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The Spectrofluorometric Determination of Anthraquinone with Hydroxylamine in the Dimethyl Sulfoxide Medium Containing Sodium Methoxide and Methanol

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Anthraquinone reacts with hydroxylamine in the dimethyl sulfoxide (DMSO) medium containing sodium methoxide and methanol. Since the reaction product is fluorescent in the medium, this reaction can be used for the fluorometric determination of anthraquinone. A DMSO solution of a sample was mixed with a DMSO solution of hydroxylamine hydrochloride and a methanolic solution of sodium methoxide. The mixture was then allowed to stand for 20 min at 60 °C in a volumetric flask with a tightly fitting stopper. After cooling and dilution to the mark with DMSO, the fluorescence intensity of the solution was measured at 597 nm, with excitation at 494 nm. The intensity was stable for at least 2 h in the tightly stoppered flask. The determination limit of anthraquinone was 100 ng. By the application of this spectrofluorimetry, anthraquinone in 9,10-phenanthrenequinone could be successfully determined over the content range from 0.2 to 4%.

Anthraquinone, which is known as an oxidation product of anthracene, is of great value as a starting material for the manufacture of vat dyes. It is also utilized in the pulp industry as a catalyst for delignification. On the other hand, trace amounts of anthraquinone may be contained as an impurity in chemicals made from coal tar because anthracene is one of the main components of tar. As a result, there is great interest in monitoring the level of the anthraquinone content in such industrial products, intermediates, and wastes. A sensitive method for the determination of anthraquinone is, therefore, of importance. Several methods for determination have been developed, including gas chromatography,[1,2] liquid chromatography,[3-5] polarography,[6,7] and spectrophotometry.[8-10] The method using spectrofluorimetry has, however, been little discussed in the literature.

In the present paper, the reaction conditions of anthraquinone with hydroxylamine are investigated for the spectrofluorometric determination. This reaction proceeded advantageously in the dimethyl sulfoxide (DMSO) medium containing sodium methoxide and methanol. Anthraquinone could be determined by measuring the fluorescence intensity of the reaction product at 597 nm, with excitation at 494 nm. The product, however, could not be identified in the present research because of difficulties in isolating it from the reaction system. As a result of the application of this fluorimetry, anthraquinone in 9,10-phenanthrenequinone could be successfully determined.

Experimental

Reagents. DMSO was cooled to a temperature below the freezing point (18.5 °C). When about 90% of the DMSO had been frozen, the liquid remaining without solidification was removed. After the solid thus obtained had been completely melted, the same process was repeated 3 times. The DMSO finally obtained was stored in a tightly stoppered vessel because of its hygroscopicity. Anthraquinone and 9,10-phenanthrenequinone were purified by zone melting. Hydroxylamine hydrochloride was of a guaranteed reagent grade purchased from Tokyo Kasei Kogyo Co., Ltd. A methanolic solution of sodium methoxide was prepared by the dilution of a commercial chemical (sodium methoxide, about 28% in methanol, purchased from Wako Pure Chemical Industries, Ltd.) with proper amounts of methanol. The exact concentration of the commercial sodium methoxide solution was determined by titration.

Apparatus. All the fluorometric measurements were carried out using a Hitachi 650-40 fluorescence spectrophotometer. A 150-W Xenon lamp was used as the exciting source.

Procedure. Five milliliters of a sample solution containing less than about 2 μg of anthraquinone in 5 ml of DMSO was transferred into a 10-ml volumetric flask. Then, 2 ml of a 0.1 M (1 M=1 mol dm^{-3}) solution of hydroxylamine hydrochloride dissolved in DMSO was added to the flask. After stirring, 0.7 ml of a 3.5 M methanolic solution of sodium methoxide was added. The mixture was allowed to stand for 20 min at 60 °C in the tightly stoppered flask, with occasional stirring, and then it was diluted to the mark with DMSO after cooling in tap water. The anthraquinone was determined by measuring the fluorescence intensity of the resultant solution at 597 nm, with excitation at 494 nm. A DMSO solution containing 2.00 μg of anthraquinone per 5 ml was treated under the same conditions and at the same time; the fluorescence was used as the standard.

