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Substituent-Group Effects on the Fluorescence of Aluminum–Schiff Base Complexes and Their Application to the Determination of Aluminum

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The effects of the substituents in the Schiff-base *N*-salicylidene-*o*-aminophenol (SOAP) on the fluorescence of its aluminum complexes were investigated. *N,N'*-Disalicylideneethylenediamine (DSED) derivatives were used as aldehyde reagents for the condensation to prepare 25 different SOAP compounds, for these reagents can be conveniently hydrolyzed to yield salicylaldehyde derivatives. *o*-Aminophenol (OAP) derivatives were used as amine reagents for the SOAP compounds. A methoxyl group at the 4-position of the benzylidene group in the Schiff base and a sulfo group at the 5-position in the amine component increased the fluorescence intensity of the complex. The relationship between the fluorescence intensity and the structure of the complex was investigated by the Free–Wilson method of estimation, and good agreements were obtained between the experimental and the calculated values of the fluorescence intensities. The fluorometric determination procedure of aluminum was established by using 4,4'-dimethyl DSED and 4-sulfo OAP. Aluminum can be accurately determined in the range from 0.001 to 3 μg/25 ml under optimum conditions.

N-Salicylidene-*o*-aminophenol (SOAP) is an excellent fluorometric reagent for aluminum, gallium, and beryllium.[1–6] Derivatives of SOAP may have even better sensitivities than the parent compound. Several substituted SOAP have been examined in connection with the fluorometric determination of aluminum. Some SOAP derivatives, which have only one substituent group in the aldehyde or amine component, have been described in previous studies.[2–4] Only a few studies involving SOAP with two or more substituent groups have been performed,[7] however, except with alkyl derivatives.[5]

In the case of 2,2'-dihydroxyazobenzene, which has a structure analogous to the SOAP structure, some derivatives with two or three substituents have been synthesized, and several derivatives, such as Superchrome Garnet Y and Lumogallion, have been found to be excellent fluorometric reagents.[8–11] In the present paper, the fluorescence properties of aluminum complexes with 25 SOAP derivatives including several combinations of various substituent groups were systematically examined in order to establish the lower detection limits for the fluorometric determination of aluminum. The relationship between the fluorescence intensities and the structures of the aluminum complexes has been explained by means of the Free–Wilson method of estimation.[12] Furthermore, the fluorometric determination of aluminum has been established by using the most sensitive reagent, which is superior to the conventional one, *N*-salicylidene-2-aminophenol-4-sulfonic acid.[2]

Experimental

Apparatus. The fluorescence spectra and the intensity were measured with a Hitachi Model 204 fluorescence spectrophotometer fitted with a 150 W Xenon lamp. The absorption spectra were measured with a Shimadzu Model UV-200S double-beam spectrophotometer. A Toa pH Meter, Model HM-20E, was used for the pH measurements.

Reagents. Standard Solution of Aluminum: A stock solution of aluminum was prepared by dissolving 0.5038 g of aluminum (99.99%) with 20 ml of concentrated hydrochloric acid and then diluting the solution to 500 ml with water. The standard solution was prepared by suitable dilution with 0.1 M hydrochloric acid (1 M=1 mol dm^{-3}) from the stock solution.

***N,N'*-Disalicylideneethylenediamine (DSED) and Its Derivatives:** DSED and its derivatives were synthesized by the condensation of salicylaldehyde and its derivatives with ethylenediamine[8] and then characterized by means of elemental analysis, their melting points, and their IR spectra. These compounds were dissolved in *N,N*-dimethylformamide (DMF). The aldehydes, which were not commercially available, were prepared by the Duff reaction.[13]

***o*-Aminophenol (OAP) and Its Derivatives:** OAP (Wako Pure Chemical Industries, Ltd.) and its derivatives (Tokyo Kasei Kogyo Co., Ltd.) were purified by the method of Charles and Freiser.[14] These compounds were then dissolved in 0.1 M ammonia or DMF, containing ascorbic acid (6.0×10^{-2} M). These solutions are subject to oxidation, but were sufficiently stable for use over a period of 8 hours.

***N*-(2-Hydroxy-4-methylbenzylidene)-2-aminophenol-4-sulfonic Acid:** This compound was synthesized by the condensation of 2-hydroxy-4-methylbenzaldehyde with 2-aminophenol-4-sulfonic acid by the method of Freeman and White.[8] The product thus obtained was purified by repeated recrystallizations from ethanol containing activated charcoal.

