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Mordant Blue 31 as a New Fluorometric Reagent for Aluminum, Gallium, and Scandium

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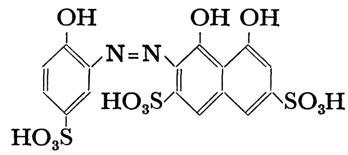
A sensitive fluorometric determination of aluminum, gallium, and scandium with Mordant Blue 31 was worked out. The reagent reacts with aluminum, gallium, scandium, yttrium, lanthanum, lutetium, indium, and magnesium in acidic or basic medium to form complexes with reddish yellow fluorescence. The complexes of aluminum (pH: 5—7), gallium (pH: 4), and scandium (pH: 6) exhibit strong fluorescence with an emission maximum at 590, 600, and 610 nm, respectively. The effects of time, pH and the concentration of the reagent were investigated. In acidic medium, aluminum, gallium, and scandium were found to form 1:1 complex with Mordant Blue 31. Under the optimum conditions, 0.1—3 μg of aluminum, 1—12 μg of gallium, and scandium in 25 m*l* sample solution could be determined. Although the fluorescence intensity of aluminum and gallium complexes was a little lower than that of the lumogallion complexes, Mordant Blue 31 was also applicable as a fluorometric reagent for aluminum and gallium. Since scandium-Mordant Blue 31 complex exhibits the strongest fluorescence of all other scandium-azo dye complexes, the reagent was used as a fluorometric reagent for scandium.

o,o'-Dihydroxyazo compounds are useful as sensitive fluorometric reagents for various metal ions.[1-6] Donald *et al.*[7] described the fluorescent properties of several metal complexes of *o,o'*-dihydroxyazo compounds. In these series chromotropic acid derivatives in particular are very sensitive and form stable fluorescent complexes with aluminum and gallium ions.

The present author describes the preparation of Mordant Blue 31 [2-(2-hydroxy-5-sulfophenylazo)-1,8-dihydroxynaphthalene-3,6-disulfonic acid], and the fluorometric determination of several metal ions.

The reagent reacts with magnesium ion to form a



Mordant Blue 31

stable fluorescent complex in alkaline solution, determination of magnesium is described.

Apparatus

Spectrofluorometric measurements were carried out with a Hitachi spectrophotometer, Model FPU-2A, equipped with a Hitachi fluorometric attachment, Model G-3. A Hitachi fluorometer, Model EPL-2, was used for fluorometric measurements and a Hitachi-Horiba glass electrode pH meter Model M-5 for pH measurements.

Reagents and Materials

Synthesis of Mordant Blue 31. 2-Amino-1-phenol-

1) A. Weissler and C. E. White, *Ind. Eng. Chem., Anal. Ed.*, **18**, 530 (1946).
2) Y. Nishikawa, K. Hiraki, K. Morishige, and T. Shigematsu, *Japan Analyst*, **16**, 692 (1967).
3) Y. Nishikawa, *ibid.*, **7**, 549 (1958).
4) R. Olsen and H. Diel, *Anal. Chem.*, **35**, 1142 (1963).
5) J. A. Radley, *Analyst*, **68**, 368 (1943).
6) Y. Nishikawa, K. Hiraki, and K. Morishige, *J. Faculty of Science and Technology, Kinki Univ.*, **2**, 15 (1967).
7) C. Donald, J. Freeman, and C. E. White, *J. Amer. Chem. Soc.*, **78**, 2678 (1956).

4-sulfonic acid dissolved in 6N hydrochloric acid was diazotized with an equivalent amount of sodium nitrite under cooling with ice. Chromotropic acid dissolved in about 6N sodium hydroxide was coupled with the diazonium salt solution under cooling below 5°C. The azo compound thus obtained was repeatedly recrystallized from 6N hydrochloric acid, and dried at room temperature in a desiccator on silica gel.

Reagent. 0.1% Mordant Blue 31 solution; 0.25 g of Mordant Blue 31 was dissolved in water and diluted with water to 250 m*l*.

Standard solution of aluminum; 1.6803 g of $Al(NH_4)(SO_4)_2 \cdot 12H_2O$ was dissolved in a small amount of water and 5 m*l* of concentrated hydrochloric acid, and diluted to 100 m*l* with water (1 mg Al/m*l*). The solution was diluted with water to prepare a solution of 1.00 μg Al per m*l*.

