Drop Based Visual Fluorometry of Aluminium at ppb Level with 2,2′-Dihydroxyazobenzene by Using Octadecylsilanized Silica Thin Layer

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A new visual fluorometry for trace aluminium has been developed. The method is based on specific immobilization of its chelate with 2,2'-dihydroxyazobenzene (H_2L) on an octadecylsilanized silica thin layer. An aqueous sample drop was evaporated into a fleck on the water-repellent surface, and chromatography was performed with acetone. The fluorescent chelate, $[AlL]^+$, remained at the spotting position, whereas the other chelates were eluted to a position close to the solvent front.

Fluorometry is one of the most frequently used simple methods as well as colorimetry. However, direct application of conventional fluorometry or colorimetry to the practical samples has often been limited because of the lack of sensitivity and selectivity in spite of progressive development of chromogenic reagents. Although solvent extraction fluorometry and colorimetry have also been long used, those methods have many disadvantages from a viewpoint of practical application. This has led to the development of a number of solid phase fluorometry and colorimetry. In our previous work, ion-pair adsorption film colorimetry[1] and micro ring fluorometry[2] were developed for visual determination of aluminium at ppb level. In the latter work, it was reported that a water-repellent surface was a great aid to concentration of an aqueous sample drop.

The aim of this work is to develop a superior visual method with a high sensitivity and selectivity for aluminium ion in a micro volume sample, which is based on a specific interaction between 1:1 aluminium chelate with 2,2'-dihydroxyazobenzene (DHAB or H_2L) and an octadecylsilanized (ODS) silica thin layer. A further stimulus is an increasing attention to this element concerning the effect of acid precipitation on terrestrial and aquatic ecosystems. The reagent, DHAB, was studied as a chelating agent for trivalent metal ions of aluminium, gallium, and indium,[3] and introduced as a fluorometric reagent for magnesium.[4] The 1:1 and 1:2 aluminium-DHAB chelates were adopted for extraction-fluorometry and extraction-spectrophotometry with tributylphosphate.[5] In our previous work, it had been investigated for the determination of trace metal ions with ion-pair reversed-phase partition HPLC,[6-9] and the visual methods mentioned above.[1,2]

In the discussion below it is shown that a simple concentration process of a tiny drop (0.001 cm^3) on an water repellent surface of ODS-silica plate and specific immobilization of the 1:1 aluminium-DHAB chelate, $[AlL]^+$, enables a simple, sensitive, and selective visual fluorometry. There do not appear to be any reports of a solid phase fluorometry or colorimetry by using an ODS-silica plate as a substrate for concentration as well as for chromatographic separation. The visual detection limit for aluminium under ultraviolet light in the dark is 1×10^{-7} mol dm^{-3} (3 ppb), which is comparable to that by graphite furnace atomic absorption spectrophotometry and much higher than that by inductively coupled plasma atomic emission spectrometry. The proposed method has been successfully applied to river water.

The standard solution of aluminium was prepared by dissolving aluminium chloride in 0.01 mol dm^{-3} hydrochloric acid (ultrapure grade) and was standardized by complexometric titration with EDTA. The solution (1×10^{-4} mol dm^{-3}) of the reagent, DHAB, was prepared by dissolving it in an slightly alkaline solution (pH 10). The buffer solution used was 0.1 mol dm^{-3} 2-morpholinoethanesulfonic acid (pH 5.5). The ODS-silica plate, RP-18 HPTLC Art. 5914, was purchased from Merck and cut in 2 x 5 cm sections. All other reagents used were of guaranteed reagent grade.

The visual fluorometry was carried out in the dark with a handy ultraviolet light, SPECTRONICS Model Q-12NF. For solid phase fluorometry, a Shimadzu Model CS-9300PC flying spot scanning densitometer was used. Fluorescence and absorbance measurements were made with a HITACHI Model F-2000 fluorescence spectrophotometer and a Hitachi U-3200 spectrophotometer, respectively.

A typical procedure is as follows: To a sample solution containing aluminium ion, add 1 cm^3 of the DHAB solution, 1 cm^3 of the buffer solution, and 2 cm^3 of ethanol and dilute the mixture to 10 cm^3 with water. Then a spot loading of 0.001 cm^3 was applied to the ODS-silica plate. The tiny drop retaining its spherical shape on the hydrophobic surface of the plate was dried in an oven at 60 °C for 3 min to form a fleck. An edge of the plate with a resulting fleck was dipped into acetone in a beaker, and chromatography was performed. The solvent path length used was approximately 1.5 cm, which took about 40 s. The plate was removed from the beaker and allowed to dry at room temperature for 1 min. The fleck was viewed under ultraviolet light in the dark. The measurement of aluminium was carried out by visual comparison with a standard series of flecks previously prepared. The fluorescent flecks were kept in the dark for at least 24 h without any change.