Results and Discussion

Fluorescence Spectra. The fluorescence spectra of the reaction product of anthraquinone with hydroxylamine are shown in Fig. 1. The excitation spectrum has maxima at 396, 471, and 494 nm. The emission spectrum exhibits a maximum at 597 nm. In order to minimize the interferences of diverse substances, 494 nm was selected as the excitation wavelength for the determination. The fluorescence intensity of the reagent blank was negligibly small. At the present stage, little is known about the structure of the fluorescent product. By a comparison of the

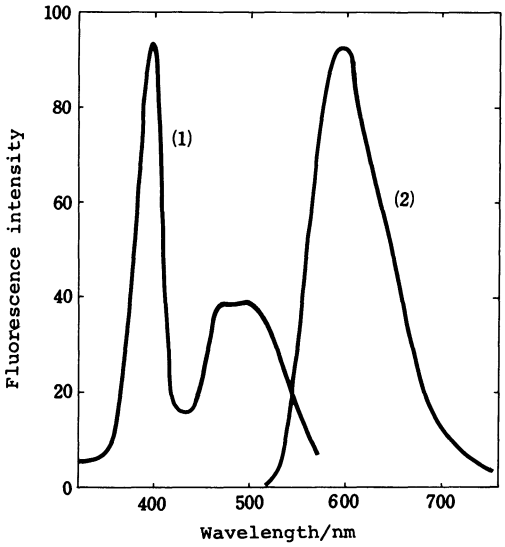

Fig. 1. Fluorescence spectra of the reaction product of anthraquinone.
(1): Excitation spectrum; (2): emission spectrum; uncorrected.

absorption and fluorescence spectra, however, the product was found not to be anthraquinone oxime, the formation of which is commonly predictable from a reaction with hydroxylamine.

Effects of Sodium Methoxide and Methanol. The reaction of anthraquinone with hydroxylamine proceeded well in DMSO containing sodium methoxide and methanol. Sodium methoxide was employed in order to make the DMSO medium strongly basic and methanol in order to increase the solubility of the sodium methoxide in the medium. The concentrations of these reagents had a relatively large influence on the fluorescence intensity of the reaction product. To study the effects of these reagents, various amounts of sodium methoxide (0.5—4.7 mmol) were added to 7 ml of a DMSO solution containing anthraquinone (2 μg) and hydroxylamine hydrochloride (0.2 mmol), together with 0.5, 0.7, and 1.0 ml of methanol. The fluorescence intensities were measured after the mixtures had stood for 20 min at 60 °C and had then been diluted to 10 ml with DMSO. The results of these experiments are shown in Fig. 2. Figure 2 indicates that an increase in the amount of methanol results in a decrease in the fluorescence intensity, and further that the increase requires more amounts of sodium methoxide for the maximum intensity to be reached. Therefore, it is necessary to take into account the simultaneous effects of these two reagents on the determination of anthraquinone. The maximum intensity observed in the presence of 0.7 ml of methanol is not so weak compared with that of 0.5 ml, and it is approximately constant over the broad range of 2—3 mmol of sodium methoxide. It is recommended, therefore, that a mixture of 0.7 ml of methanol and approximately 2.5 mmol of sodium

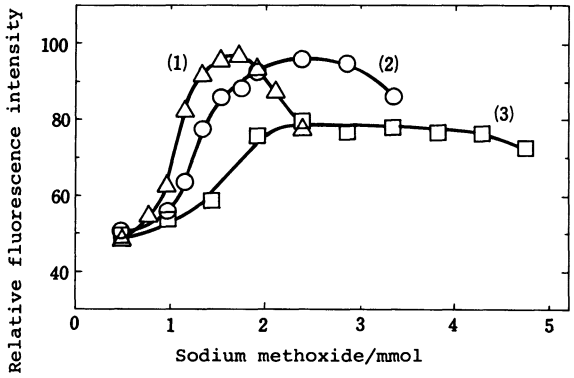

Fig. 2. Effects of the amounts of sodium methoxide and methanol.
Standard: fluorescence intensity of an ethanolic solution of Rhodamine B (20 ng ml^{-1}) was taken as 90.0 div.; anthraquinone: 2.00 mg/10 ml; (1): added along with 0.5 ml of methanol; (2): 0.7 ml; (3): 1.0 ml.

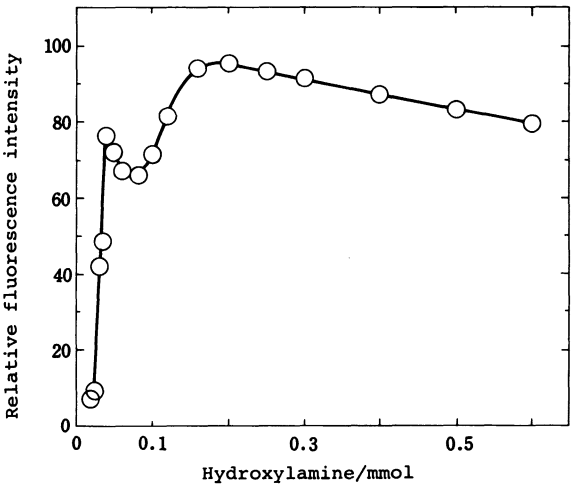

Fig. 3. Effect of the amount of hydroxylamine hydrochloride.
Standard: the intensity of the solution of Rhodamine B (20 ng ml^{-1}) was taken as 90.0 div.; anthraquinone: 2.00 μg/10 ml.

methoxide be used for the determination. In the determination procedure, 0.7 ml of a 3.5 M methanolic solution of sodium methoxide was added to a sample solution.