Uranine Standard Solution: 0.1 g of uranine (Wako Pure Chemical Industries, Ltd.) was dissolved in 100 ml of water, after which the solution was diluted appropriately with water to give solutions containing $(5.0\times10^{-2}$—$1.0)$ μg ml^{-1}. These solutions were employed as reference standards of the fluorescence in adjusting the sensitivity of the instrument.

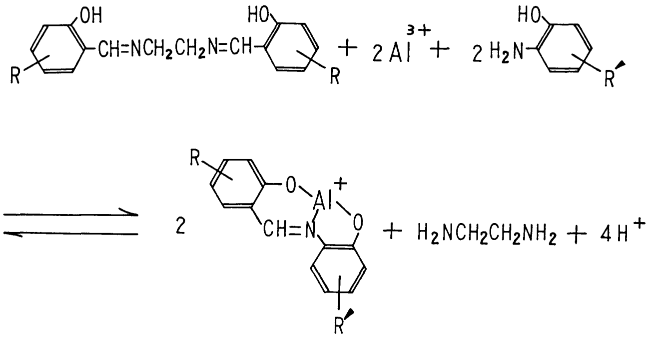
All the other reagents used were special-grade or equivalent.

General Procedure. To a sample solution containing an appropriate amount of aluminum ions, add 1 ml of a DSED-derivatives solution, 1 ml of an OAP-derivatives solution, and 1 ml of a 20% ammonium acetate solution. Adjust the pH to the required value with a diluted hydrochloric acid or an ammonia solution; then dilute the solution to 25 ml with water. After mixing thoroughly, warm the solution at 50 °C for 10 min and then cool to room temperature. Measure the fluorescence intensity at the optimum excitation and emission wavelengths, using an uranine solution as the reference standard.

As has been described by Yamamoto et al.,[15] the correction of the experimental fluorescence intensity for variations in photomultiplier response and the source intensity with the wavelength were not performed because the maximum excitation and emission wavelengths of this series of the complexes lay within a narrow region. The excitation and emission spectra and the maximum wavelengths of the complexes were the corrected values in the present paper.[16]

Results and Discussion

Formation of an Aluminum-Schiff Base Complex. According to the general procedure, DSED was hydrolyzed in an aqueous solution to liberate salicylaldehyde (SAL), which then reacted with aluminum and OAP to form a fluorescent Schiff-base complex (Scheme 1). Such a reaction had previously been utilized for the fluorometric determination of beryllium,[17] zinc,[18] aluminum,[19] and magnesium.[20] Although the same complex is formed when SOAP is used instead of DSED and OAP, the procedure in this study has the advantage that the most suitable concentrations of SAL and OAP can be selected in order to obtain the optimum fluorescence conditions, and so the fluorescence intensity of the reagent blank can be decreased.



Scheme 1.

In all cases, the fluorescence intensity of the aluminum complex required 80 min to reach its maximum at 20 °C, but readily reached its maximum intensity at 50 °C within 5 min. The fluorescence intensity is stable for at least 2 h.

The absorption spectra of the reagents alone indicated that a free Schiff base was not formed under the optimum conditions for the formation of the aluminum complex. This suggests a mechanism for this formation of the aluminum–Schiff base complex in which SAL and OAP first coordinate to the aluminum ion, thus forming a ternary chelate compound, this compound is then transformed into a Schiff-base complex by the kinetic template mechanism.[19—22]

The molar ratio of aluminum to ligand in the complex was determined fluorometrically by the continuous-variation method. The results indicate that the metal–ligand ratio is 1 : 1.

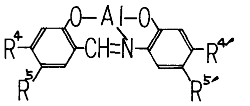
Fluorescence Properties of Aluminum-Schiff Base Complexes. The fluorescence properties of aluminum complexes with 25 SOAP derivatives were examined. Their fluorescence characteristics, as obtained under the optimum conditions, are summarized in Table 1.

Aluminum formed fluorescent complexes with a Schiff base in a weakly acidic solution. The optimum pH's for the fluorescence intensity were near pH 5. The optimum concentrations of DSED derivatives and OAP derivatives were about 1.5×10^{-4} M and $(2$—$5)\times10^{-4}$ M respectively in the final solution. The excitation and emission maxima of aluminum complexes occur at wavelengths of 395—420 nm and 485—535 nm respectively. Some typical excitation and emission spectra of aluminum complexes are shown in Fig. 2.