Standard solution of gallium; 0.1344 g of Ga_2O_3 (99.99%) was dissolved in concentrated hydrochloric acid, and diluted to 100 m*l* with water (1 mg Ga/m*l*). The stock solution was diluted with 0.1N hydrochloric acid to prepare a solution of 1.00 μg Ga per m*l*.

Standard solution of scandium; 0.1534 g of Sc_2O_3 (99.9%) was dissolved in concentrated hydrochloric acid and diluted to 100 m*l* with water (1 mg Sc/m*l*). The solution was diluted with water to make a standard solution containing 1.00 μg of Sc per m*l*.

Standard Rhodamine B solution; 0.100 g of Rhodamine B was dissolved in water and diluted to 100 m*l*. The solution was diluted with water to prepare a solution of 0.168 μg per m*l*.

Experimental

Fluorescence Spectra of Metal Mordant Blue 31 Complexes. Mordant Blue 31 reacts with aluminum, gallium, scandium, yttrium, lanthanum, lutetium, indium, and magnesium in acidic or basic medium to form complexes with reddish yellow fluorescence. The excitation and emission spectra of aluminum-, gallium-, and scandium-Mordant Blue 31 complexes, which show strong fluorescence, are given in Figs. 1 and 2, respectively.

The reddish yellow fluorescent aluminum complex exhibited excitation maxima at 330 and 540 nm and fluorescence emission maximum at 590 nm. The red fluorescent gallium complex showed excitation maxima at 340 and 580 nm, and fluorescence maximum at 600 nm. The scandium complex showed excitation maxima at 340 and 535 nm, and emission maximum at 610 nm.

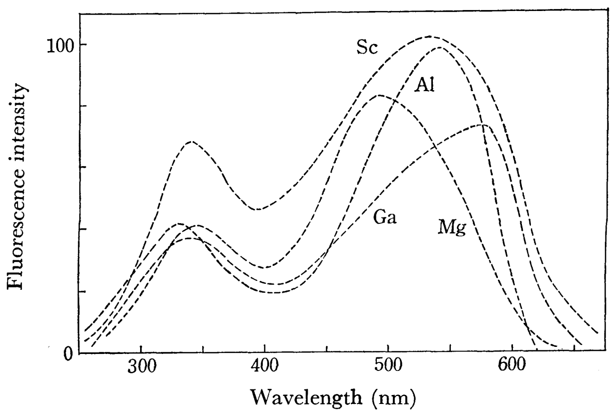


Fig. 1. Excitation spectra of metal Mordant Blue 31 complex
Al: 5×10^{-6}M, emission monochromator set at 590 nm;
Ga: 5×10^{-6}M, emission monochromator set at 600 nm;
Sc: 1×10^{-5}M, emission monochromator set at 610 nm;
Mg: 1×10^{-4}M, emission monochromator set at 585 nm

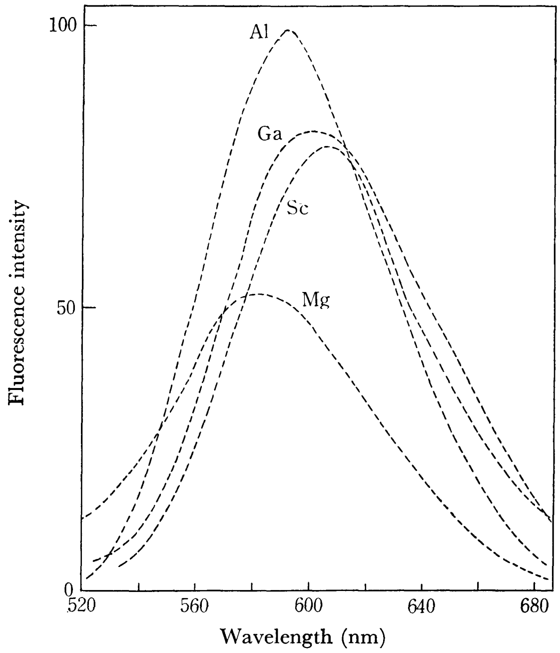


Fig. 2. Emission spectra of metal Mordant Blue 31 complex
Al: 5×10^{-6}M, excitation monochromator set at 330 nm;
Ga: 5×10^{-6}M, excitation monochromator set at 340 nm;
Sc: 1×10^{-5}M, excitation monochromator set at 340 nm;
Mg: 1×10^{-4}M, excitation monochromator set at 340 nm

