAN050 gas-tight syringe (Ito; Fuji, Japan), a Model FP920 fluorescence detector (Jasco, Tokyo, Japan), a Model 7520 microvalve injector with an injection volume of 0.2 μL (Rheodyne, Cotati, CA, USA), and a 100 × 0.32 mm microcolumn. The data were handled by a Computer Aided Chromatography data processor (Nippon Filcon, Tokyo, Japan). The microcolumn was immersed in a water-bath to avoid variation of ambient temperature. Silica-base anion-exchange resin, TSK_{gel} IC-Anion-SW (5 μm particle diameter; Tosoh, Tokyo, Japan), was packed into the fused-silica tubing with 0.32

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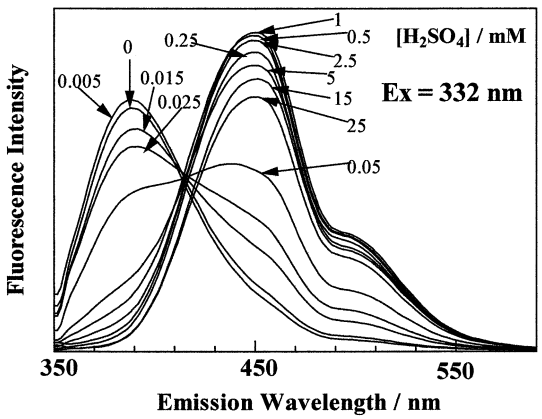

Fig. 1 Fluorescence spectra of quinine sulfate at different sulfuric acid concentrations. Excitation wavelength: 332 nm. Concentration of quinine sulfate: 10 μM.

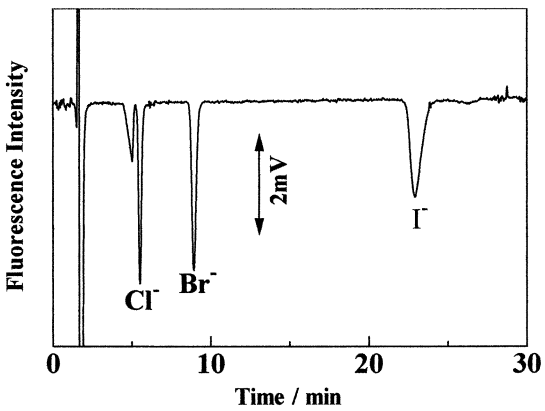

Fig. 2 Fluorimetric detection of halide ions *via* quenching. Column: TSK_{gel} IC-Anon-SW, 100 × 0.32 mm i.d. Eluent: 20 mM sodium sulfate containing 0.2 mM quinine sulfate and 1 mM sulfuric acid. Flow rate: 4.2 μL/min. Wavelengths of detection: Ex = 332 nm; Em = 448 nm. Analytes: 1 mM each (0.2 μL).

mm i.d., as previously reported.[13] Spectra of quinine sulfate were measured in the stopped-flow mode by using a Model FP920 fluorescence detector. The pH of aqueous solutions was measured with a Model IM-20E ion meter (TOA Electronics, Tokyo, Japan).

Reagents

Purified water was produced in the laboratory by using a GS-590 water distillation system (Advantec, Tokyo, Japan). Other reagents were of reagent grade and were obtained from Nacalai Tesque, unless otherwise noted. The reagents were used as received.

Results and Discussion

Fluorescence spectra of quinine sulfate in the presence of different concentrations of sulfuric acid

Both the profile and the intensity of fluorescence of quinine sulfate varied depending on the pH of the solution. Figure 1 shows the fluorescence spectra of quinine sulfate dissolved in different concentrations of sulfuric acid when the excitation wavelength was kept at 332 nm. At sulfuric acid concentrations higher than 0.25 mM, *i.e.*, at pH values lower than 3.44, nearly the same spectrum profiles with slightly different intensities were observed, where quinine is protonated. On the other hand, at pH values higher than 5.80, a different profile was observed, where quinine is not protonated. Considering the spectrum profiles, quinine is partially protonated at pH between 3.44 and 5.80. In addition, the pK_a of quinine is 4.13 and 8.26.[14] The variation of the spectrum profiles is caused by the protonation of quinine molecules. Since the strongest fluorescence intensity was observed at 1 mM sulfuric acid (pH 2.95), the concentration of sulfuric acid was kept at 1 mM in the following experiments.

Fluorescence quenching of quinine sulfate due to halide ions

The principle of dynamic fluorescence quenching is well-known. Dynamic (collisional) quenching results from diffusive encounters between the excited state fluorophore and the quenching agent.[15] The relationship between fluorescence intensity and the quenching agent concentration is represented by the following Stern–Volmer equation:

$$F_0/F - 1 = K_{SV}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and the presence of the quenching agent, K_{SV} is the Stern–Volmer constant, and [Q] is the concentration of the quenching agent, respectively. When dynamic quenching is involved, a linear relationship between $(F_0/F - 1)$ and [Q] should be observed.

