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Fluorometric Determination of Chloride with 2-(5-Nitro-2-furyl)benzothiazole[1]

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A new fluorometric method for determination of the chloride ion was established. Chloride reacted with 2-(5-nitro-2-furyl)benzothiazole in 60% sulfuric acid upon ultraviolet irradiation to form 2-(5-chloro-2-furyl)benzothiazole, which gave intense violet fluorescence with an excitation maximum at 375 nm and emission maximum at 422 nm. Chloride could be determined in the range of 0.1—10 μg/ml by measuring the fluorescence intensity.

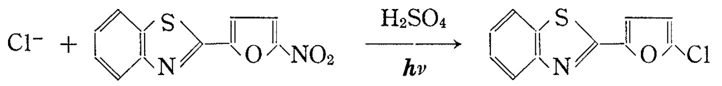
This method can be applied to the determination of chloride in tap water.

Keywords——fluorometric determination; chloride; 2-(5-nitro-2-furyl)benzothiazole; 2-(5-chloro-2-furyl)benzothiazole; UV-irradiation

A colorimetric determination of chloride using mercuric thiocyanate and the ferric ion[3-8] has been reported, and a method based on the use of iron(III) perchlorate to form the chlorocomplex of iron(III) was proposed by West and Coll.[9] Some organic reagents for mercury and copper, such as diphenylcarbazone,[10,11] Methylthymol Blue,[12] diethyldithiocarbamate[13] and dithizone[14] have also been utilized for the colorimetric determination of chloride. As a fluorometric method, a titrimetric procedure using mercury(II) with a fluorescence indicator, Anisidine Blue[15] (tetrasodium *o*-dianisidine-N,N,N′,N′-tetraacetate), was proposed for determination of the chloride ion, based on the quenching of the blue fluorescence of the indicator.

We found that 2-(5-nitro-2-furyl)benzothiazole (I) changed into 2-(5-chloro-2-furyl)benzothiazole (II), giving an intense violet fluorescence on ultraviolet irradiation in a hydrochloric acid medium,[16] as shown in Chart 1.

The fluorescence reaction was applied for the microdetermination of chloride.



I II

Chart 1

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[2] Location: *1-35-23 Nozawa, Setagaya-ku, Tokyo.*

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Experimental

Apparatus——(1) Spectrofluorophotometric and spectrophotometric measurements were carried out with a Hitachi MPF-2A spectrofluorophotometer and a Hitachi EPS-3T spectrophotometer, respectively.

(2) A Toshiba Black Light Fluorolamp (FL 20S BLB, peak at 360 nm) was used for ultraviolet irradiation.

(3) Gas chromatograms were recorded with a Shimadzu GC-6AM gas chromatograph.

Reagents and Materials——0.01% (w/v) I-Dioxane Solution: Compound I (10 mg), synthesized according to the method reported in the previous paper,17) was dissolved in 100 ml of dioxane (super special grade). This solution was stable for three days in the dark at room temperature.

Chloride Stock Solution: Sodium chloride (standard reagent; 0.1649 g) was placed in a 200 ml volumetric flask and the flask was filled with redistilled H_2O to the mark. One ml of the solution thus prepared contains 500 μg of chloride ions.

Chloride Standard Solution: The chloride stock solution was diluted with redistilled H_2O to give 0.1—10 μg/ml as chloride.

60% Sulfuric Acid: Sulfuric acid (super special grade; 60 g) was added to 40 g of redistilled H_2O.

Quinine Sulfate (reagent grade) Solutions: Solutions of 15 μg/ml and 2 μg/ml of quinine sulfate in 0.1 N H_2SO_4 were prepared.

Recommended Procedure——One ml of a sample solution containing 0.1—10 μg/ml of chloride is placed in a light-resistant test tube. Next, 3 ml of 60% sulfuric acid and 1 ml of 0.01% I-dioxane are added and the solution is mixed well. All the above procedures are performed in an ice bath. The mixture is transferred into a quartz cell and irradiated with ultraviolet light for 40 min at 10 cm from the light source (Toshiba Black Light Fluorolamp: FL 20S BLB). The fluorescence intensity of the reaction solution obtained is measured with excitation at 375 nm and emission at 422 nm (Fig. 1).