Fluorometric Determination of metals. *(1) Aluminum*: To about 15 m*l* of the sample solution containing 0.1—3 μg of aluminum, 0.15 m*l* of 0.1% Mordant Blue 31 solution and 2 m*l* of 20% ammonium acetate solution were added. The pH value of the solution was adjusted to 6 with dilute hydrochloric acid or ammonia water and the solution was diluted to 25 m*l* with water. After being stood for 60 min at room temperature, the fluorescence intensity of the solution was measured at 590 nm (excitation: 330 or 540 nm) against the standard Rhodamine B solution.

(2) Gallium: To the sample solution containing 1—12 μg of gallium, 0.25—0.3 m*l* of 0.1% Mordant Blue 31 solution and 2 m*l* of 20% ammonium acetate solution were added. The pH was adjusted to 4, and diluted to 25 m*l*. After being stood 15 min, the fluorescence intensity of the solution was measured at 600 nm (excitation: 340 or 580 nm) against the standard Rhodamine B solution.

(3) Scandium: Scandium was determined by the same procedure as for gallium, adjusting the pH to 6 (excitation: 340 or 535 nm, emission: 610 nm).

Results and Discussion

Effect of Time. The fluorescence of the gallium- and the scandium-complex in aqueous medium was fully developed within a few minutes after mixing the solutions, and was stable for at least 2 hr. On the other hand, the fluorescence of aluminum complex required 120 min to reach the maximum as shown in Fig. 3. The fluorescence of some metal-azo-complex

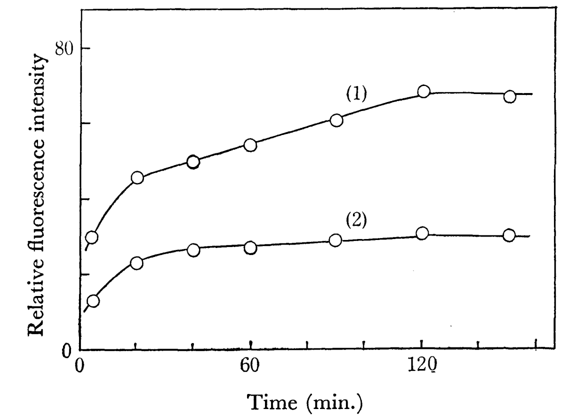


Fig. 3. Stability of fluorescence intensity of aluminum Mordant Blue 31 complex
(1) Al 2 μg, 0.1% reagent solution 0.15 m*l*, pH 6.0, 20 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)
(2) Al 2 μg, 0.1% reagent solution 0.15 m*l*, pH 6.0, 10 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 546, secondary filter 61)

readily reached the maximum intensity on warming at 80°C for several minutes. The aluminum complex was unstable on heating and the fluorescence intensity was measured after standing for 1 hr.

Effect of pH. The effect of pH on the fluorescence intensity of the metal-complexes is shown in Fig. 4. The pH values were adjusted with hydrochloric acid or ammonia water, except in the case of the magnesium complex where ammonium chloride-ammonia buffer solution and sodium hydroxide solution were used for pH control.