Linear Stern–Volmer plots for quinine sulfate in the presence of halide ions such as chloride, bromide and iodide were observed. In other words, the dynamic quenching is involved in quenching of quinine sulfate due to the halide ions. The largest quenching was observed for iodide, whereas the smallest quenching was observed for chloride. It should be noted that the fluorescence intensity was not altered in the presence of 100 mM fluoride. This means that fluoride cannot be detected by the present method. In addition, the Stern–Volmer constants of the halide ions could be calculated from the slopes of the straight lines.

Detection of halide ions

The above results indicate that the halide ions can be visualized by fluorimetric detection based on fluorescence quenching of quinine sulfate. Figure 2 demonstrates a fluorimetric detection of 1 mM each of chloride, bromide and iodide in μIC, where the eluent contains 20 mM sodium sulfate, 0.2 mM quinine sulfate and 1 mM sulfuric acid. The relative peak heights of the analytes were 100, 95 and 54 for chloride, bromide and iodide, respectively. On the contrary, the relative peak areas were 100, 137 and 193 for chloride, bromide and iodide, respectively.

The quenching of fluorescence is often observed for metal cations. The influence of metal cations may not be of relevance in the present detection system because free metal cations are not retained on the anion-exchange column, whereas complexes with metal cations may be separated from the analyte halide ions.

Under the conditions in Fig. 2, the detection limits at a signal-to-noise ratio of 3 were 0.87, 2.12 and 5.77 mg/L for chloride, bromide and iodide, respectively. The present detection method was quantitative, and the peak height of each halide ion was linear up to the concentration of 1 mM. However, the sensitivity of the present system is poor. The detection limit of 0.071 mg/L was achieved for chloride by μIC with indirect photometric detection.[16] In order to improve the sensitivity of

Table 1 Relative signal intensity and the retention time of inorganic anions

	IO_3^-	NO_2^-	BrO_3^-	Cl^-	ClO_3^-	Br^-	NO_3^-	I^-
Relative peak height	4.9	22.5	0.0	100	1.5	95.3	1.1	54.0
Relative peak area	3.3	NM[a]	0.0	100	1.8	137	2.0	193
Peak direction	Negative	Negative	ND[b]	Negative	Positive	Negative	Positive	Negative
Retention time, min	2.94	4.70	—	5.59	7.29	9.05	9.95	23.04

Operating conditions are as given in Fig. 2. a. NM: not measured. b. ND: not detected.

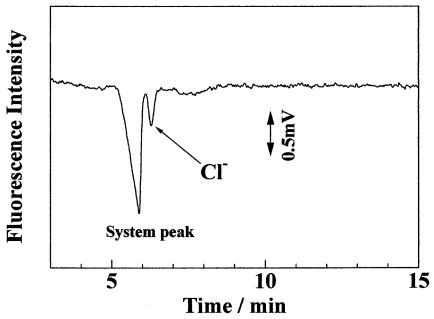

Fig. 3 Fluorimetric detection of chloride in tap water. Operating conditions are as in Fig. 2 except for the sample. Sample: 0.2 µL tap water.

the present detection method, one should increase the background-to-noise ratio. The ratio of the background to its noise achieved under the operating conditions in Fig. 2 was 3.7×10^3. This value is within the range normally observed in fluorimetric detection.[17] If fluctuation of the light source energy can be reduced, the ratio will be improved. On-column[12] or precolumn[18] enrichment will effectively improve the concentration sensitivity of the present system.

The reproducibility of the retention time and the signal intensity under the conditions in Fig. 2 was estimated for six successive measurements. The relative standard deviations (RSDs) for the retention time were 0.48 – 0.72%, whereas those for the peak height and peak area were 0.61 – 1.9% depending on the analytes.

Response of other ions

Although inorganic anions other than halide ions also gave signals but the signal intensities were smaller than that of the chloride ion. Table 1 compares the relative peak height observed under the conditions in Fig. 2. Nitrite gave a relatively larger signal, but it was overlapped with the system peak eluting before chloride as seen in Fig. 2. Bromate gave no signal, whereas chlorate and nitrate gave small positive peaks. The results in Table 1 show that the present detection system is relatively sensitive to halide ions except for fluoride. In addition, the reason why chlorate and nitrate gave positive peaks is not yet certain.

Determination of chloride in water samples

The present system was quantitative and applicable to the determination of chloride in water samples. Figure 3 demonstrates the detection of chloride in tap water based on fluorescence quenching of quinine. The concentration of chloride was determined to be 0.10 mM or 3.7 mg/L.

Conclusion

It was proved that halide ions could be visualized based on fluorescence quenching of quinine under acidic conditions. Chloride in water samples could be determined by the present system. The detection sensitivity should be improved by decreasing the noise level as well as by using precolumn or on-column enrichment technique.

Acknowledgements

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