[Chem. Pharm. Bull. 25(6)1237—1242(1977)]

UDC 546.175.08 : 543.426.06

Fluorophotometric Method for the Determination of Nitrate with 2-Phenylbenzothiazole[1]

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(Received September 7, 1976)

Nitrate reacts with 2-phenylbenzothiazole in concentrated sulfuric acid to form 6-nitro-2-phenylbenzothiazole, which changes to 6-amino-2-phenylbenzothiazole by the irradiation with ultraviolet light in an ammoniacal dioxane solution and the 6-amino-2-phenylbenzothiazole gives the intense fluorescence with excitation maximum at 358 nm and emission maximum at 457 nm. By using this reaction, 0.01—3.0 μg/ml of nitrate ion can be determined fluorophotometrically.

In the application of this method for the analysis of tap water, the interferences from nitrite ion, residual chlorine and chloride ion can be eliminated by using sulfamic acid, sodium sulfite and silver sulfate, respectively.

Keywords——fluorometry; nitrate; 2-phenylbenzothiazole; 6-nitro-2-phenylbenzothiazole; 6-amino-2-phenylbenzothiazole; irradiation

The fluorophotometric methods for the determination of nitrate have been reported by using reagents such as disodium fluoresceine,[3] 2,3-diaminonaphthalene,[4] and 2,2-'dihydroxy-4,4'-dimethoxybenzophenone.[5]

It was found that 2-phenylbenzothiazole (I) reacted with nitrate in a sulfuric acid solution to form 6-nitro-2-phenylbenzothiazole (II), and it was converted into 6-amino-2-phenylbenzothiazole (III), which exhibited the intense blue fluorescence by irradiation with ultraviolet light in an ammonia alkaline solution. I exhibited higher fluorescence intensity than 2-(*p*-bromo-, *p*-chloro-, or *p*-cyanophenyl)benzothiazole.

On the basis of these results, the fluorometric determination of nitrate with I was established.

The fluorescence reaction is shown in Chart 1.

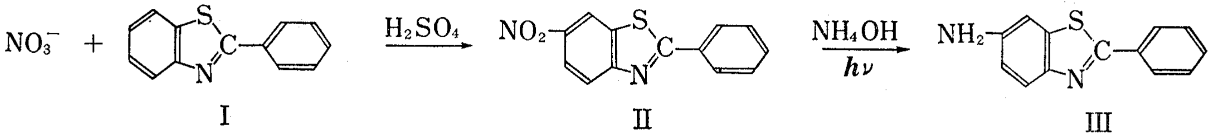

Chart 1

Experimental

Apparatus——(1) Spectrofluorometric and spectrophotometric measurements were made 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 irradiation with ultraviolet light.

Reagents and Materials——0.3% I-Sulfuric Acid Solution: I (0.3 g) synthesized according to the previous paper[6] was dissolved in 100 ml of concentrated sulfuric acid (super special grade).

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1) This work was presented at the 96th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April, 1976.
2) Location: *1-35-23 Nozawa, Setagaya-ku, Tokyo.*
3) H.D. Axelrod, J.E. Bonelli, and J.P. Lodge, Jr., *Anal. Chim. Acta*, **51**, 21 (1970).
4) C.R. Sawicki, *Analytical Letters*, **4**, 761 (1971).
5) B.K. Afgham, and J.F. Ryan, *Anal. Chem.*, **47**, 2347 (1975).
6) Saburo Nakano, Hirokazu Taniguchi, and Kazuo Mikoshiba, *Yakugaku Zasshi*, **93**, 344 (1973).

Nitrate Stock Solution: Potassium nitrate (0.0163 g) was dissolved in 100 ml of redistilled water, this solution contains 100 μg/ml as nitrate ion.

Nitrate Working Standard Solution: The stock solution was diluted with redistilled water to give 0.01—3.0 μg/ml as nitrate ion.

Quinine Sulfate (Japanese Pharmacopoeia VIII) Solution: The solutions of 10 μg/ml, 5.0 μg/ml, and 0.5 μg/ml in 0.1N H_2SO_4 were prepared.