The fluorescence intensities of the aluminum complexes were markedly changed by the substituent groups on the Schiff base. A methoxyl group at the 4-position of the benzylidene group in the Schiff base (No. **3** in Table 1) and a sulfo group at the 5-position in the amine component (No. **9**) increased the fluorescence intensity of the complexes. These results were consistent with those reported by Morishige.[5] However, the combination of a methyl or a methoxyl group at the 4-position in the aldehyde component and a sulfo group at the 5-position in the amine component (No. **16** or **19**) further increased the fluorescence intensity. The fluorescence enhancement upon the introduction of these substituent groups was due to the increase in the molar absorptivity and the fluorescence quantum yield of its aluminum complex, as will be described below.

The relationship between the fluorescence intensity and the structure of the complex was investigated by means of the Free–Wilson method of estimation, which has been known as a mathematical technique describing the structure-biological activity relationships of a series of chemical analogs.[12] The mathematical model used is based upon the assump-

Table 1. Optimum Fluorescence Conditions for Aluminum-Schiff-Base Complexes



No.	Substituent				DSED Derivative concn. (10^{-4} M)	OAP Derivative concn. (10^{-4} M)	pH	Relative fluorescence intensity[b]		Wavelength[c](nm)	
	R^4	R^5	$R^{4'}$	$R^{5'}$				Net intensity[a]	Blank	Ex.	Em.
1	H	H	H	H	1.5	6.9	5.0	38.8	4.0	405	520
2	CH_3	H	H	H	1.4	6.9	5.3	51.5	6.5	400	505
3	OCH_3	H	H	H	1.3	6.9	5.3	70.0	10.0	400	500
4	OH	H	H	H	1.3	8.3	5.1	49.0	7.5	400	500
5	H	CH_3	H	H	1.0	6.2	5.3	49.0	7.0	405	510
6	H	OH	H	H	1.4	6.9	5.3	7.5	6.7	415	520
7	H	H	CH_3	H	1.5	4.4	5.1	52.5	6.0	405	520
8	H	H	OH	H	1.5	3.0	4.6	65.0	12.0	400	500
9	H	H	H	SO_3H	1.1	2.4	5.3	93.0	6.5	400	490
10	H	H	H	COOH	1.5	4.3	5.1	67.0	11.5	400	500
11	H	H	H	Cl	1.5	4.2	5.3	23.5	3.5	415	535
12	CH_3	H	OH	H	1.4	2.4	4.6	59.5	11.5	400	500
13	OCH_3	H	OH	H	1.3	1.8	5.0	38.5	10.0	400	495
14	OCH_3	H	CH_3	H	1.3	4.4	5.3	68.5	10.0	400	500
15	OH	H	OH	H	1.3	1.8	4.8	31.5	9.0	395	490
16	CH_3	H	H	SO_3H	1.4	3.2	5.3	110.5	6.5	400	490
17	CH_3	H	H	COOH	1.4	6.4	5.0	91.5	20.5	400	500
18	CH_3	H	H	Cl	1.1	6.3	5.3	49.0	5.0	420	530
19	OCH_3	H	H	SO_3H	1.3	4.8	5.5	113.5	12.5	400	490
20	OCH_3	H	H	COOH	1.3	6.4	5.0	111.0	26.5	395	485
21	OH	H	H	SO_3H	1.0	4.0	5.5	92.5	6.5	400	485
22	OH	H	H	COOH	1.0	6.4	5.0	81.5	21.5	395	485
23	OH	H	H	Cl	1.3	5.3	5.5	50.2	4.8	410	520
24	H	CH_3	H	SO_3H	1.4	2.4	5.5	83.7	6.8	420	490
25	H	CH_3	H	Cl	1.1	6.3	5.3	49.0	7.0	420	530

a) Al(III): 0.25 μg/25 ml (3.7×10^{-7} M). b) 70 div. vs. 0.5 μg ml^{-1} uranine (setting 405 nm/500 nm). c) Corrected value.

tion that the fluorescence intensity [I] in a series of analogs can be given as the sum of the "overall average" values (μ) and the contribution of each substituent [R]; i.e.,

$$\mu + \Sigma[R] = [I] \qquad (1)$$

where [R] is the specific value determined by a substituent group at a certain position. Furthermore, the sum of [R] at each position is restricted to zero in this model; i.e.,

$$\Sigma[R] = 0 \quad \text{(at each position).} \qquad (2)$$

The application of this method of estimation to the fluorescence intensities of aluminum complexes of 9 SOAP derivatives, which have substituent groups at the 5-position on the benzylidene group in each component of the Schiff base, has been investigated by Yamamoto et al.[15] They reported that this method could be applied to that series of Schiff bases.