Effect of Hydroxylamine. Hydrochloride of hydroxylamine was used as the reaction reagent because of facility in handling. The effect of hydroxylamine was measured by varying the amount over the wide range from 20 μmol to 1 mmol in the presence of 2 μg of anthraquinone (approximately 10 nmol) and 2.5 mmol of sodium methoxide. These experiments showed that a large excess of hydroxylamine caused the fluorescence intensities to decrease. A milky turbidity appeared when more than 0.5 mmol of hydroxylamine hydrochloride had been added. In the region less than about 0.1 mmol, on the other hand, the fluorescence spectra were distinct from those

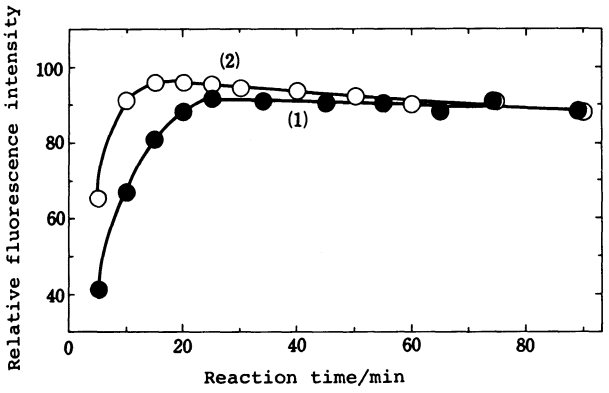

Fig. 4. Effects of reaction time and temperature. Standard: the intensity of the solution of Rhodamine B (20 ng ml^{-1}) was taken as 90.0 div.; anthraquinone: 2.00 mg/10 ml; (1): reacted at 50 °C; (2): 60 °C.

shown in Fig. 1. The fluorescence intensities of the reaction product, as measured over the range of hydroxylamine hydrochloride up to 0.6 mmol, are illustrated in Fig. 3. The maximum fluorescence intensity was obtained when approximately 0.2 mmol of the hydrochloride was added. Another unique peak is further observed at the approximate amount of 0.04 mmol in Fig. 3. Judging from the spectra, it seems likely that the peak was due to the formation of the mono-reacted species. The species, however, was unstable under these conditions, and the intensity decreased rapidly. Therefore, 2 ml of a 0.1 M DMSO solution of hydroxylamine hydrochloride was used in the determination procedure.

Effects of Reaction Temperature and Time. The reaction temperature and the time are important variables for the determination. The effects of these variables on the fluorescence intensity of the product were studied under various conditions. Figure 4 illustrates the results obtained by varying the standing time from 5 to 90 min at the reaction temperatures of 50 and 60 °C. All the measurements were carried out in a 10-ml volumetric flask with a tightly fitting stopper. As is shown in Fig. 4, the reaction of anthraquinone with hydroxylamine at temperatures of 50 and 60 °C is completed by letting it stand for approximately 25 and 15 min respectively. If the flask was left open, however, the fluorescence intensity was unstable and in some cases increased with the increase in the standing time without arriving at a constant value until at least 2 h. Therefore, this reaction was performed by letting the mixture stand for 20 min at 60 °C in the tightly stoppered flask.

Stability of the Fluorescence. In order to investigate the stability of fluorescence of the reaction product, 5 ml of a DMSO solution containing 2 μg of anthraquinone was allowed to react according to the determination procedure. The solution was cooled in tap water, diluted to the mark with DMSO, and then allowed to stand at room temperature. The fluorescence intensity of this solution was measured at adequate time intervals. The intensity was consequently constant for at least 2 h in the tightly stoppered flask. When the stopper was opened, however, the intensity became relatively unstable. This unstability may be attributable to atmospheric oxygen, carbon dioxide, and/or moisture, but no obvious conclusion could be drawn even from additional experiments.