Sodium hypochlorite was purchased from Wako Pure Chemical Co., Ltd. (Japanese Standards of Food Additives III).

All other chemicals and solvent were of reagent grade.

Recommended Procedure——One ml of a sample solution containing 0.01—0.3 μg/ml as nitrate ion is placed in a tube and cooled in ice-cold water. To the solution, 1 ml of 0.3% I-sulfuric acid and 5 ml of concentrated sulfuric acid are added under cooling and shaking. The reaction mixture is allowed to stand for 10 min at room temperature, after the addition of 30 ml of redistilled water, the mixture is cooled in ice-cold water, and then extracted twice with 10 ml of ether for 2 min. The ethereal solution is evaporated to dryness, the residue is dissolved in 3 ml of 95% (v/v) dioxane and 1 ml of concentrated ammonium hydroxide, and the solution placed in a quartz cell is irradiated with ultraviolet light for 15 min at 10 cm from light source (Toshiba Black Light Fluorolamp: FL 20S·BLB). The fluorescence intensity of the obtained measurement solution is measured with excitation at 358 nm and emission at 457 nm (Fig. 1). The spectrofluorophotometer is set to read with a quinine solution (excitation: 358 nm, emission: 457 nm).

Calibration Curve——The calibration curves are made by the same way as described above. A linear relationship existed between the concentration of nitrate ion and fluorescence intensity in the range of 0.01—3.0 μg/ml (Fig. 2).

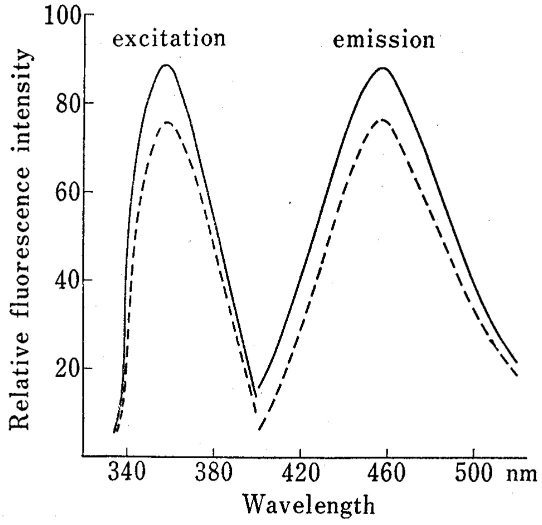

Fig. 1. Excitation and Emission Spectra

——: sample solution of determination of nitrate
----: 6-amino-2-phenylbenzothiazole in blank solution

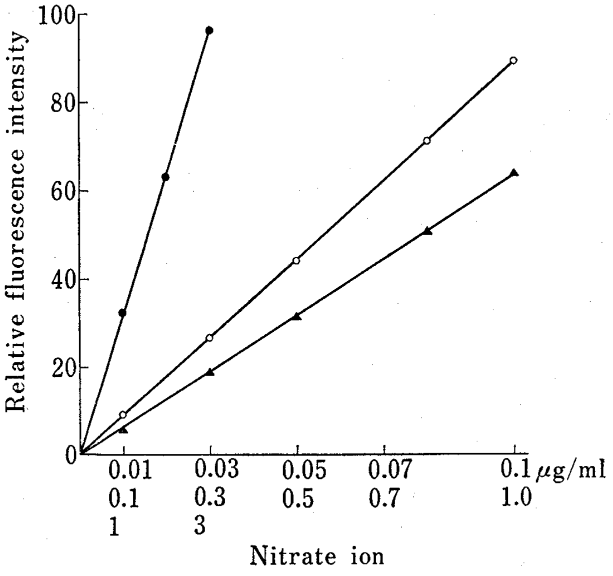

Fig. 2. Calibration Curves for Nitrate

—▲—: 0.01—1.0 μg/ml
—○—: 0.1—1.0 μg/ml
—●—: 1.0—3.0 μg/ml

Application to Tap Water——One ml of 0.5% sulfamic acid solution, 1 ml of 5% sodium sulfite solution and 2 ml of 1N sulfuric acid solution are added to 2 ml of tap water (sample), and the mixture is heated on a water bath for 5 min to eliminate the interferences from nitrite and hypochlorite ion. To the reaction mixture is added 2 ml of 0.5% silver sulfate solution to eliminate chloride ion, and diluted to 20 ml in a measuring flask with redistilled water. After the centrifugation of the reaction mixture, 1 ml of the supernatant solution is used as the sample solution in the recommended procedure.

