2084 © 1989 The Chemical Society of Japan NOTES Bull. Chem. Soc. Jpn., 62, 2084—2086 (1989) [Vol. 62, No. 6

The Fluorometric Determination of Magnesium with 2,2′-Dihydroxy-4,4′-dimethylazobenzene

Izuo Aoki,*,†Atsushi Saito, and Kunihiro Watanabe
Faculty of Science and Technology, Science University of Tokyo, 2641, Yamazaki, Noda-shi, Chiba 278
†R. & D. Laboratory for Speciality Chemicals, Nippon Soda Co., Ltd., 12-54, Goi-minamikaigan, Ichihara-shi, Chiba 290

(Received December 24, 1988)

Synopsis. In an alkaline medium, magnesium reacts with 2,2′-dihydroxy-4,4′-dimethylazobenzene to emit a strong fluorescence, which is about 5 times as intense as that obtained with its parent compound (2,2′-dihydroxyazobenzene). Nonextraction (water-ethanol-dioxane, a mixed solvent) and extraction (an isopentyl alcohol solvent) procedures for the fluorometric determination of magnesium with this reagent were established. 0.1 μg to 4.0 μg of magnesium can be rapidly determined by the nonextraction procedure. In the extraction procedure, 1000-fold excesses of calcium did not interfere even when no masking agent was used.

Among a large number of 2,2′-dihydroxy azo compounds available for use in colorimetric and fluorometric methods for metal ions, 2,2′-dihydroxyazobenzene (DHAB), which has the most fundamental structure, is an excellent fluorometric reagent for magnesium in the presence of calcium.[1] 8-Quinolinol,[2] methyl salicylate,[3] and calcein[4] give fluorescence reactions with magnesium, but they also react with calcium to emit a strong fluorescence. Dagnall et al.[5] proposed 2,3-bis(salicylideneamino)benzofuran (SABF) as a specific reagent for magnesium without any interference from calcium. Although SABF seems to be more sensitive than DHAB, DHAB is superior to SABF with regard to the stability of its magnesium complex, for SABF gradually hydrolyzes in aqueous methanol solutions.

One of the present authors has previously reported that 2,2′-dihydroxy-4,4′-dimethylazobenzene (4,4′-DDAB) is more sensitive than the parent compound, DHAB, for the fluorometric determination of gallium.[6] In this study, it was found that 4,4′-DDAB reacted quantitatively with magnesium to emit a strong fluorescence in an alkaline medium. The detection limit for magnesium with this reagent was as good as that obtained with SABF. On the other hand, the magnesium–DHAB complex is extractable into isopentyl alcohol; however, the extraction procedure is not so useful for the determination of magnesium because the distribution coefficient of the complex between isopentyl alcohol and water is only about 9.[7] The present authors have, though, found that a remarkable enhancement of the distribution coefficient could be achieved by using 4,4′-DDAB instead of DHAB.

Experimental

Apparatus. The fluorescence spectra and the intensity were measured with a Hitachi Model 204 fluorescence spectrophotometer fitted with a 150 W Xenon lamp. A Shimadzu fluorescence spectrophotometer Model RF-540 (exciting source; 150 W Xenon lamp, photomultiplier; R924F) was used for the measurements of the fluorescence quantum efficiencies.

Reagents. Standard Solution of Magnesium. A stock solution of magnesium was prepared by dissolving 0.5012 g of magnesium (99.9%) with 30 cm^3 of 6 mol dm^{-3} hydrochloric acid and then diluting the mixture to 1 dm^3 with water. The standard solution was prepared by suitable dilution with 0.1 mol dm^{-3} hydrochloric acid from the stock solution.

Synthesis of Azo Compounds. DHAB, 4,4′-DDAB, and 2,2′-dihydroxy-5,5′-dimethylazobenzene (5,5′-DDAB) were synthesized by the conventional method[8] and were identified by means of their melting points and IR spectra. These azo compounds were used as the 1,4-dioxane solution.

Rhodamine B Reference Standard Solution. 0.1 g of rhodamine B was dissolved in ethanol to give a solution containing 10 μg cm^{-3}. This solution was then diluted with water.

All the other reagents and solvents were of an analytical grade and were used without further purification.

Determination Procedure

(A) Nonextraction Procedure (Water-Ethanol-Dioxane, a Mixed Solvent). (a) Preparation of Buffer Solution. Add 20 cm^3 of ethylenediamine (en) and 10 cm^3 of 6 mol dm^{-3} hydrochloric acid to 200 cm^3 of ethanol, and then allow the mixture to cool. Dilute the solution to 500 cm^3 with 1,4-dioxane. **(b) Determination Procedure of Magnesium.** To a sample solution containing from 0.1 μg to 4 μg of magnesium, add 15 cm^3 of the buffer solution and 1 cm^3 of a 0.01% 4,4′-DDAB solution. Dilute the solution to 25 cm^3 with water and allow the mixture to stand for 10 minutes at room temperature. Measure the fluorescence intensity at 560 nm with an excitation wavelength of 465 nm by using a rhodamine B solution as the reference standard.

