Detection of Hydrophobic Stain on Cotton Surface by Fluorescent Probe*

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The minute amount of hydrophobic substances $10^{-8}\sim10^{-7}$ mol/cm^2 (geometric area) on cotton fabric surface can effectively be detected by fluorescent probe using magnesium-8-anilino-1-naphthalenesulfonate (ANS). The cotton fabrics were dyed in aqueous ANS solution (1×10^{-4} mol/l), before and after the staining with stearic acid, palmitic acid, tristearin, oleic acid, and squalene, and the intensities of fluorescence from the cotton surface was measured by reflectance spectrophotometer under the varying wavelength of ultra-violet (200—300 mμ). The intensity of fluorescence increased with the increase of fatty stain on the surface, but showed the tendency of saturation by exceeding the closest packing of the fatty acid monolayers. Correlation was also found between the fluorescent intensity and the yellowness (*b*-value) of naturally soiled cotton fabrics. The bleaching of the yellowed fabrics by sodium hypochlorite effectively reduced the *b*-value but did not change the fluorescence appreciably, and suggested that the hydrophobic substances still remained on the fabric surfaces in spite of their white appearance.

The surface reflectance of the whiteness of the cloths might be the most practical and fundamental standard of the cleanliness of the fabrics which agrees most with the human sense. Thus, the surface reflectometry is now the widely accepted method to detect the soils on fabric in the study of detergency. The reflectometry is versatile for its easy measurement in the practical systems, but its application is limited to the case where the amount of the soil adhering to the fabric is enough to change the reflectance of incident light by absorption. Generally speaking, the monomolecular layer of fatty acid adsorbed on soiled surface is too little in amount to change the intensity of light by a single reflection at solid/air interface.

A number of studies have been reported to estimate the trace amount of fatty substances on fabric surfaces, by extracting them with solvent followed by equilibrium lens[1] or gravimetric method[2,3] of analyses, as well as gas, liquid, thin layer or gel-filtration chromatography followed by spectroscopic analyses.[4–6] The radiotracer technique is also useful especially in detecting minute amounts of multi-component soils, without extraction procedure, by dual-lebelling and measuring the residual soil with a liquid scintillation counter,[7–11] or useful in the kinetic studies of detergency using an end-windowed dip-counter.[12] All these methods are excellent and have offered much useful information on the molecular mechanism of detergency, however, they always accompany some limitations in their application. For chromatographic analysis, all the soils should have such nature that is extractable by the certain solvent, which is not necessarily the case. And the latter radiotracer method, which utilizes artificial soils labelled with radioisotope, is only significant in the model system. Any physicochemical method which detects minute stains and gives a strict measure of cleanliness or dirtiness of the fabrics without an extraction procedure, should be helpful at present in the study of detergency as a measure of cleansing efficiency.

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Some derivatives of naphthalenesulfonate are practically non-fluorescent when dissolved in water but fluorescent strongly when dissolved in organic solvent,[13] or one bound to the hydrophobic part of molecules.[14] These fluorescent dyes are utilized by a number of investigations in the determination of hyper-structure of proteins[15–17] or detergent micelles,[18] and in the analysis of various biological phenomena,[19] and are called as "fluorescent probe", or "hydrophobic probe."

The present paper deals with some experimental trials, using magnesium-8-anilino-1-naphthalenesulfonate as an adsorption indicator,[20] to detect minute fatty stain on cotton fabric stains, which are too small in amount to be detected by ordinary reflectance spectroscopy, or to be extracted for successive physicochemical analyses.

The results suggest that the fluorescent probe might offer a new measure of cleanliness of fabrics useful for the study of detergency.

Experimental

Materials. *Probe*: The fluorescent probe grade magnesium-8-anilino-1-naphthalenesulfonate (ANS) obtained from Eastman-Kodak Co., N. Y. was dissolved in distilled water to make a stock solution of 1×10^{-3} mol/l. The stock solution was kept in a dark and cool place until needed. The concentration of the dyeing solution about 1×10^{-4} mol/l was found optimal from the preliminary experiment to visualize the stain by naked eye under the ultraviolet light.

Hydrophobic Stains: Stearic acid, palmitic acid, oleic acid, tristearin, and squalene, all analytical grade (Fischer Scientific Co., N. J.) were dissolved in benzene to make the staining solution. The concentration ranged between $2\times10^{-4}\sim1\times10^{-2}$ mol/l.