Results and Discussion

Identification of 6-Nitro-2-phenylbenzothiazole (II)

After nitrate had reacted with I in a sulfuric acid solution by the method described in the recommended procedure, the reaction mixture was extracted with ether. An ultraviolet spectrum of the ethereal extract agreed with that of the ethereal solution of the authentic sample (II) prepared by the method of Bogert, and Abrahamson[7] and as shown in Fig. 3, they showed the absorption maximum at 321 nm.

The reaction product of nitrate with I was detected on the thin-layer chromatogram [TLC, silica gel: Wako Gel B-5, developed by acetone-cyclohexane (3: 25), *Rf*: 0.57], sprayed with ammonium hydroxide and irradiated with ultraviolet light (Toshiba Black Light Fluorolamp: FL 20S-BLB).

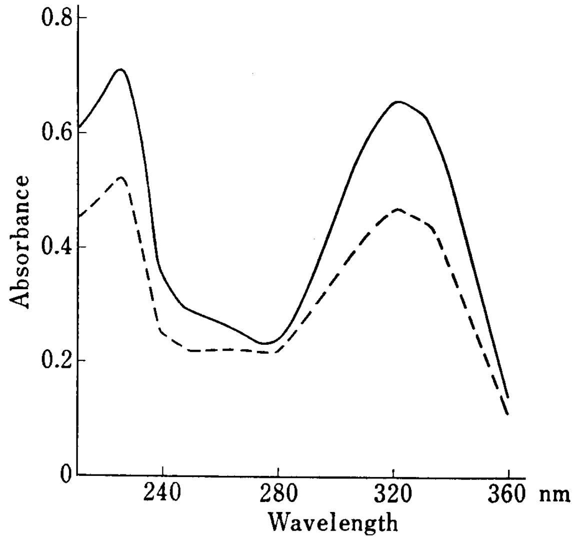

Fig. 3. Ultraviolet Spectra

——: 6-nitro-2-phenylbenzothiazole in ether
----: reaction product of nitrate with 2-phenylbenzothiazole in ether

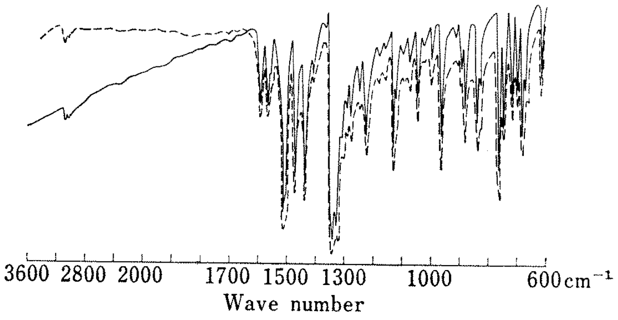

Fig. 4. IR Spectrum of 6-Nitro-2-phenylbenzothiazole (KBr)

——: authentic sample of 6-nitro-2-phenylbenzothiazole
----: nitration product in analytical procedure

The reaction product was also identified as II by comparison with the authentic sample (II)[7] in the IR spectrum (Fig. 4), and by the elemental analysis (*Anal.* Calcd. for $C_{13}H_8O_2N_2S$: C, 60.93; H, 3.15; N, 10.93. Found: C, 61.15; H, 3.22; N, 11.20) and the mixing melting point test (mp 192—193°, recrystallized from benzene).

On the basis of these results, it was confirmed that nitration of I occurred by the presence of nitrate in the sulfuric acid solution to give II.