This method was applied to the experimental fluorescence intensities of a total of 25 aluminum complexes in our study. The μ and [R] values were calculated by means of multiple regression analysis using a computer. Then the calculated values were substituted into Eq. 1; the fluorescence intensity of each complex was thus obtained. The results are presented as the correlation of the calculated values with the experimental values of the fluorescence intensities in Fig. 1 (Key, ○). Good agreements were obtained between these two values, with the relation of $y=1.00x+0.021$ and a correlation coefficient of 0.940. These results indicated that this method could also be applied to the series of Schiff bases which have substituent groups at the 4- and 5-positions in each component.

Moreover, the μ and [R] values were calculated by using the experimental values of 11 kinds of complexes which have only one substituent group in either the aldehyde or amine component of the Schiff base (μ;50.2, $[H]_{R^4}$;−5.4, $[CH_3]_{R^4}$;7.3, $[OCH_3]_{R^4}$;25.8, $[OH]_{R^4}$;4.8, $[H]_{R^5}$;2.1, $[CH_3]_{R^5}$;12.3, $[OH]_{R^5}$;−29.2, $[H]_{R^{4'}}$;−1.4, $[CH_3]_{R^{4'}}$;12.3, $[H]_{R^{5'}}$;−6.8, $[SO_3H]_{R^{5'}}$;47.9, $[COOH]_{R^{5'}}$;21.4, $[Cl]_{R^{5'}}$;−22.1). The fluorescence intensities of the complexes which have a combination of two substit-

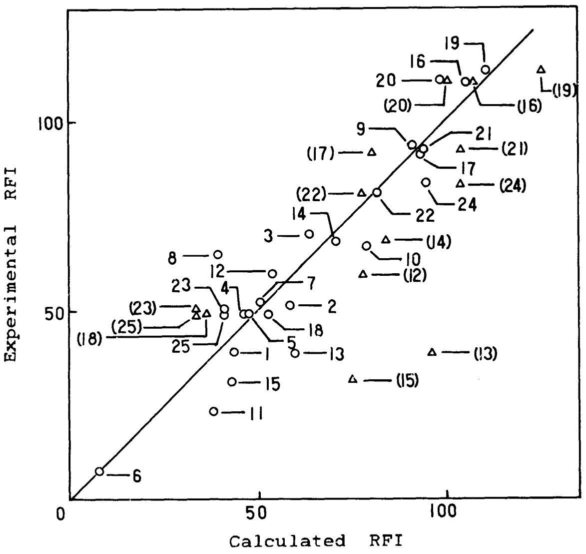


Fig. 1. Relationship between experimental and calculated values of fluorescence intensities of aluminum complexes (for key to substituents, see Table 1). O; calculated from all of 25 aluminum complexes. Δ: calculated from 11 aluminum complexes, which have only one substituent.

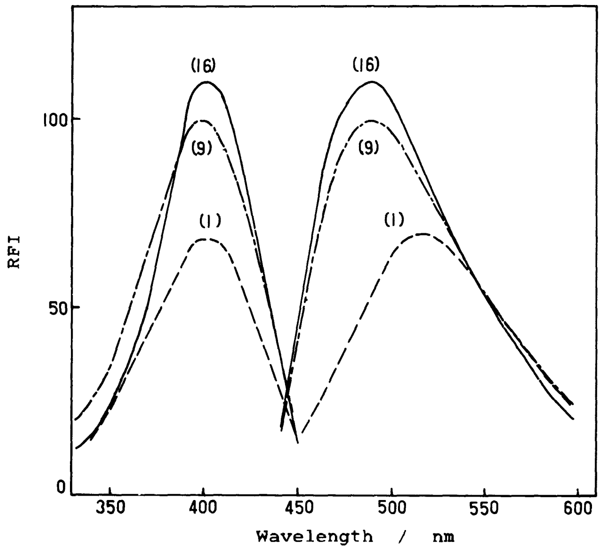


Fig. 2. Corrected excitation and emission spectra of aluminum complexes with Schiff bases; (1) SOAP (*N*-salicylidene-*o*-aminophenol), (9) *N*-salicylidene-2-aminophenol-4-sulfonic acid, (16) *N*-(2-hydroxy-4-methylbenzylidene)-2-aminophenol-4-sulfonic acid. Conditions for (1), (9), and (16); reference to Table 1.