Cotton: The cotton cloth for the specimen was obtained from the Test Fabric Inc., N. Y. in the form of plain-woven fabric without an optical brightner (20×20 threads/cm, 17.7 mg/cm^2 at the standard condition of the present experiment). In order to eliminate any residual greasy substance originally contaminating the fabrics, the specimens were purified by the following procedure.[11] The cotton cloth, cut into 45×45 cm, was washed in a Terg-O-Tometer in 0.25% Calgon solution for 10 min at 50 °C, rinsed twice for 5 min in distilled water, and air dried. The samples were further cut into a smaller 6×6 cm size and extracted with ethyl ether for

24 hr by Soxhlet extracting apparatus, and stored in a greasless glass container after being dried in clean air.

Procedure. After the prolonged exposure of cotton sample to a standard condition (20±2 °C, 65±2% R.H.) to equilibrate it, 0.2 ml of the staining benzene solution was applied to the center of cotton cloth from a micro-syringe. The cloth stained was conditioned again after the evaporation of benzene, then dyed in 100 ml of ANS dyeing solution for 20 min, rinsed in 250 ml of 1/100 M NaCl aqueous solution, and damp dried between absorbing filter papers. The aqueous NaCl solution helped to wash off any extra ANS in the cloth without losing a large portion of the ANS bound to the hydrophobic stain.

The fluorescence from the cloth dyed with ANS was measured by a Bausch & Lomb 505 spectrophotometer equipped with reflectance attachment under varying wavelength of incident ultraviolet light (280~300 mμ). The intensity of fluorescence of the test specimen is expressed in percent to that of control cloth, which is the sample with a known amount of fatty stain dyed with ANS. All these procedures were carried out at room temperature (24—28 °C), and precautions were taken to avoid unnecessary exposure of the specimen to the light which might accelerate the decomposition of ANS.

Results and Discussion

Figures 1a and b demonstrate the hydrophobic stain of stearic acid on cotton fabrics visualized by fluorescent probe. Under an ultra-violet light the hydrophobic stain shows as a white circular area (Fig. 1a), which can not be observed under the ordinary white light from an incandescent source (Fig. 1b). The cotton cloth in Fig. 1 was stained with stearic acid by dropping an aliquot of benzene solution of stearic acid to the center of the cloth to make a circular stain, and dyed with ANS by the procedures mentioned above. The pictures were taken, one (Fig. 1a) under the ultra-violet light with a yellow filter on the lens, and the other (Fig. 1b) under the ordinary white light without a filter using panchromatic B & W film (Tri-X, Kodak). These experiments show the fluorescent probe being as effective as to detect minute 8.61×10^{-8} mol/cm² amount of stearic acid, by leaving it on the fabric surface without applying any extraction processes.

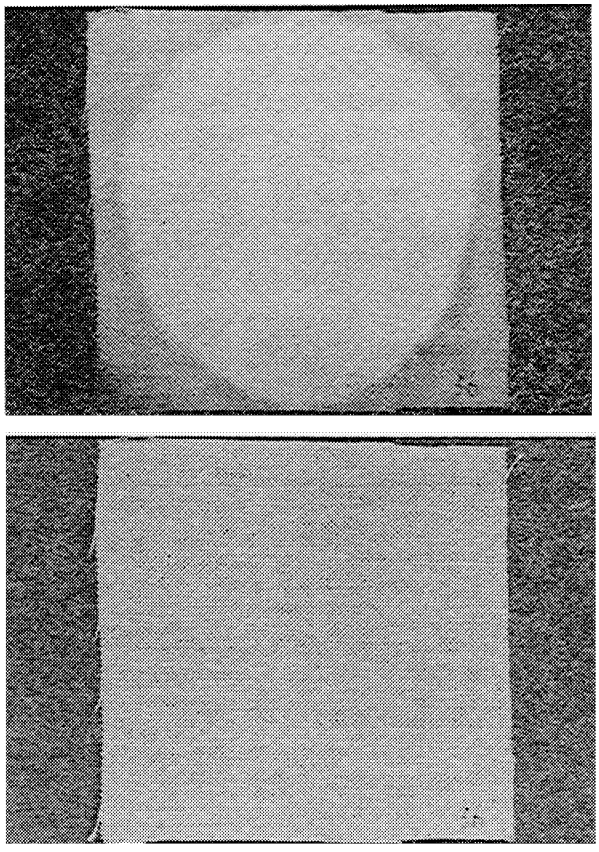

Fig. 1. Stearic acid stain on cotton fabrics visualized by fluorescent probe.
(a): Picture taken under the ultra-violet light using yellow filter.
(b): Picture of the same sample (a) taken under the white light.