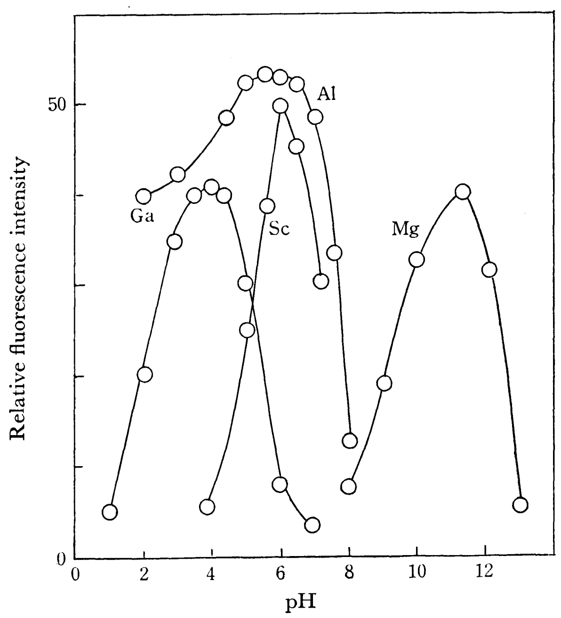


Fig. 4. Effect of pH of solution on fluorescence intensity
Al: 2 μg, 0.1% reagent solution 0.15 m*l*, 40 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)
Ga: 10 μg, 0.1% reagent solution 0.25 m*l*, 40 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)
Sc: 10 μg, 0.1% reagent solution 0.30 m*l*, 60 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)
Mg: 30 μg, 0.1% reagent solution 1.00 m*l*, 100 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)

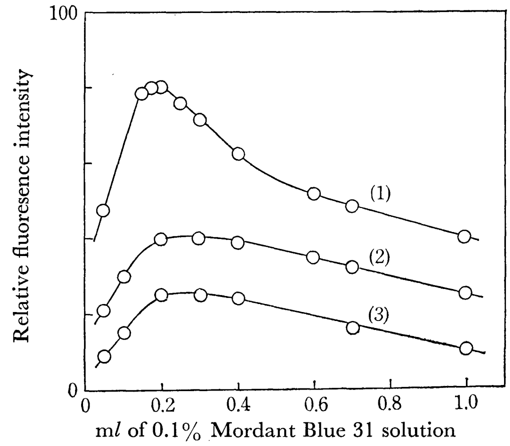


Fig. 5. Effect of reagent concentration
(1) Al 3 μg, pH 6.0, 20 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)
(2) Ga 10 μg, pH 4.0, 40 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)
(3) Sc 10 μg, pH 6.0, 30 div. (*viz.* 0.168 μg Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61)

The aluminum and scandium complexes show the maximum fluorescence intensity at pH 6. The pH-intensity curve for the aluminum complex shows maximum value at pH 5—7. The maximum fluorescence intensity of the gallium complex is obtained at pH 4, while the magnesium complex fluoresces in basic medium and gives the maximum intensity at pH 11.

Effect of Concentration of Mordant Blue 31. The effect of concentration of the reagent was examined by adding the different amounts of Mordant Blue 31 to the solution containing 3 μg of aluminum, 10 μg of gallium or 10 μg of scandium, followed by adjusting the pH to the optimum value for the metal complex.

As shown in Fig. 5, the maximum fluorescence intensity was achieved with the use of 0.15 m*l* of 0.1% Mordant Blue 31 solution for the determination of 0.1—3 μg of aluminum. For 1—12 μg of gallium, and 1—12 μg of scandium, respectively 0.25 m*l*, 0.3 m*l* of the reagent solution were required. In all cases, the fluorescence intensity decreases gradually in proportion to the amount of the reagent added. This is due to inner filter effects. Thus a definite amount of reagent was used.

Calibration Curves. Figure 6 shows the calibration curves for aluminum, gallium, and scandium obtained by the above procedures by setting up the fluorometer at 20—40 division using the standard Rhodamine B solution. A linear relationship holds between the fluorescence intensity and the metal ion concentration, showing that aluminum, gallium, and scandium could be determined in the following range: Al; 0.1—3 μg, Ga; 1—12 μg, Sc; 1—12 μg, with an average error of 3 percent.