Identification of 6-Amino-2-phenylbenzothiazole (III)

As shown in the spectra in Fig. 1, the spectrum of III (the authentic sample prepared by the method of Bogert, and Abrahamson[7]) in a blank solution obtained by the recommended procedure exhibited an excitation at 358 nm and an emission maximum at 457 nm, and these spectra agreed with those of a measurement solution obtained by the same procedure.

After evaporation of the solvent of its measurement solution, to the residue was added an alkaline solution and extracted with ether. The ultraviolet spectrum of the fluorescent product which was obtained from the ethereal solution exhibited an absorption maximum at 348 nm (in 3 ml of 95% dioxane and 1 ml of concentrated ammonium hydroxide) and its spectrum agreed with that of the authentic sample (Fig. 5).

The fluorescent product in the measurement solution was also identified as III by comparison with the authentic sample (III)[7] in the IR spectrum (Fig. 6) and the *Rf* value (0.20) of TLC developed similarly to the case of II, and by the elemental analysis (*Anal.* Calcd. for $C_{13}H_{10}N_2S$: C, 68.99; H, 4.55; N, 12.48. Found: C, 69.08; H, 4.45; N, 12.48.) and the mixing melting point test (mp 203—204°, recrystallized from benzene).

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[7] M.T. Bogert and E.M. Abrahamson, *J. Am. Chem. Soc.*, **44**, 826 (1922).

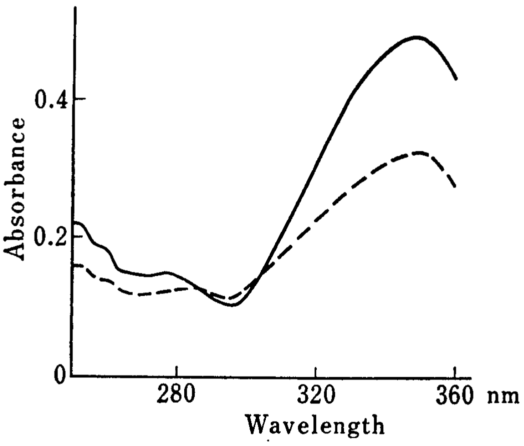

Fig. 5. Ultraviolet Spectra

——: 6-amino-2-phenylbenzothiazole in 95% (v/v) dioxane (3 ml) and ammonia water (1 ml)
----: fluorescent product of nitrate with 2-phenylbenzothiazole in 95% (v/v) dioxane (3 ml) and ammonia water (1 ml)

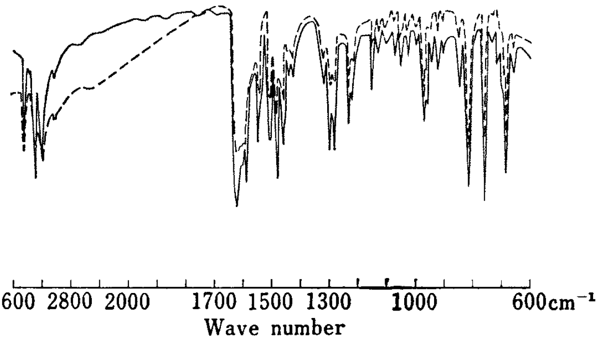

Fig. 6. IR Spectrum of 6-Amino-2-phenylbenzothiazole (KBr)

——: authentic sample of 6-amino-2-phenylbenzothiazole
----: fluorescent product in analytical procedure

The nuclear magnetic resonance (NMR) spectrum of the fluorescent product (in $CDCl_3$) exhibited the proton signal at 2.70 ppm, and its signal disappeared in deuterium oxide. Therefore, the presence of amino group was suggested.

These results indicated that II was converted into III in an ammonia alkaline solution by irradiation with ultraviolet light.

Choice of Experimental Conditions for Fluorometry

Effect of Reagent Concentration——The constant fluorescence intensity was obtained in the range of 0.1—1.0% (w/v) of I (Fig. 7), therefore 0.3% I-sulfuric acid was chosen as the reagent concentration.