uent groups were estimated by the use of the μ and [R] values obtained. As is shown in Fig. 1 (Key, Δ), a linear relationship was observed between the estimated and the experimental values, with the relation of $y=0.771x+20.1$ and a correlation coefficient of 0.917, except for the complexes which have a hydroxyl group at the 4-position in the amine component of the Schiff base (Nos. **12**, **13**, and **15**). These results suggest that, if the fluorescence intensities of several compounds among these series of Schiff base are experimentally measured, a better structure for the fluorometric reagent can be predicted by means of this method of estimation. In general, the combination of two substituent groups, both of which increase the fluorescence intensity, will provide a much stronger fluorescence. On the other hand, the combination of substituent groups, one of which increases the fluorescence intensity, while the other decreases it, will cause their properties to cancel each other out. The exception of the hydroxyl group at the 4-position in the amine component suggests that this group interacts with a substituent group in the aldehyde component to contribute to the fluorescence intensity.

The molar absorptivity (ε), the fluorescence quantum yield (ϕ_f), and the fluorescence sensitivity index (FSI) of the aluminum-SOAP complex (No. **1**) have been reported by Morishige (ε=20400, ϕ_f=0.205, and FSI=0.309).[4] The substitution of a sulfo group at the 5-position in the amine component (No. **9**) caused a remarkable increment in ϕ_f (0.802) and a slight decrement of ε (14100), so that FSI was raised to 0.653.[4] In this study a much higher FSI (0.766) was achieved by the combination of a methyl group and a sulfo group (No. **16**), where ϕ_f and ε were 0.810 and 18100 respectively.

Fluorometric Determination of Aluminum

Procedure I. The combination of *N*,*N'*-bis(4-methylsalicylidene)ethylenediamine [4,4'-dimethyl DSED] and 2-aminophenol-4-sulfonic acid [4-sulfo OAP], which form Complex No. **16** (in Table 1), was the most suitable reagent for the fluorometric determination of aluminum because of its high sensitivity and its reproducibility, with a low blank value. The analytical conditions were, therefore, investigated further.

The excitation and emission spectra of the aluminum complexes of these reagents are shown in Fig. 2. The optimum excitation and emission wavelengths are 400 (uncorrected value: 410) nm and 490 (480) nm respectively.

The effect of the pH on the fluorescence intensity is indicated in Fig. 3. The aluminum complex showed maximum fluorescence in the pH region of 5.1—5.5.

Figure 4 shows the effect of the concentration of the reagent at the optimum pH. The maximum fluorescence intensity was achieved with 1.4×10^{-4} M of 4,4'-dimethyl DSED and 3.2×10^{-4} M of 4-sulfo OAP in the final solution. These concentrations correspond to the addition of 1 ml of 0.1% 4,4'-dimethyl DSED and 1 ml of 0.15% 4-sulfo OAP in a 25-ml solution for the determination of aluminum.

0.1% 4,4'-dimethyl DSED dissolved in DMF was stable for over one month. 0.15% 4-sulfo OAP in a

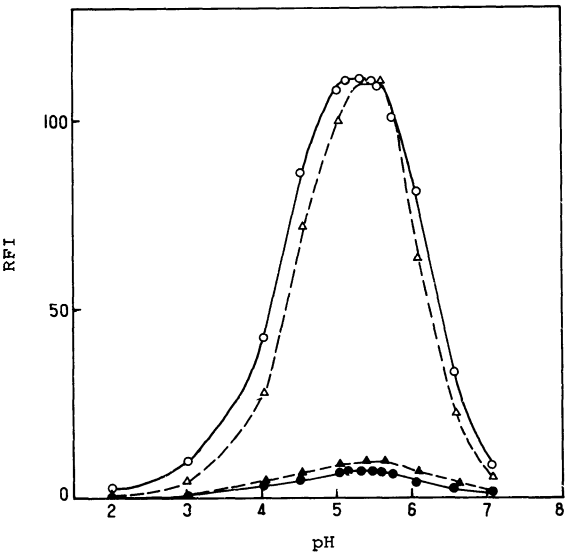


Fig. 3. Effect of pH on fluorescence intensity. Al(III): 0.25 μg/25 ml (3.7×10^{-7} M), Wavelength: 400 nm/490 nm, 70 div. vs. 0.5 μg ml^{-1} uranine (setting 405 nm/500 nm), Reagent: ○; 4,4′-dimethyl DSED (1.4×10^{-4} M)+4-sulfo OAP (3.2×10^{-4} M): △; *N*-(2-hydroxy-4-methylbenzylidene)-2-aminophenol-4-sulfonic acid (4.8×10^{-4} M): ●; reagent blank of ○: ▲; reagent blank of △.