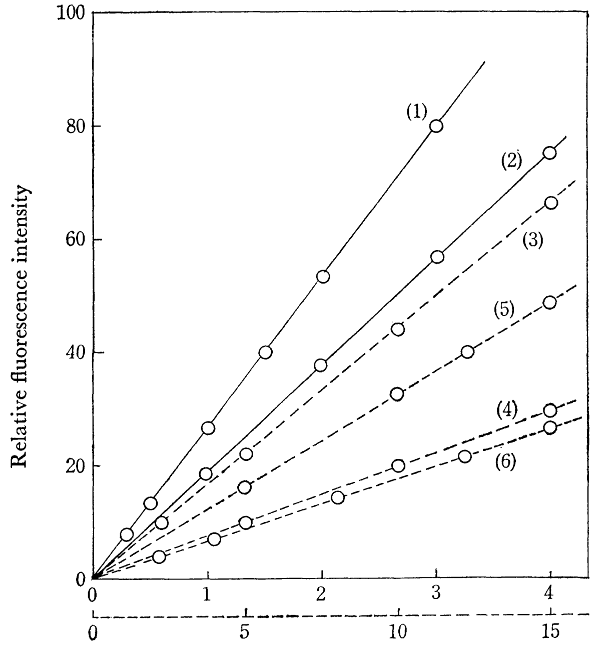


Fig. 6. Calibration curves for aluminum, gallium, and scandium
(1) Al: 20 div. (0.168 μg Rhodamine B/m*l* standard solution), 0.1% reagent solution 0.15 m*l*, FPL-2 (primary filter; 365, secondary filter; 61)
(2) Al: 20 div. (0.168 μg Rhodamine B/m*l* standard solution), 0.1% reagent solution 0.15 m*l*, G-3 (330 nm/590 nm)
(3) Ga: 40 div. (0.168 μg Rhodamine B/m*l* standard solution), 0.1% reagent solution 0.25 m*l*, FPL-2 (primary filter; 365, secondary filter; 61)
(4) Ga: 40 div. (0.168 μg Rhodamine B/m*l* standard solution), 0.1% reagent solution 0.25 m*l*, G-3 (340 nm/600 nm)
(5) Sc: 25 div. (0.168 μg Rhodamine B/m*l* standard solution), 0.1% reagent solution 0.30 m*l*, FPL-2 (primary filter; 365, secondary filter; 61)
(6) Sc: 25 div. (0.168 μg Rhodamine B/m*l* standard solution), 0.1% reagent solution 0.30 m*l*, G-3 (340 nm/610 nm)

Figure 7 shows the calibration curves for some ions forming fluorescent complexes with Mordant Blue 31. The conditions for the fluorometric determinations are summarized in Table 1.

We see that magnesium, yttrium, lanthanum, lutetium, and indium can also be determined with Mordant

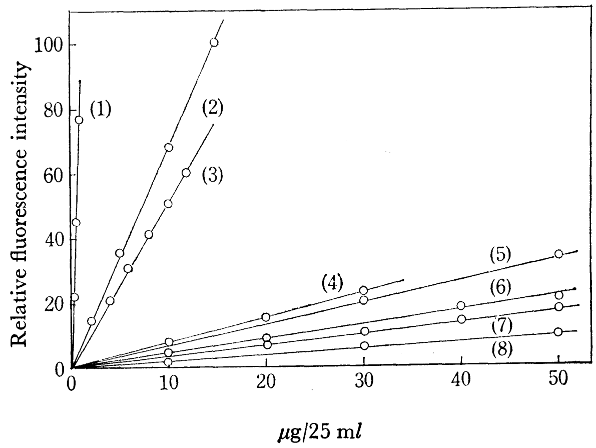


Fig. 7. Calibration curves for some fluorescent complex forming ions at 60 div. (*viz.* 0.168 Rhodamine B/m*l* standard solution, FPL-2-primary filter 365, secondary filter 61).
(1) Al, (2) Ga, (3) Sc, (4) Mg, (5) Y, (6) La, (7) Lu, (8) In

Blue 31 but with low sensitivities. Thus Mordant Blue 31 is excellent as a fluorometric reagent for aluminum, gallium, and scandium.

Effect of Diverse Ions. The effect of 26 cations on the determination of aluminum, gallium, and scandium was studied. The results are summarized in Table 2.

Determination of aluminum. Cobalt(II), iron(III), hafnium(IV), and vanadium(V) reduced the fluorescence intensity. Indium, yttrium, and scandium ions gave a positive error. The following ions did not interfere: magnesium, calcium, nickel(II), copper(II), manganese(II), zinc, cadmium, beryllium, tin(II, IV), thallium(III), arsenic(III), zirconium(IV), selenium(IV), titanium(IV), tungstate(VI), chromate(VI), and molybdate(VI) ions.

Determination of gallium. Fifty micrograms of magnesium, calcium, nickel(II), manganese(II), zinc, cadmium, cobalt(II), beryllium, indium, yttrium, thallium(III), iron(III), arsenic(III), hafnium(IV), zirconium(IV), selenium(IV), titanium(IV), and tungstate(VI) ions did not interfere. Scandium and aluminum ions gave a positive error. Copper(II), tin(II, IV), vanadate(V), chromate(VI), and molybdate(VI) ions reduced the fluorescence of gallium.