In this study, it was found that an ODS-silica plate was a very great aid to concentration as well as to specific immobilization and chromatographic separation of foreign ions. In conventional reversed-phase thin layer chromatography (TLC), a sample solution with high water content was avoided for loading because hydrophobic surface repelled an aqueous sample. In the proposed method, when 0.001 cm^3 of 1:4 methanol-aqueous solution is loaded on the ODS-silica plate, the tiny drop retained its spherical shape and was concentrated in a fleck with 1 mm diameter after evaporation on the water repellent surface. A spectral study showed that the 1:1 chelate, $[AlL]^+$, is significantly fluorescent, whereas the 1:2 chelate,

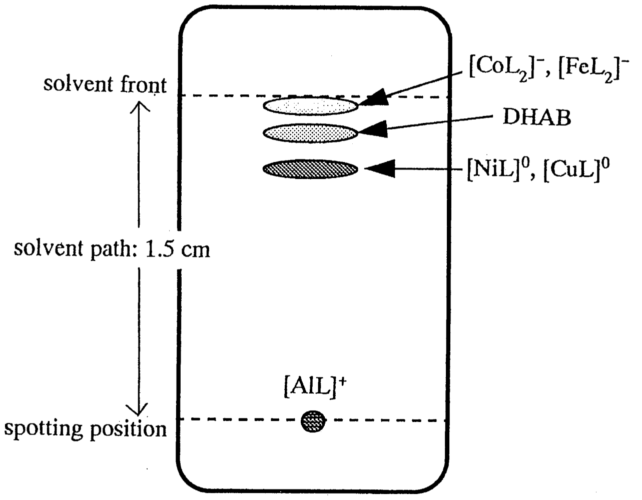

Figure 1. Specific immobilization of aluminium. Developing solvent: acetone; Plate: RP-18 HPTLC Art. 5914; Volume of sample loading: 0.001 cm^3; Drying; 60 °C, 1 min.

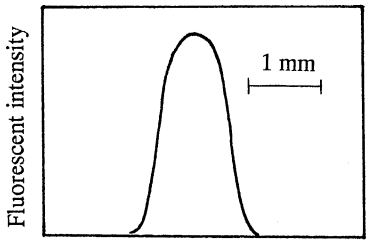

Figure 2. Typical densitogram for aluminium fleck. Excitation wavelength: 510 nm. The other conditions are the same as those in Figure 1.

$[AlL_2]^-$, and DHAB are not fluorescent.[2] When chromatography was performed with one of the organic solvents tested; acetone, chloroform, acetonitrile, dimethylsulfoxide, methanol, 1,2-dichloroethane, toluene, and cyclohexane, the aluminium chelate remained as a sharp fleck at the spotting position, whereas the excess reagent, DHAB, and the chelates of other metal ions such as iron(III), cobalt(III), copper(II), and nickel(II) were eluted to a position close to the solvent front (Figure 1). Acetone was chosen as a developing solvent over the others that were evaluated because of its resolving power. Magnesium ion, which forms a fluorescent DHAB chelate in alkaline region,[4] and zinc ion do not react with the reagent at pH 5.5. Consequently, this method is essentially free from the interferences of other common metal ions. The pattern of aluminium fleck was extremely consistent. Therefore, contrary to conventional TLC separation, strict control of running conditions, such as temperature, volume of solvent, exact path length, and prior equilibration of the plate with the solvent, is not required to achieve a high degree of reproducibility between plates in different runs.

Table 1. Determination of aluminium in river water

Sample	Al found / $\mu g\ dm^{-3}$		
	This method		Certified value
	Visual method	Densitometry	
JAC 0031	15	19.4	13.4 ± 0.7
JAC 0032	60	64.4	61 ± 2

The linearity of fluorescence intensity was assessed with the densitometric responses up to 30 ppb. A typical densitogram was shown in Figure 2. The detection limit by densitometry, 1 x 10^{-7} mol dm^{-3} (3 ppb), was the same as that by visual measurement. The sensitivity is limited by the blank contribution from the reagents used. Pre-cautions to reduce the contamination was discussed in our previous reports on HPLC of aluminium at ppb level.[8,9] The practical blank value for aluminium is less than 1 ppb in our laboratory. The values by the proposed method for the reference materials, River water JAC 0031 and JAC 0032, provided by the Japan Society for Analytical Chemistry were in fair agreement with the certified values (Table 1).

Although the mechanism of specific immobilization of the aluminium chelate still remains to be investigated, the main points underlying the high sensitivity and selectivity of the method described would be readily interpreted as a significant interaction between the 1:1 aluminium-DHAB chelate and the ODS-silica layer, the specific fluorescence, and chromatographic separation. The proposed method provides a rapid, simple and inexpensive technique for drop based determination of aluminium at ppb level, requiring no sophisticated instrumentation and high laboratory skill.

References and Notes

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