Effect of the Amount of Sulfuric Acid——As shown in Fig. 8, the constant fluorescence intensity was obtained in the range of 0.5—8.0 ml, therefore, 5 ml of concentrated sulfuric acid was chosen.

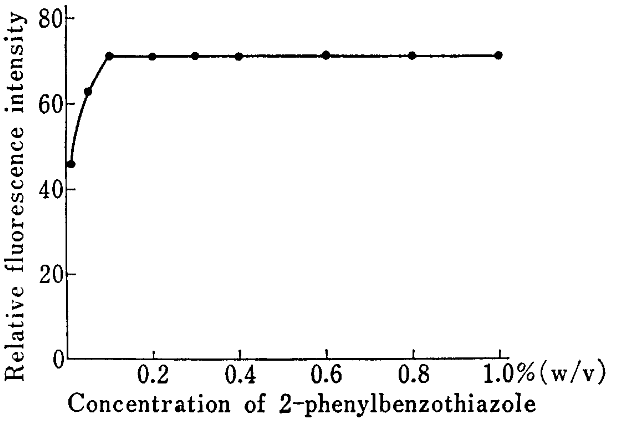

Fig.7. Effect of 2-Phenylbenzothiazole Concentration in 2-Phenylbenzothiazole Reagent Solution

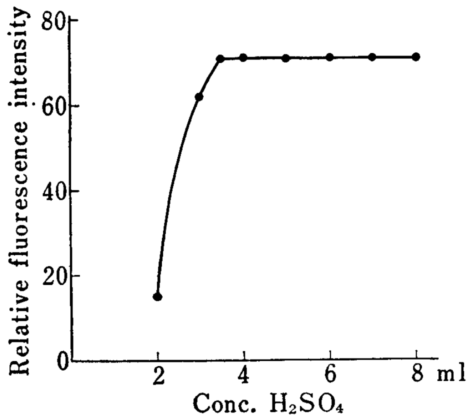

Fig. 8. Effect of the Amount of Sulfuric Acid

Effect of Reaction Temperature and Time——The fluorescence intensity was constant in the range of 0—100° and 1—60 min. Therefore, room temperature and 10 min were chosen, respectively.

Choice of Conditions on the Extraction with Ether——To the number of the extraction with 10 ml of ether for 2 min, twice was found to be enough for the extraction of the fluorescent product.

Effect of Organic Solvent on Fluorescence Intensity——As shown in Table I, when 95% dioxane was used, the highest fluorescence intensity was obtained. Therefore, 95% dioxane was chosen as the solvent.

TABLE I. Solvent Effects on Fluorescence Intensity of Reaction Product

Solvent	λ_{ex}[a] (nm)	λ_{em}[b] (nm)	R_{FI}[c]
Methanol	354	460	51.0
Ethanol	354	460	61.0
Propanol	354	460	62.0
Isopropanol	354	460	65.0
Pyridine	362	467	52.0
Dioxane(95% v/v)	358	457	71.0
Acetone	357	461	69.0

a) excitation maximum
b) emission maximum
c) relative fluorescence intensities, uncorrected; fluorescence readings at same sensitivity of instrument

Effect of the Concentration of Ammonium Hydroxide——The fluorescence intensity was constant in the range of 10—28% (Fig. 9). Therefore, concentrated ammonium hydroxide was used.

Effect of Irradiation Time——A reaction solution was irradiated with ultraviolet light at 10 cm from light source. As shown in Fig. 10, the fluorescence intensity was constant in the range of 4—40 min. Therefore, 15 min was chosen as the irradiation time.