The sensitivity of the fluorometer is adjusted with 15 μg/ml or 2 μg/ml quinine sulfate solution (excitation, 375 nm; emission, 422 nm).

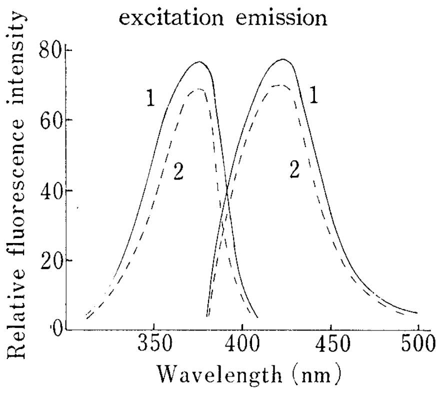

Fig. 1. Excitation and Emission Spectra

1: measurement solution for chloride,
2: 2-(5-chloro-2-furyl)benzothiazole in reagent blank solution.

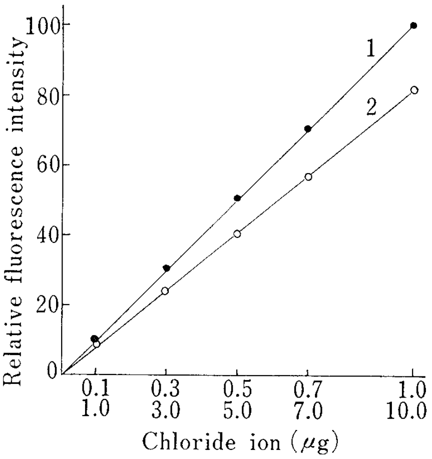

Fig. 2. Calibration Curves for Chloride

1: 1.0—10 μg/ml,
2: 0.1—1.0 μg/ml.

Calibration Curve——The calibration curves were prepared by the recommended procedure. A linear relationship was observed between the fluorescence intensity and the concentration of chloride in the range of 0.1—10 μg/ml (Fig. 2).

Determination of Chloride in Tap Water——Tap water was diluted with redistilled H_2O to bring the chloride ion concentration within the range of 0.1—10 μg/ml. One ml of the diluted solution was analyzed by the recommended procedure.

Results and Discussion

Identification of 2-(5-Chloro-2-furyl)benzothiazole (II)

The excitation and emission spectra for the determination of chloride are shown in Fig. 1. The excitation maximum was at 375 nm and the emission maximum was at 422 nm. The

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17) S. Nakano, H. Taniguchi, and K. Mikoshiba, *Yakugaku Zasshi*, **93**, 344 (1973).

spectra coincided with those of an authentic sample of II which was synthesized by the method described previously.[17]

The reaction product formed in the fluorescent solution was extracted with ether. After removal of the ether, the fluorescent product was isolated by means of column chromatography on alumina (Aluminum Oxide Woelm neutral) with chloroform eluent. The absorption spectrum of the fluorescent eluate coincided with that of a solution (chloroform) of authentic II, as shown in Fig. 3. I and II gave absorption maxima at 370 nm and 330 nm, respectively.

The fluorescent product was also identified as II by thin-layer chromatography [silica gel (Wako Gel B-5), developed with benzene-chloroform (1: 1); *Rf*, 0.65].

In addition, the fluorescent product was again extracted with benzene and analyzed by means of gas chromatography (Fig. 4). Its retention volume coincided with that of a benzene solution of authentic II.[16]

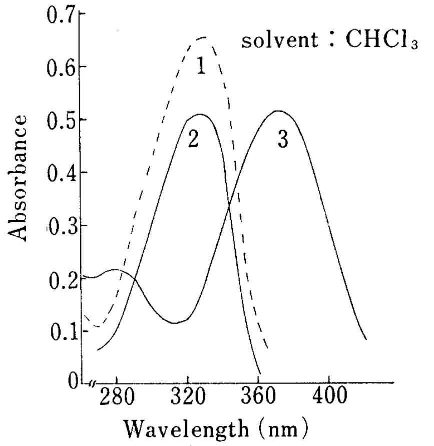

Fig. 3. Absorption Spectra

1: 2-(5-chloro-2-furyl)benzothiazole,
2: reaction product,
3: 2-(5-nitro-2-furyl)benzothiazole.