Calibration Curve and Reproducibility. A calibration curve for the determination of anthraquinone was prepared from its DMSO solutions containing 0—2.00 μg per 5 ml according to the determination procedure. The solution with the highest concentration was used as the standard. The calibration curve thus prepared was linear between the fluorescence intensity and the concentration of anthraquinone. According to this curve, 0.1—2 μg of anthraquinone could be determined. The reproducibility of this method was measured by using 2.00 μg of anthraquinone. The relative standard deviation was 1.7% on 20 measurements over a several-day period. In these experiments, an ethanolic solution of Rhodamine B was used as the standard.

Behavior of Other Quinones and Polynuclear Aromatic Carbonyl Compounds. Since it seemed reasonable to assume that this reaction is applicable to other quinones, additional research was undertaken. Several aromatic compounds which have carbonyl groups were selected in order to investigate their reactivities with hydroxylamine. The spectral data of the resulting fluorescent products of these compounds are shown in Table 1. In this research, approximately 10—20 μg of each compound was treated under the same conditions as anthraquinone. *p*-Benzoquinone and 9-fluorenone were indistinct where the fluorescent substances were produced under the present conditions. Table 1 indicates that most of the quinones react with hydroxylamine much like anthraquinone, even though the conditions are not the optimum ones. Therefore, there is a good possibility that such nonfluorescent compounds can be determined by fluorimetry using hydroxylamine as the reaction reagent.

Effects of Diverse Substances. The effects of 16 different substances were studied for the determination of anthraquinone. Table 2 summarizes the effects on the fluorescence intensity of the reaction product of anthraquinone. The measurements of the intensity are not interfered with by the presence of even 100-fold amounts of biphenyl, naphthalene, acenaphthene, phenanthrene, dibenzofuran, dibenzothiophene, and carbazole. Pyrene and fluoranthene give negative errors, and fluorene, a positive one. Since anthracene changes in part to anthraquinone by oxidation during the determination process, the presence of large

Table 1. Fluorescence Excitation and Emission Maxima of the Reaction Products of a Variety of Quinones and Polynuclear Aromatic Carbonyl Compounds

Substances	Excitation maxima	Emission maxima
	nm	nm
2-Methylanthraquinone	396*, 472, 493	601
2-Ethylanthraquinone	397*, 471, 492	601
9,10-Phenanthrenequinone	291, 314, 367*, 420(s)	532
Acenaphthenequinone	322, 375, 460(s), 469*	545
1,2-Naphthoquinone	353	476
1,4-Naphthoquinone	292, 349*, 395	494
Vitamin K_1	297, 363*, 390(s)	509
Vitamin K_2	297, 364*, 395(s)	512
Vitamin K_3	295, 358*, 390(s)	501
Anthrone	393*, 471, 485(s)	567, 600(s)
9-Fluorenone	obscure	
p-Benzoquinone	obscure	

The most intense maximum in each spectrum is indicated by *, and the shoulder, by (s); uncorrected.

Table 2. Effects of Diverse Substances

Substances added		Relative fluorescence intensity[a]	Difference
	μg		
None		100.0	
Biphenyl	206	100.7	+0.7
Naphthalene	208	102.2	+2.2
Acenaphthene	207	100.7	+0.7
Phenanthrene	219	98.8	−1.2
Dibenzofuran	200	103.5	+3.5
Dibenzothiophene	212	100.3	+0.3
Carbazole	209	99.3	−0.7
Pyrene	22	95.5	−4.5
	45	94.2	−5.8
9-Fluorenone	11	97.5	−2.5
	22	95.2	−4.8
Anthracene	1.1	99.4	−0.6
	4.2	96.5	−3.5
	21	102.9	+2.9
	42	121.2	+21.2
1,4-Naphthoquinone	1.1	102.5	+2.5
	4.2	105.9	+5.9
Acenaphthenequinone	1.1	96.4	−3.6
	2.2	93.9	−6.1
Fluoranthene	0.42	96.3	−3.7
	1.1	94.3	−5.7
Fluorene	0.41	103.0	+3.0
	1.0	105.7	+5.7
Acridine	0.35	96.6	−3.4
	0.70	94.8	−5.2
Anthrone	0.045	102.2	+2.2
	0.11	105.5	+5.5

a) The fluorescence intensity of a solution of the reaction product (2.00 μg anthraquinone per 10 ml) was taken as 100 div.

amounts of anthracene gives a positive error. Quinones and the other carbonyl compounds have an observable effect on the determination of anthraquinone, for these substances also react with hydroxylamine. Acridine has a relatively large influence, much as do fluoranthene, fluorene, and the quinones. Anthrone is the most influential of all these substances.

