Highly Sensitive Visual Fluorometry of Aluminium at ppb Level with Ring-like Solid Phase of Poly(vinyl alcohol)

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By constructing a self-ordered nearly perfect ring system, a new type of visual test method has been developed. Poly(vinyl alcohol) forms a sharp ring–like solid phase (10.6 mm diameter) when a 100-μl drop of its aqueous solution was evaporated on a poly(vinyl chloride) plate. Aluminium ion of 1 – 30 ppb has been determined visually as a fluorescent 1:1 chelate of 2,2'-dihydroxyazobenzene concentrated into the ring.

Simple, rapid, and cost-effective methods for determination of trace elements are urgent needs in various fields. Spot test[1] with filter paper is one of the promising approaches to fulfil these demands, in which colored spots can be seen in the form of flecks or rings. However, the classical methods often lack sensitivity because sample solutions diffuse into the body of filter paper. We have developed a visual test method based on concentration of analyte (aluminium chelate) into a ring-like solid phase formed automatically on a hydrophobic substrate. An expression of the analytical results by the use of a sharp ring geometry greatly assists visual detection for human eyes. Self-ordered ring formation has received keen interests in the field of analytical chemistry[2,3] and printing technology.[4] Weisz had proposed ring oven technique.[2] The ring oven has a cylindrical heating block with a vertical hole at its center. A test solution and an appropriate solvent are dropped onto the filter paper placed on the oven. The analyte is migrated concentrically toward the heated edge, where the solvent evaporates and the analyte is concentrated in the ring. We had reported a spot test with a hydrophobic filter paper.[3] A drop of solution containing an analyte, chromogenic reagent, and pH buffer components such as piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was dried in an ordinary oven. The buffer components deposited along a drop-substrate-air contact line to form an asymmetric ring-like solid phase, where the analyte was concentrated. These results suggest that self-ordered ring formation system should be constructed by a suitable combination of ring-forming substances and substrates. However, surface roughness of the solid ring and the filter paper had eventually limited its ring shape reproducibility.

First of all, survey for ring-forming substances that produce a flat surface has been conducted. Poly(vinyl alcohol) (PVA) is the most effective one among several kinds of water soluble polymers, which gave a highly transparent and flat ring-like solid adhesive to the hydrophobic substrate. A highly reproducible clear-cut ring was obtained. Its shape was a nearly perfect circle. A ppb level of aluminium ion was distinctly visualized in the ring as a fluorescent 1:1 chelate of 2,2'-dihydroxyazobenzene (DHAB).

The recommended procedure is as follows: to 20 μl of 2-morpholinoethanesulfonic acid (MES) buffer on a white poly(vinyl chloride) plate (PVC, plasticizer: 2–3% dioctyl phthalate), 30 μl of DHAB (Dojin Laboratories, Japan) aqueous solution containing 0.833% PVA (n = 500; degree of saponification, 98.3–98.7%; Wako Pure Chemical Industries, Japan) and 50 μl of test solution were added. The drop was evaporated to dryness at 70 °C on a heating plate (Iuchiseieido TP-80, Japan). The fluorescent ring was measured by visual comparison with the standard series under irradiation of UV light in the dark. A handy UV lamp Spectroline Q-12NF/J (Spectronics Corporation, USA) of wavelength 365 nm with 3-power magnifier lens was used. A densitometer Shimadzu CS-9300PC equipped with a xenon lamp was also used for the digital evaluation (excitation 505 nm, beam size 0.4 × 0.4 mm).

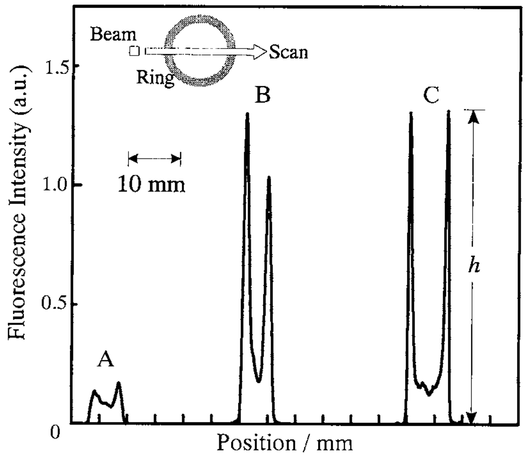

Figure 1. Fluorometric densitograms of rings. $[Al]_T$, 1×10^{-6} M; $[DHAB]_T$, 3×10^{-6} M; volume of drop, 100 μl. A, pH 5.0 (acetate buffer); B, pH 6.5 (PIPES buffer); C, pH 5.0 (acetate buffer) with PVA; substrate, ADVANTEC 2S filter paper (Advantec Toyo, Japan). 1 M = 1 mol dm^{-3}.

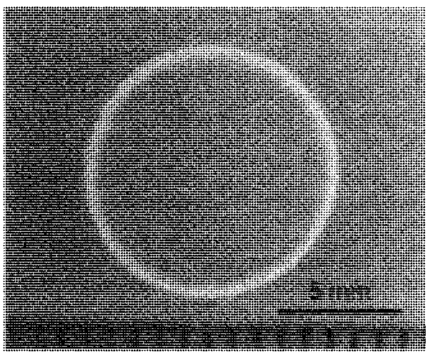

Figure 2. Fluorescence photograph of a PVA ring containing a fluorescent aluminium chelate. Constitution of drop: $[Al]_T$, 1×10^{-6} M; $[DHAB]_T$, 3×10^{-6} M; [MES], 5×10^{-3} M; pH 5.5; PVA, 0.25%; volume of drop, 100 μl. Substrate, PVC plate. Evaporation, 70 °C. The handy UV lamp was used for excitation.