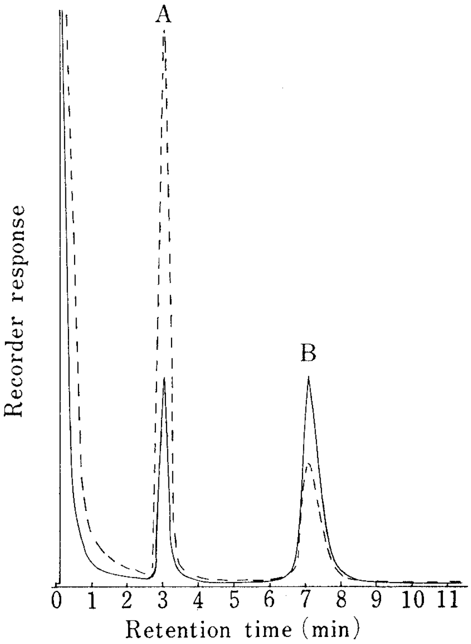

Fig. 4. Gas Chromatograms of the Fluorescent Product

A: 2-(5-chloro-2-furyl)benzothiazole,
B: 2-(5-nitro-2-furyl)benzothiazole,
– –: authentic sample,
——: reaction solution of chloride,
column, 3 mmφ × 2 m; packing, 5% Silicone DC 550 on Chromosorb W (60—80 mesh); carrier gas, He; flow rate, 60 ml/min; column temp., 179°; chart speed, 10 mm/min.

These results indicate that chloride reacted with I to form II in a sulfuric acid medium upon ultraviolet irradiation.

Conditions for the Determination of Chloride

Effect of Reagent Concentration——As intense fluorescence was obtained in the range of 0.008—0.01% (w/v) I-dioxane solution (Fig. 5), 0.01% (w/v) I-dioxane solution was used as the reagent solution.

Effect of the Concentration of Sulfuric Acid——As the fluorescence intensity was found to be constant in the range of 57.5—62.5% sulfuric acid, 60% sulfuric acid was used.

Effect of Light Source and Irradiation Time——In order to select an effective light source, the Toshiba Black Light Fluorolamp (EL 20S BLB, peak at 360 nm), the Toshiba Neo Line (FLR 20S W/M, light in the visible region) and the Toshiba Kenkosen-Yo Fluorolamp (FL 20S E, peak at 310 nm) were examined (Fig. 6). The results indicated that the Toshiba Black Light Fluorolamp (FL 20S BLB) was the most effective for the determination of chloride, and the most effective irradiation time was 40 min.

Stability of Fluorescence

The fluorescence under the usual conditions was stable for 90 min at room temperature after the reaction.

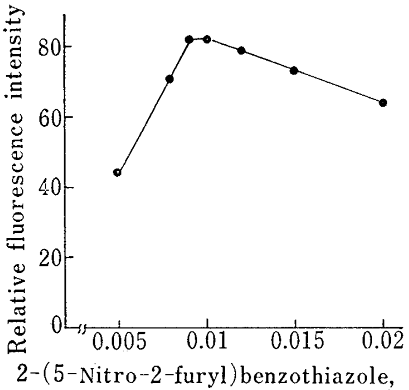

Fig. 5. Effect of 2-(5-Nitro-2-furyl)-benzothiazole Concentration

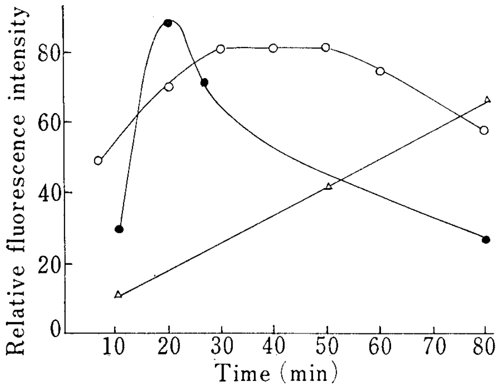

Fig. 6. Effect of Lamp Type and Irradiation Time on the Relative Fluorescence Intensity

—○—: Toshiba Black Light Fluorolamp (FL 20S BLB, peak at 360 nm),
—△—: Toshiba Neo Line (FLR 20S W/M, light in the visible region),
—●—: Toshiba Kenkosen-Yo (FL 20SE, peak at 310 nm).