Determination of scandium. Cobalt(II), iron(III), hafnium(IV), zirconium(IV), and chromate(VI) ions

Table 1. Conditions for fluorometric determination

		Ion							
		Al	Ga	Sc	Mg	Y	La	Lu	In
Fluorescence excitation band (nm)		330 (540)	340 (580)	340 (535)	340 (500)	335 (540)	340	340 (550)	340 (500)
Fluorescence emission band (nm)		590	600	610	585	590	590	590	620
Optimum pH (pH range)		6.0 (2—8)	4.0 (1—6)	6.0 (4—8)	11.0 (8—13)	7.0 (5—9)	6.0 (5—8)	6.0 (5—8)	6.0 (4—7.5)
Used amounts of 0.1% reagent soln. (m*l*)		0.15	0.25	0.30	1.00	0.25	0.50	0.50	1.00
Standing time (min)		60	15	15	15	15	15	15	15
Fluorescence intensity at 60 div./1μg	G-3	66.0	3.02	2.7	0.63				
	FPL-2	79.5	6.78	5.05	0.77	0.66	0.37	0.36	0.19
Fluorescence intensity ratio	weight	421	35.9	26.7	4.09	3.49	1.94	1.90	1.00
	molar	114	25.2	12.1	1.00	3.12	2.70	3.35	1.15

Table 2. Effect of diverse ions

Ion	Taken (μg)	Al Found (μg)	Taken (μg)	Ga Found (μg)	Taken (μg)	Sc Found (μg)
Be^{2+}	10	2.1	50	10.0	—	—
Mg^{2+}	10	2.0	50	10.0	50	10.0
Ca^{2+}	10	2.0	50	10.0	50	10.0
Co^{2+}	10	1.0	50	10.0	50	3.3
Ni^{2+}	10	2.1	50	10.5	—	—
Mn^{2+}	10	2.1	50	10.5	50	10.7
Cu^{2+}	10	1.9	50	0.6	—	—
Zn^{2+}	10	2.1	50	10.3	—	—
Cd^{2+}	10	2.0	50	10.6	—	—
Sn^{2+}	10	1.9	50	6.6	—	—
Al^{3+}	—	—	2	13.7	2	⧻
Sc^{3+}	10	2.5	50	11.5	—	—
Fe^{3+}	10	0.5	50	9.1	50	8.1
Ga^{3+}	10	1.9	—	—	50	12.0
As^{3+}	10	2.0	50	10.0	—	—
Y^{3+}	10	2.4	50	10.0	50	10.0
In^{3+}	10	2.4	50	10.7	50	9.0
Tl^{3+}	10	2.1	50	10.4	50	10.8
Ti^{4+}	10	2.0	50	9.1	—	—
Se^{4+}	10	2.1	50	9.5	50	10.7
Zr^{4+}	10	1.8	50	10.0	50	6.2
Sn^{4+}	10	2.0	50	7.7	—	—
Hf^{4+}	10	1.3	50	10.8	50	8.0
VO_3^-	10	1.7	50	0.9	—	—
CrO_4^{2-}	10	2.1	50	8.0	50	6.5
MoO_4^{2-}	10	2.1	50	6.5	—	—
WO_4^{2-}	10	2.0	50	10.0	—	—

Al taken: 2 μg, Ga, Sc taken: 10 μg respectively.
⧻ Serious positive intereference.