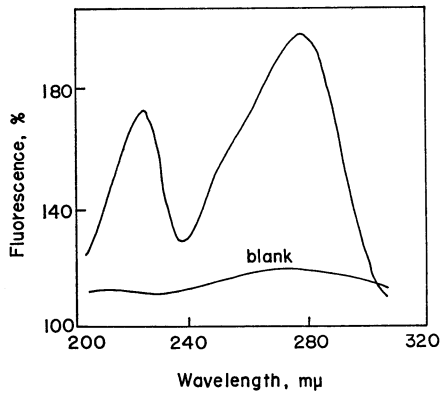

Fig. 2. Fluorescence of cloths with and without stearic acid stain against the wavelength of incident light.

Figure 2 shows the typical fluorescence intensity *vs.* wavelength of incident light curves of cotton dyed by ANS with and without the stearic acid stain. The amount of stearic acid put on cloth is 8.61×10^{-8} mol/cm². The intensity of fluorescence increased with stearic acid stain, and the two characteristic maxima were observed at 225 and 275 mμ of ultra-violet irradiation compared with the blank cotton surface which showed very weak fluorescence. Any appreciable differences in the fluorescence spectra have never been observed also for the other fatty acids as well as tristearin and squalene. The intensity of fluorescence increased with the amount of fatty stains, and the two characteristic maxima appeared around 225 and 275 mμ respectively.

The fluorescence intensities at these maxima were plotted against the amount of fatty stain on the cloth surface, and are shown in Figs. 3~6. The increase of fluorescence in these figures indicates the net increase of intensity from the blank cotton surface which is the clean specimen dyed with ANS without the oily stains. The saturation of fluorescence is observed for stains applied in excess of certain levels which seemed to be specific to the kind of stain. The amount of stain in these figures is expressed in moles per apparent (geometric) unit area of the fabrics (mol/cm²). The true surface area of the cloth, however, should be far larger than the geometric one,

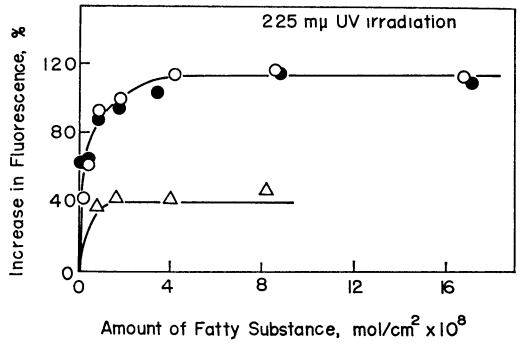

Fig. 3. Relation between fluorescence and amount of fatty substances on cotton fabrics.
○: Palmitic acid, ●: Stearic acid, △: Tristearin.

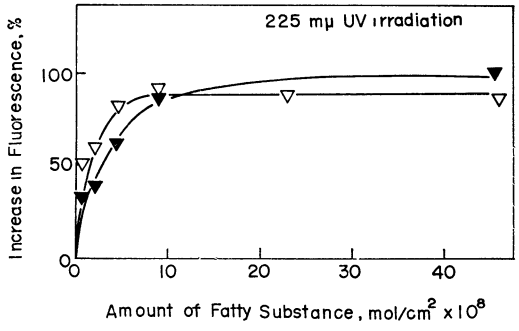

Fig. 4. Relation between fluorescence and amount of fatty substances on cotton fabrics.
▽: Oleic acid, ▼: Squalene

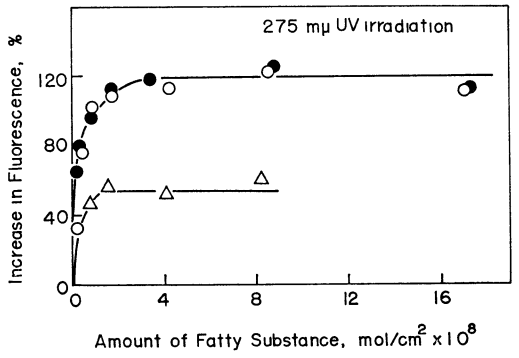

Fig. 5. Relation between fluorescence and amount of fatty substances on cotton fabrics.
○: Palmitic acid, ●: Stearic acid, △: Tristearin.