Table I. Effects of Foreign Ions on Chloride Determination

Foreign ions	Added as	Chloride used (μg/ml)	Amount of foreign ion (μg/ml)	Chloride found (%)
Ag^+	Nitrate	0.5	0.5	77.8
		5.0	5.0	78.0
Hg_2^{2+}	Nitrate	0.5	0.5	83.6
		5.0	5.0	92.8
Hg^{2+}	Acetate	0.5	0.5	77.8
		5.0	5.0	68.0
Pb^{2+}	Nitrate	0.5	0.5	88.9
		5.0	5.0	80.0
Cu^{2+}	Nitrate	0.5	0.5	75.9
		5.0	5.0	54.4
Fe^{2+}	Ammonium sulfate	0.5	0.5	100.0
			5.0	76.3
		5.0	5.0	100.0
			50.0	100.0
			500.0	86.7
Fe^{3+}	Nitrate	0.5	0.5	100.0
			50.0	66.7
		5.0	5.0	100.0
			50.0	44.4
Co^{2+}	Nitrate	0.5	0.5	94.8
			5.0	85.9
		5.0	5.0	98.5
			50.0	92.9
Ni^{2+}	Nitrate	0.5	0.5	88.2
			5.0	83.7
NO_2^-	Sodium salt	0.5	0.5	99.6
			5.0	126.9
		5.0	5.0	100.0
			50.0	132.3
CN^-	Potassium salt	0.5	0.5	106.3
		5.0	5.0	100.0
			50.0	112.9
CrO_4^{2-}	Potassium salt	0.5	5.0	101.0
		5.0	50.0	101.0
			500.0	66.7

Foreign ions	Added as	Chloride used (μg/ml)	Amount of foreign ion (μg/ml)	Chloride found (%)
AsO_4^{3-}	Sodium salt	0.5	0.5	124.4
		5.0	5.0	105.3
I^-	Potassium salt	0.5	0.5	122.7
		5.0	5.0	107.1
Br^-	Potassium salt	0.5	0.5	149.3
		5.0	5.0	118.4
SCN^-	Potassium salt	0.5	0.5	114.9
		5.0	5.0	110.2
ClO_3^-	Potassium salt	0.5	0.5	100.0
			5.0	80.9
		5.0	50.0	100.0
			500.0	99.5
BrO_3^-	Potassium salt	0.5	0.5	87.0
		5.0	5.0	78.3
ClO_4^-	Potassium salt	0.5	0.5	87.1
		5.0	5.0	76.9

Effect of Foreign Ions

The effects of various ions were examined in the presence of 0.5 μg/ml and 5.0 μg/ml of chloride. The foreign ions which interfere with the proposed method are shown in Table I. Among these ions, bromide, iodide, thiocyanate and arsenate caused large positive errors and silver, mercurous, mercuric, lead and cupric ions caused large negative errors.

Foreign ions such as Zn^{2+}, Co^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+, NO_3^-, IO_3^-, $H_2PO_4^-$, $B_4O_7^{2-}$, I^- did not interfere with the determination of 0.5 μg/ml of chloride and 5 μg/ml of chloride when present in 10-fold and 100-fold excess, respectively.

The proposed method could be used for the determination of chloride in the range of 0.1—10 μg/ml as chloride ion. The coefficients of variation for sample solutions having chloride ion concentrations of 0.1 μg/ml, 1.0 μg/ml and 10 μg/ml were calculated to be 6.14, 1.79 and 1.64, respectively (ten measurements each).

Table II. Determination of Chloride in Tap Water by the Proposed and Official Methods

	Proposed method as chloride ion (μg/ml)	Official method[18] as chloride ion (μg/ml)
	20.18	22.94
	19.82	22.68
	19.69	22.42
	19.17	22.68
	20.00	22.85
	19.89	22.16
	19.09	22.85
	19.66	22.68
	18.71	22.42
	19.15	22.94
Mean	19.536	22.640
σ	0.476	0.257

The results of the determination of chloride levels in tap water are shown in Table II; the data obtained by the present method are compared with those obtained by the official method (Mohr method for chloride).18)

In conclusion, the method proposed is a sensitive and effective method for the microdetermination of chloride.

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18) Pharmaceutical Society of Japan (ed.), "Standard of Analysis for Hygienic Chemist with Commentary," Kanehara Shuppan Co., Tokyo, 1973, p. 717.