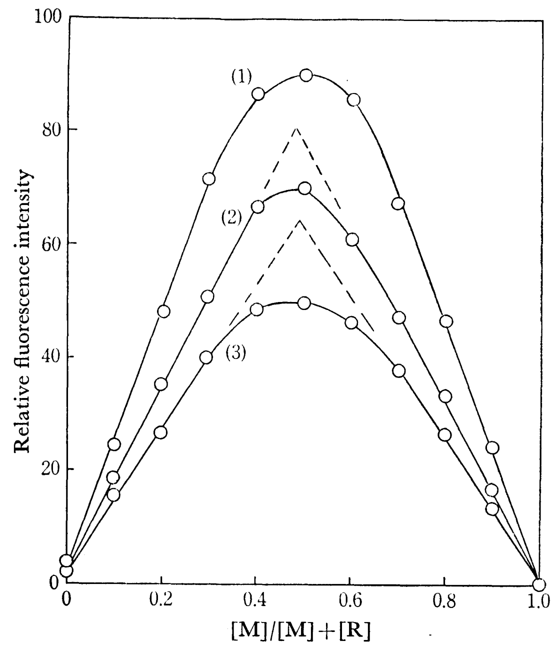


Fig. 8. Continuous variation method
(1) [Ga]=[R]=1.92×10^{-3} mol, 20 div.
(2) [Al]=[R]=5×10^{-4} mol, 10 div.
(3) [Sc]=[R]=1.92×10^{-3} mol, 30 div.

gave negative error. Gallium and aluminum ions gave a positive error.

Determination of the Metal to Ligand Ratio. The mole ratio of metal to the reagent in the metal-Mordant Blue 31 complexes was fluorometrically determined by

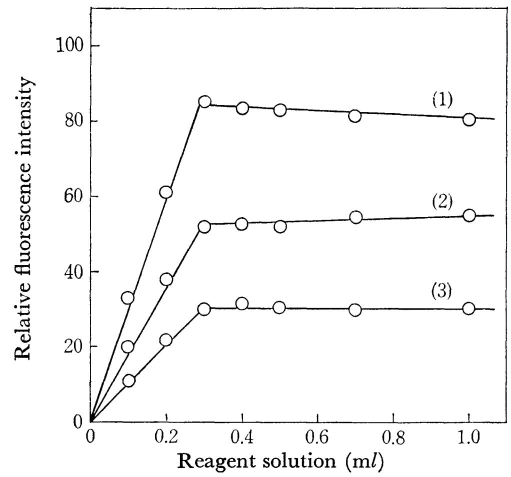


Fig. 9. Mole ratio method
(1) [Al]=[R]=5×10^{-4} mol, Al 0.3 m*l*, 20 div.
(2) [Ga]=[R]=1.92×10^{-3} mol, Ga 0.3 m*l*, 20 div.
(3) [Sc]=[R]=1.92×10^{-3} mol, Sc 0.3 m*l*, 30 div.

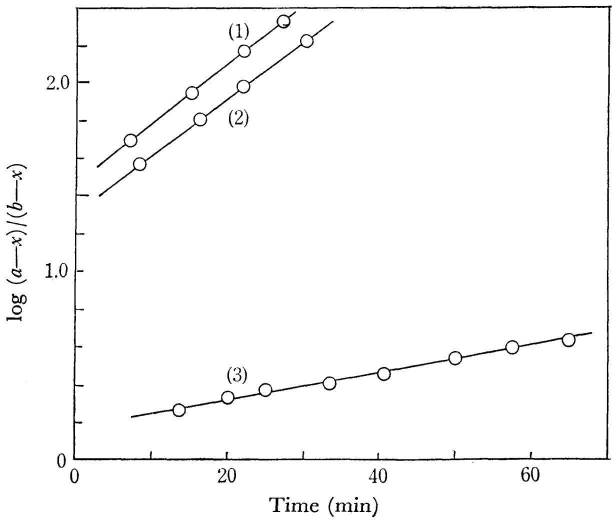


Fig. 10. Kinetics of chelate formation as indicated by fluorescence, *a* is initial concentration of aluminum, *b* is initial concentration of Mordant Blue 31, and *x* is concentration of chelate as indicated by fluorescence.
(1) Al 50 μ mol, reagent 5 μ mol (2) Al 5 μ mol, reagent 50 μ mol (3) Al 50 μ mol, reagent 50 μ mol

continuous variation and mole ratio methods. Figure 8 shows the results of the continuous variation method indicating the formation of a 1:1 complex. Mole ratio studies (Fig. 9) also gives the evidence of a 1:1 complex. The metal to ligand ratio can be kinetically determined when the complexes are formed with a measurable rate. Figure 10 shows the second order kinetics in the fluorescence development of aluminum-Mordant Blue 31 complex. Kinetic studies also indicate the formation of a 1:1 complex. The formation constants of aluminum and gallium complexes were found to be 1×10^6 at 15°C from the curves in Fig. 8.

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