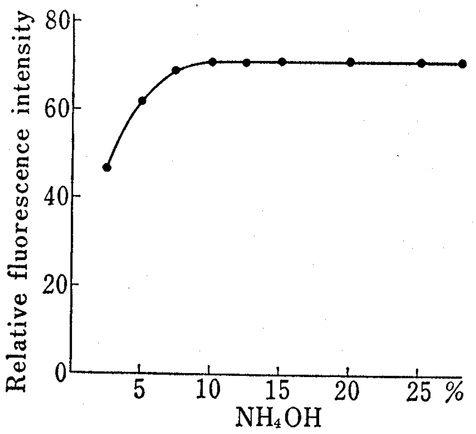

Fig. 9. Effect of Ammonia Concentration in Ammonia Reagent Solution

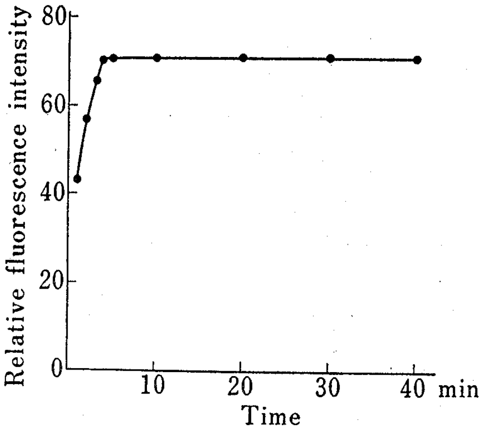

Fig. 10. Effect of Irradiation Time with Ultraviolet Light

Light Source——A Toshiba Black Light Fluorolamp (FL 20S-BLB) was the most effective light source for irradiation in comparison with other tested lamps [Toshiba Sakkin Lamp (GL 15, peak at 253.7 nm); Toshiba Kenkosenyo Fluorolamp (FL 20S, peak at 310 nm)].

Stability of Fluorescence

The fluorescence under the usual conditions was stable for 24 hr at room temperature after reaction.

Interfering Ions

Iterferences of various ions on the recommended procedure were examined by measuring the fluorescence intensity in the presence of foreign ions.

TABLE II. Effect of Diverse Ions

Diverse ions	Added as (μg/ml)	Amount of ion (μg/ml)	I found (%)
Ca^{2+}	pantothenate	5000	99.3
Cd^{2+}	sulfate	20	100.0
Cu^{2+}	sulfate	20	99.5
Fe^{2+}	ammonium sulfate	1	99.3
		2	87.3
Fe^{3+}	ammonium sulfate	500	100.0
Mg^{2+}	sulfate	5000	100.0
Mn^{2+}	sulfate	20	100.0
Zn^{2+}	sulfate	200	99.5
NH_4^+	sulfate	2000	100.0
Cl^-	sodium salt	100	92.9
ClO^-	sodium salt	1.5	122.6
$H_2PO_4^-$	sodium salt	5000	100.0
NO_2^-	sodium salt	10	121.1

I ion taken: 1.0 μg/ml

TABLE III. Determinations of Nitrate in Tap Water by Proposed and Official Methods

	Proposed method as NO_3^- (μg/ml)	Official method[8] as NO_3^- (μg/ml)
	12.5	11.6
	12.5	11.6
	12.6	11.7
	12.3	11.6
	12.0	11.6
$\bar{x}$(%)	12.38	11.62
s(%)	0.47	0.04

As shown in Table II, interfering ions were chloride, hypochlorite, nitrite, and ferrous ion.

The proposed fluorometric method was applied to the determination of nitrate in the range of 0.01—3.0 μg/ml of nitrate ion. The coefficients of variation from solutions containig 0.01 μg/ml, 0.8 μg/ml, and 3.0 μg/ml were 1.04%, 0.25%, and 0.45%, respectively. Also, the results of the determination of nitrate in tap water were shown in Table III, and similar results were obtained from the proposed method and sodium salicylate method.[8] In conclusion, the proposed method can be applied for a microdetermination of nitrate.

Acknowledgement The authors are very grateful to Professor Toyozo Uno of Kyoto University for the encouragement throughout this work and the helpful suggestion. Thanks are also due to Miss Yuriko Takayama and the staffs of Center of Instrumental Analysis for the measurement of the IR and NMR spectra, and elemental analysis.

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[8] Pharmaceutical Society of Japan (ed.), Standards of Analysis for Hygienic Chemist with Commentary, Kinbara Shuppan Co., Ltd., Tokyo, 1973, p. 707.