The fluorometric densitograms obtained by light scanning across the ring is shown in Figure 1. The curve A in Figure 1 was observed without any ring-forming substances. With PIPES buffer an asymmetric ring was obtained as shown in curve B. By the use of PVA, however, the two sharp symmetric peaks are observed as shown in curve C and the fluorescent aluminium-DHAB chelate was effectively concentrated into the circular ring (Figure 2).

The ring formation process of PVA on a PVC plate was followed by fluorescence microscopic observations. Results are shown in Figure 3. As the drop was evaporated, the contact angle of the drop decreased and its height decrease (Figure 3, A ~ C). But the radius of the drop did not shrink, because its contact line was pinned at the original point. Thus PVA deposited along the contact line. Concentration of the analyte into the ring was almost simultaneous with the ring formation by partition between aqueous solution and solid PVA phase. The aluminium-DHAB chelate was the best fitted compounds to this system among several kinds of azo-dyes, triphenylmethane dyes, and their metal chelates. Details of such survay for substances will be reported elsewhere. After almost all the PVA had deposited, the drop left from the ring and shrunk in the shape of the liquid film (Figure 3, D and E). Finally, highly water-soluble species are deposited at the center.

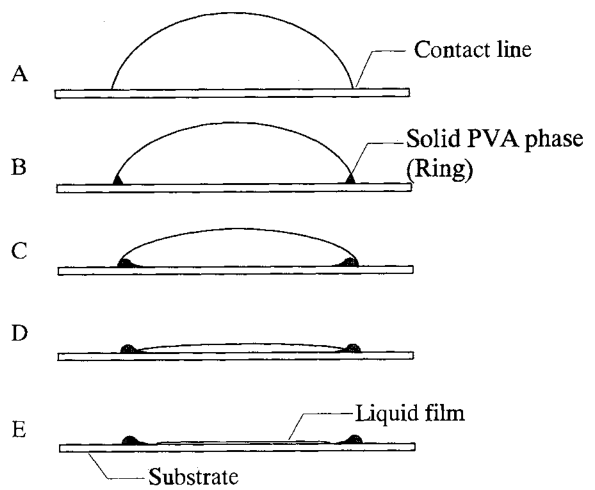

Figure 3. Scheme of a ring formation on a hydrophobic substrate during evaporation.

Both of the wettability and the water-repellent nature of the surface of the substrate were significant for the formation of the ring. The water-repellent nature was essential for the drop on the substrate to maintain the spherical cap shape as shown in Figure 3, A. The wettability was also important for the contact line of the drop to be pined on the substrate through the evaporation process. The water-repellent nature of various substrates was evaluated with the critical inclination angle θ_c ; at that angle a 100-μl drop of water on the tilted substrate rolls down. The water-repellent nature decreased with increase in θ_c. The value of θ_c for films of Parafilm (America National Cans) and Sealonfilm (Fuji Photo Film Co., Ltd., Japan) were 2.0, 3.8, and those for plates of poly(tetrafluoroethylene), poly(methyl methacrylate), poly-styrene, PVC, and polypropylene were 6.2, 6.9, 7.4, 9.4, and 10.1 degree, respectively. By the use of substrates having the critical angle less than 6.2 degree, radiuses of the drop shrunk during the evaporation and a portion of the analyte remained inside of the circular perimeter. The efficiency of the concentration of analyte into the ring increased with an increase in θ_c. The boundary of the ring was the most sharp on PVC plate among all substrates examined. Consequently, the white PVC plate was a suitable substrate for the proposed procedure.

The geometrical profile of the PVA-ring was investigated. The diameter of the ring obtained from a 100-μl drop of sample solution was 10.6 mm and its coefficient of variation (C.V.) was 2.2% (n = 8). The annulus width of the ring was 0.5 mm. The C.V. of the peak height, *h* (see Figure 1 curve C), on the densitogram was 5.1% (n = 8) for 1×10^{-6} M of aluminium. These results confirm a good reproducibility of the ring formation.

The visual detection limit of aluminium distinguished from a reagent blank ring was 5×10^{-8} M. The detectable intervals were 5×10^{-8} M for the concentration range (5–20) $\times 10^{-8}$ M, 7×10^{-8} M for the range (20–60) $\times 10^{-8}$ M, and 20×10^{-8} M for the range (60–100) $\times 10^{-8}$ M. Aluminium ion was quantitatively determined with the densitometer; peak height, *h* was proportional to aluminium concentration in the range of 2×10^{-8} M to 1×10^{-6} M. The fluorescence intensities of rings remained constant for at least one day at room temperature.

This method was applied to the visual determination of aluminium in six kinds of tealeaves (aluminium content, 0.2–2 mg/g). The results were in good agreement with those obtained by densitometry and graphite furnace atomic absorption spectrometry.

The solid ring of PVA on the PVC plate provides a favorable medium for concentration of various kinds of analyte. In the previous work,[2] compounds such as crystal violet, fluorescein, and brilliant green were not concentrated into the ring but those were strongly adsorbed on the hydrophobic filter paper used as a substrate. Even by the use of PVA in the previous system, these dyes have not been concentrated in the ring, however, the proposed method has been successfully used to them.

This technique will be useful for simple determination of trace substances in tiny samples without any special instruments. Expression of analytical results by self-ordered ring provides a powerful means for the detection of ultra-trace substances by human eye perception.

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References and Notes

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