Determination in 9,10-Phenanthrenequinone. The fluorometric method was applied to the determination of anthraquinone in 9,10-phenanthrenequinone (9,10-PQ). The influence of 9,10-PQ on the determination was first studied. A DMSO solution containing 2.00 μg of anthraquinone per 5 ml was treated, according to the determination procedure, in the presence of 0—110 μg of 9,10-PQ. Blank experiments were also carried out in the presence of 9,10-PQ at the same time. The results of these experiments are

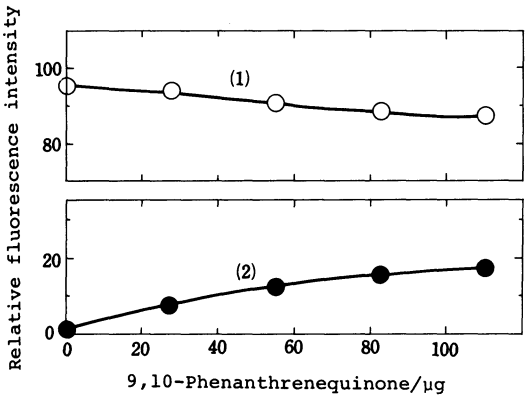

Fig. 5. Effect of the amount of 9,10-phenanthrenequinone.
Standard: the intensity of the solution of Rhodamine B (20 ng ml^{-1}) was taken as 90.0 div.; (1): 9,10-phenanthrenequinone solution containing anthraquinone (2.00 mg/10 ml); (2): 9,10-phenanthrenequinone alone.

Table 3. Analytical Results for Anthraquinone in Synthetic Mixtures of 9,10-Phenanthrenequinone

Content %	Relative fluorescence intensity[a)]	Found %	
0.20	15.0, 15.9	0.2_0, 0.2_5	
0.40	19.3, 19.2	0.4_2, 0.4_1	
0.80	27.8, 26.9	0.8_4, 0.8_0	
1.60	42.0, 42.0	1.5_5, 1.5_5	
2.40	57.8, 58.9	2.3_4, 2.4_0	
3.20	74.4, 74.7	3.1_7, 3.1_9	
4.00	91.0, 92.5	4.0_1, 4.0_8	
4.00	89.6, 90.9	3.9_4, 4.0_0	
4.00	88.1, 88.9	3.8_6, 3.9_0	$\bar{\chi}=3.9_8$
4.00	89.0, 90.3	3.9_1, 3.9_7	$\sigma=0.06_3$
4.00	91.2, 89.9	4.0_2, 3.9_5	R.S.D.=1.6%
4.00	91.0, 92.0	4.0_1, 4.0_6	

a) The fluorescence intensity of the product (2.00 μg anthraquinone per 10 ml) was taken as 95.0 div.

shown in Fig. 5. Figure 5 indicates that the fluorescence intensity of the reaction product of anthraquinone decreases with an increase in the amount of 9,10-PQ, but that the intensity of the blank solution increases. This apparently conflicting influence of 9,10-PQ is because the negative interference is greater than the positive one, which is based on the fluorescence of the reaction product of 9,10-PQ with hydroxylamine. For the determination of anthraquinone it is necessary to reduce such interference due to 9,10-PQ. In case it is present in small amounts, however, the effect can be corrected by the use of a calibration curve prepared in the presence of 9,10-PQ. Therefore, a calibration curve for anthraquinone (0—2 μg) was prepared in the presence of 50 μg of 9,10-PQ according to the procedure. The solution obtained by treating 2.00 μg of anthraquinone was used as the standard. The curve thus obtained was linear between the fluorescence intensity and the concentration of anthraquinone. However, the curve did not intersect at the point of origin, and its gradient was smaller than that for anthraquinone alone because of the influence of 9,10-PQ. The analytical results for anthraquinone in synthetic mixtures of 9,10-PQ are shown in Table 3. Each synthetic mixture contains 2% of phenanthrene, carbazole, and 9-fluorenone, as well as 0.2% of 1,4-naphthoquinone, acenaphthenequinone, dibenzofuran, dibenzothiophene, acridine, biphenyl, naphthalene, acenaphthene, anthracene, fluoranthene, and pyrene. Table 3 indicates that the anthraquinone in 9,10-PQ can be determined over the range from 0.2 to 4%. In addition, anthraquinone in 3 different chemicals of 9,10-PQ was determined; the resulting contents were 0.4, 4.0, and below 0.2%. The emission spectrum of the solution used for the determination agreed well with that of the solution prepared in the presence of 9,10-PQ. From the results of these experiments, the present fluorimetry can be applied to the determination of small amounts of anthraquinone in 9,10-PQ.

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