(B) Extraction Procedure. To a sample solution containing from 0.04 μg to 3 μg of magnesium, add 1 cm^3 of a 0.5 mol dm^{-3} sodium hydrogencarbonate aqueous solution. Adjust the pH to 11.7 with a dilute hydrochloric acid or a dilute sodium hydroxide and then add 1 cm^3 of a 0.02% 4,4′-DDAB solution. Transfer the mixture into a 100 cm^3 separatory funnel and adjust its volume about 50 cm^3 with water. Add 10 cm^3 of isopentyl alcohol to the solution and shake it for 10 minutes. Discard the aqueous layer after the separation of the layers. Centrifuge the organic layer at 2000 rpm for 10 minutes, if necessary. Measure the fluorescence intensity at 560 nm with an excitation wavelength of 480 nm by using a rhodamine B solution as the reference standard.

Results and Discussion

Fluorescence Properties of the Magnesium-4,4′-DDAB Complex. The fluorescence properties of the magnesium-4,4′-DDAB complex in several solvents were investigated and compared with those of magnesium-DHAB and -5,5′-DDAB complexes. The fluorescence properties of these three complexes are listed in Table 1.

The molar extinction coefficients at the maximum absorption wavelength (ε) of the magnesium complexes were affected by the methyl groups in the ligands (azo compounds), while they changed only slightly in different solvents. The ε value of the 4,4′-DDAB complex was the largest among the three complexes. The fluorescence quantum efficiencies (ϕ_f) of the complexes were remarkably affected by both methyl groups and solvents. The ϕ_f values of the 4,4′-DDAB complex in the mixture of water-ethanol-dioxane (volume ratio: 3 : 2 : 3) were about 4 times and 9 times larger than that of the DHAB and 5,5′-DDAB complexes in the same solvent respectively.

The above-mentioned ε and ϕ_f values shown that the absolute sensitivities ($\varepsilon \cdot \phi_f$)[10] of the magnesium-4,4′-DDAB complex in both water-ethanol-dioxane and isopentyl alcohol were about (3—5) times larger than that of the DHAB complex in these solvents.

Structure of the Magnesium-4,4′-DDAB Complex. The mole ratio of magnesium to 4,4′-DDAB in the complex was determined fluorometrically by the continuous variation method to be 1 : 1 in both the mixture of water-ethanol-dioxane and isopentyl alcohol. These results were consistent with those obtained by the equilibrium-shift method.

Knoeck et al.[11] have proposed that the fluorescence enhancement by the addition of alcohols to aqueous magnesium-DHAB complex solutions probably comes from a direct magnesium coordination by the alcohols. The present authors have determined the mole ratio of magnesium to isopentyl alcohol in the extracted 4,4′-DDAB complex by the equilibrium-shift method to be 1 : 2. The complete composition of the magnesium-4,4′-DDAB complex in isopentyl alcohol is, therefore, Mg(4,4′-DDAB)(isopentyl alcohol)$_2$, which is consistent with the five-coordinate structure proposed for the DHAB complex.[11]

Effect of Organic Solvent. In Determination Procedure A (a nonextraction procedure), the effect of adding organic solvents to aqueous solutions on the fluorescence intensity of the magnesium-4,4′-DDAB complex was investigated. A remarkable increase in the fluorescence intensity of the complex was observed upon the addition of ethanol and 1,4-dioxane, while the intensity of the reagent blanks was not changed. The maximum fluorescence intensity of the complex, which was about 5 times as intense as that of its aqueous solution, was obtained with more than 11 cm^3 for dioxane or with 20 cm^3 for ethanol in a final volume of 25 cm^3. Other organic solvents, i.e., acetone, the mixtures of ethanol-methyl isobutyl ketone (MIBK), -ethyl acetate, or -tributyl phosphate (TBP), and the mixtures of ethanol and nonpolar organic solvents, were less effective than ethanol and dioxane. For the determination procedure of magnesium, it was convenient to add these organic solvents as a buffer solution by mixing ethylenediamine (en) and hydrochloric acid. As far as the enhancement ability of the fluorescence intensity of the magnesium complex is concerned, dioxane was superior to ethanol, but dioxane caused a turbidity when en and hydrochloric acid were mixed. A mixture of dioxane and ethanol (3:2, v/v) was, therefore, chosen as a solvent of the buffer solution. In consideration of the volume of the sample solution, it was suitable for the determination procedure to add 15 cm^3 of this solution in a final volume of 25 cm^3.