if we take into account the true surface area of each cotton fibers which compose the fabrics. The specific surface area of cotton fibers has been determined by several workers to be around $2.37\sim2.75\times10^3$ cm²/g.[21,22] Using these values, we can estimate the actual amount of the stains on cotton fiber in moles per true unit area. The closest monolayer coverage of cotton fibers in the cloth by fatty acid molecules should be attained at $3.00\sim3.98\times10^{-8}$ mol/cm² (geometric), assuming the molecular area of fatty acids to be 20.5 Å²/molecule.[23] The saturation of fluorescence in Figs. 3 and 5 occurred for the stain of both stearic and palmitic acids around at 4×10^{-8} mol/cm² which agreed with the monolayer value given above. The similar agreement between

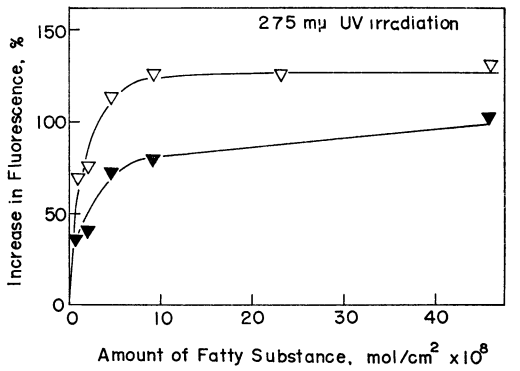

Fig. 6. Relation between fluorescence and amount of fatty substances on cotton fabrics.
▽: Oleic acid, ▼: Squalene

observed and calculated monolayer values is also observed for tristearin which has a molecular area[12] nearly three times larger than that of fatty acid. The saturation of fluorescence is observed for tristearin at smaller amount of stains (about 1/3 of fatty acid) as expected.

The fluorescent probe was also applied to naturally soiled cotton cloths (pillow cases) of different degree of yellowness, which were obtained by the repeated soiling and washing procedures. In Fig. 7, the intensity of fluorescence at 275 mμ ultra-violet irradiation from yellowed fabrics are plotted against the degree of yellowness (b-value) of the fabrics. Here, the b-values are estimated by using a Hunter D25 Color and Color-Difference Meter with the ultra-violet filter in place to eliminate any fluorescent effects of the cloths.[11] Stronger fluorescence was observed for more yellowed fabrics. The intensity of fluorescence

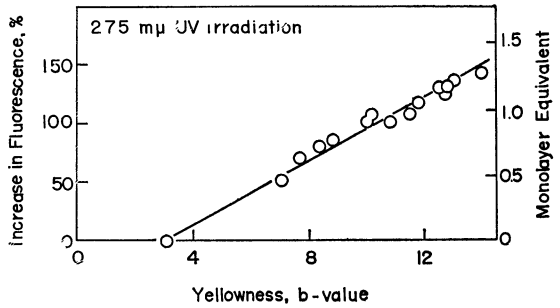

Fig. 7. Relation between b-value and fluorescence.

Table 1. Effects of bleaching on both b-value and fluorescence at 275 mμ ultra-violet irradiation

b-Value		Fluorescence	
Before bleaching	After bleaching	Before bleaching	After bleaching
9.47	3.54	85%	74%
10.44	3.74	100	80
11.84	3.87	120	100
12.89	4.01	125	81
12.99	3.65	130	110
13.58	4.47	140	112

is also expressed conveniently in monolayer equivalent of fatty acid as seen in the right hand ordinate.

The bleaching of yellowed fabrics by 0.5% aqueous sodium hypochlorite effectively reduced the *b*-value, but fluorescent probe still showed for these bleached white fabrics a fluorescence nearly as strong as that of unbleached cloths, as shown in Table 1. This might mean that any hydrophobic substances are still remaining on the fabrics in spite of their whiteness. It may be generally recognized that the yellowing of the cotton fabrics during the recycling process of soiling and washing in everyday life, is probably caused by the oxidation or polymerization of fatty substances which have not been removed by laundering.[3,24] The residual fatty substances after washing might be a so small amount as that of monomolecular layer, and might not be detected until accumulated and yellowed by usual reflectance measurements hitherto been utilized.

The fluorescent probe should offer an useful measure of cleanliness in the study of detergency not only for its simple and inexpensive background.

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