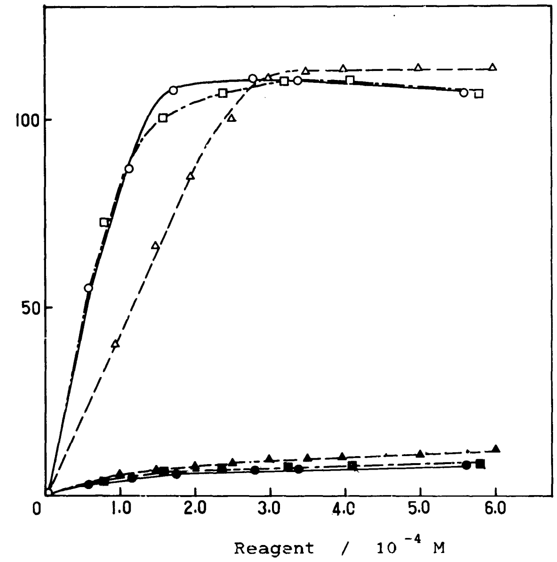


Fig. 4. Effect of concentration of 4,4′-dimethyl DSED (○), 4-sulfo OAP (□) and *N*-(2-hydroxy-4-methylbenzylidene)-2-aminophenol-4-sulfonic acid (△) on fluorescence intensity. Al(III): 0.25 μg/25 ml (3.7×10^{-7} M), pH: 5.3, Wavelength: 400 nm/490 nm, 70 div. vs. 0.5 μg ml^{-1} uranine (setting 405 nm/500 nm), ○: containing 4-sulfo OAP (3.2×10^{-4} M): □: containing 4,4′-dimethyl DSED (1.4×10^{-4} M), ●: reagent blank of ○: ■: reagent blank of □, ▲: reagent blank of △.

0.1 M ammonia solution containing ascorbic acid (6.0×10^{-2} M) was stable for use over a period of 24 h, while this solution without ascorbic acid was subject to oxidation and exhibited a brown color. This solution should, therefore, be made up daily.

According to the general procedure, aluminum can be determined in the range from 0.001 to 3 μg/25 ml with a relative error of 3% under optimum conditions, as has been described above.

The effects of diverse ions were also studied. The following cations in 100-fold excesses did not interfere with the determination of aluminum: silver(I), arsenic(III), barium(II), boron(III), cadmium(II), calcium(II), magnesium(II), manganese(II), mercury(II), molybdenum(VI), thallium(I), and zinc(II). Cobalt(II), chromium(VI), copper(II), indium(III), iron(II), iron(III), and nickel(II) caused negative errors in 10-fold excesses. Beryllium(II) and gallium(III) in 10-fold excesses did not interfere at all.

Procedure II. As has been mentioned, the same complex is formed by using only the Schiff base [i.e., *N*-(2-hydroxy-4-methylbenzylidene)-2-aminophenol-4-sulfonic acid] instead of those two components. Another procedure was also investigated by using the Schiff base as a fluorometric reagent, because the Schiff base in a DMF solution is more stable than 4-sulfo OAP.

The excitation and emission spectra, and the optimum wavelengths of the aluminum complex with this reagent, were just the same as those obtained by Procedure I.

The effect of the pH on the fluorescence intensity is indicated in Fig. 3. The aluminum complex showed its maximum fluorescence at pH 5.3—5.5.

Various amounts of a 0.075% (2.4×10^{-3} M) Schiff-base solution were added to a solution containing 0.25 μg of aluminum, after which the fluorescence intensity was measured at the optimum pH. As is shown in Fig. 4, 4.8×10^{-4} M is adequate. This concentration is equivalent to the addition of 5 ml of a 0.075% reagent solution in 25 ml for the procedure of determining aluminum. The 0.075% reagent solution is stable for over one month.

Aluminum can be determined in the same range as in Procedure I, i.e., from 0.001 to 3 μg/25 ml.

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