According to Determination Procedure B, the magnesium-4,4′- DDAB complex was extracted with TBP and alcohols, such as isopentyl alcohol, pentyl alcohol, and isobutyl alcohol, but not with MIBK, ethyl acetate, and other nonpolar organic solvents, such as hexane, benzene, and chloroform. Although the extracts with TBP and the alcohols were nearly equal in fluorescence intensity, isopentyl alcohol was chosen for the extraction solvent because it is less soluble in water than the other alcohols and because TBP is inferior to isopentyl alcohol in its separability from water because of its specific gravity (0.97). The extractability of the magnesium-4,4′-DDAB complex was 91.8% (isopentyl alcohol : aqueous phase=1 : 5, v/v), and the distribution coefficient of the complex was 56.3, while the corresponding values of the magnesium-DHAB complex were 66.4% and 9.9 respectively.

Table 1. Fluorescence Properties of Magnesium Complexes with DHAB, 4,4′-DDAB, and 5,5′-DDAB[a]

Ligand	Solvent[b]	$\lambda_{ex(max)}/\lambda_{em(max)}$(nm)		ε	ϕ_f[c]	$\varepsilon \cdot \phi_f$[d]
		Apparent	Corrected	dm^3 mol^{-1} cm^{-1}		dm^3 mol^{-1} cm^{-1}
DHAB	W/E/D	465/565	480/590	1.6×10^4 (480 nm)	0.013	2.1×10^2
	I	475/570	485/595	1.6×10^4 (485 nm)	0.019	3.0×10^2
4,4′-DDAB	W	465/570	480/595	2.2×10^4 (480 nm)	0.013	2.9×10^2
	W/E/D	465/560	480/590	2.2×10^4 (480 nm)	0.049	1.1×10^3
	I	480/560	485/590	2.0×10^4 (485 nm)	0.062	1.2×10^3
5,5′-DDAB	W/E/D	490/575	505/605	1.8×10^4 (505 nm)	0.0055	1.0×10^2

a) The mole ratio of magnesium to ligands is 1 : 1. b) W/E/D: a mixture of water-ethanol-dioxane (3 : 2 : 3, volume ratio); I: isopentyl alcohol; W: water. c) Reference standard solution: 1 μg cm^{-3} rhodamine B ethanol solution (ϕ_f=0.76).[9] d) Absolute sensitivity: $\varepsilon \cdot \phi_f$.

Effect of pH. A maximum fluorescence intensity was obtained at pH values from 10.3 to 11.3 in the mixture of water-ethanol-dioxane (Fig. 1). This pH value can be adequately maintained with the buffer solution described above. In the case of isopentyl alcohol extraction, the magnesium complex showed its maximum fluorescence in the pH range from 11.5 to 12.0. A 0.5 mol dm^{-3} sodium hydrogencarbonate solution was chosen as the buffer because the buffer consisting of en and hydrochloric acid caused an unfavorable separability between isopentyl alcohol and the aqueous phase.

Effect of the 4,4′-DDAB Concentration. The effect of the variation in the 4,4′-DDAB concentration on the fluorescence intensity was also investigated. The maximum intensity was obtained in the range of $(2—4)\times10^{-7}$ mol for 1 μg (4.1×10^{-8} mol) of magnesium in the mixture of water-ethanol-dioxane, where 4×10^{-7} mol corresponded to adding 1 cm^3 of a 0.01% 4,4′-DDAB solution. The addition of 1 cm^3 of a 0.02% 4,4′-DDAB solution was suitable in the isopentyl alcohol-extraction procedure.

Effects of Standing Time and of Shaking Time. The fluorescence of the magnesium complex developed fully within a few minutes after the preparation of the solution in the nonextraction procedure and after shaking in the extraction procedure. The intensity was stable for at least 2 hours.

Calibration Curves. According to Determination Procedure A, magnesium can be determined in the range of (0.1—4.0) μg. Linear relationships are observed between the fluorescence intensity and the magnesium concentration in this range. The coefficient of variation (c.v.) for 1 μg and 0.1 μg of magnesium were 1.2% and 3.2% (n=10) respectively. In Determination Procedure B, the determinable range of magnesium is (0.04—3.0) μg. The c.v. values for 1 μg and 0.04 μg of magnesium were 2.0% and 7.3% (n=10) respectively.

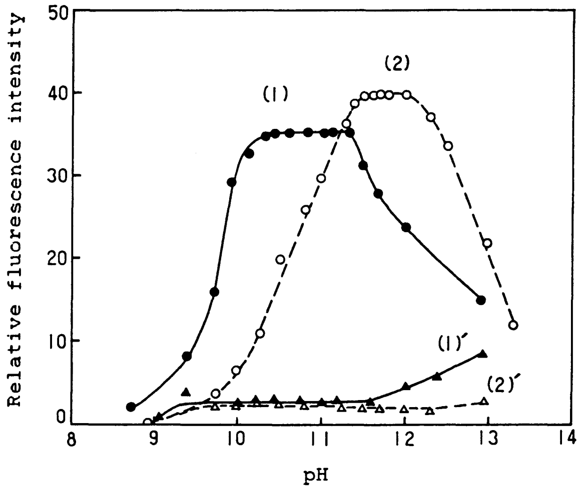

Fig. 1. Effect of pH on fluorescence intensity of magnesium-4,4′-DDAB complex.
(1) *Nonextraction procedure*; solvent: water-ethanol-dioxane (3:2:3, vol. ratio), Mg(II): 1 μg, 0.01% 4,4′-DDAB: 1 cm^3, wavelength: 465 nm/560 nm, 50 div vs. 0.5 μg cm^{-3} rhodamine B (setting 465 nm/560 nm).
(2) *Extraction procedure*; solvent: isopentyl alcohol, Mg(II): 1 μg cm^{-3}, 0.02% 4,4′-DDAB: 1 cm^3, wavelength: 480 nm/560 nm, 80 div vs. 0.5 μg cm^{-3} rhodamine B (setting 480 nm/560 nm).
(1)′ Reagent blank of (1).
(2)′ Reagent blank of (2).

Effect of Diverse Ions. The effects of diverse ions on the fluorescence intensity produced by 1 μg of magnesium were also investigated. In the nonextraction procedure, the following cations in 1000-fold excesses did not interfere: silver(I), arsenic(III), gold(III), barium(II), bismuth(III), cadmium(II), mercury(II), molybdenum(VI), nickel(II), palladium(II), antimony(III), selenium(IV), and thallium(I). Calcium(II), tin(IV), and zinc(II) caused no interference in 100-fold excesses. 1000-fold amounts of chromium(VI), indium(III), and zirconium(IV), 100-fold amounts of iron(II), lanthanum(III), and tungsten(VI), and 10-fold amounts of aluminum(III), cobalt(II), cupper(II), gallium(III), and hafnium(IV) could be tolerated by adding 3 cm^3 of 40% (2.7 mol dm^{-3}) triethanolamine as a masking agent. 1000-fold amounts of cobalt(II) and copper(II), and 500-fold amounts of iron(II) and iron(III) could be tolerated by adding 3 cm^3 of 10% (1.5 mol dm^{-3}) potassium cyanide. In the extraction procedure, 1000-fold excesses of calcium(II), 100-fold excesses of aluminum(III), and 10-fold excesses of chromium(VI), gallium(III), and titanium(IV), did not interfere even when no masking agent was used.

In conclusion, the authors recommend the nonextraction procedure (Determination Procedure A), because it is simple and rapid, and therefore yields highly reproducible results.

References

1) F. D. Snell, "Photometric and Fluorometric Methods of Analysis, Metals. Part 2," John Wiley & Sons, New York (1978), p. 1928.

2) S. Watanabe, W. Frantz, and D. Trottier, *Anal. Biochem.*, **5**, 345 (1963).

3) B. Aurivillius and P. Stenson, *Ark. Kemi.*, **23**, 551 (1965); *Chem. Abstr.*, **63**, 1680f (1965).

4) D. F. H. Wallach and T. L. Steck, *Anal. Chem.*, **35**, 1035 (1963).

5) R. M. Dagnall, R. Smith, and T. S. West, *Analyst*, **92**, 20 (1967).

6) K. Watanabe, *Bunseki Kagaku*, **32**, 698 (1983).

7) H. Diehl, R. Olsen, G. I. Spielholtz, and R. Jensen, *Anal. Chem.*, **35**, 1144 (1963).

8) D. C. Freeman, Jr., and C. E. White, *J. Org. Chem.*, **21**, 379 (1956).

9) H. V. Drushell, A. L. Sommers, and R. C. Cox, *Anal. Chem.*, **35**, 2166 (1963).

10) Y. Nishikawa and K. Hiraki, "Keikou・Rinkou Bunseki-hou (Fluorimetry and Phosphorimetry)," Kyoritsu (1984), p. 97.

11) J. Knoeck and J. A. Buchholz, *Talanta*, **18**, 895 (1971).