

# Spectrophotometric Determination of the Critical Micelle Concentration of Some Alkyldimethylbenzylammonium Chlorides Using Fluorescein

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SOME DYES are known to alter their color when in solution with cationic or anionic surfactants (1, 2). Using this knowledge, Corrin and Harkins (3) introduced a visual spectral dye method for the determination of the critical micelle concentration (CMC). Nicols and Kindt (4) then utilized spectrophotometric techniques instead of visual observations to determine the CMC. Since the introduction of the method, numerous papers have been published using various dyes to determine the CMC (5, 6).

Although many investigators favor the spectral dye method, Colichman (7) using spectrophotometric techniques with brom phenol blue reported the CMC of quaternary ammonium salts to vary directly with a change in dye concentration. A re-evaluation of the spectral dye method was done with pinacyanol to determine the CMC of sodium laurylsulfate by Mukerjee and Mysels (8). They concluded that this method gives only a rough approximation of the CMC. Using the same surfactant and pinacyanol chloride, Goddard and Jones (9) found that the CMC was lowered in the presence of the dye. Malik and Verma (10) using the spectrophotometric method determined the CMC of cationic surfactants with a very sensitive dye, alizarin red S, and observed a range of values for the CMC which were comparable to reported literature values.

The accuracy of the spectral method is obviously debatable, but CMC values defined as an abrupt change in surface tension measurements have long been accepted (5, 6, 11). Both the surface tension method and a spectrophotometric method utilizing the absorbance of a soluble dye-micelle complex have been herein used to determine the accuracy of fluorescein as a dye for determining the CMC of alkyldimethylbenzylammonium chlorides. The effects of surfactants on the fluorescence properties of fluorescein have been observed earlier (3). Evidence has been found supporting the use of the spectral dye method. However, one limitation was observed.

## EXPERIMENTAL

**Reagents.** Those alkyldimethylbenzylammonium chlorides studied were lauryl, myristyl, cetyl, and stearyl. The lauryl derivative was obtained from I. C. I. Organics, Inc., the cetyl derivative was obtained from Pfaltz and Bauer, and the myristyl and stearyl derivatives were obtained from Fine Organics, Inc. These compounds were recrystallized from methylethylketone, dried, and the analysis confirmed with a Hewlett-Packard Model 185 Carbon, Hydrogen, and Nitrogen Analyzer. Fluorescein disodium salt (white label quality) was obtained from Eastman Organic Chemicals and used as received. All water used was distilled and deionized.

**Apparatus.** The absorbance measurements were made on a Cary Model 14 Recording Spectrophotometer at 503  $m\mu$  using a 1.00-cm flow cell. Flow was maintained at 13 cc/min by a Buchler Polystaltic pump and titrations were performed with a Class A Kimax buret. Surface tension measurements were obtained by a Fisher Model 20 DuNouy Surface Tensiometer using a 6.00-cm platinum-iridium ring. The pH measurements were determined with a Corning Model 7 pH meter using a combination electrode.

**Procedure.** To obtain the CMC spectrophotometrically, stock dye solutions of  $5.00 \times 10^{-6}M$  and  $1.00 \times 10^{-3}M$  fluorescein disodium salt were prepared. Solutions of the surfactants were then prepared using each of the dye solutions as the solvent. Using the method of Corrin and Harkins (3) the respective stock dye solution was then titrated into 25 ml of the surfactant dye solution and the absorbance measured at 503  $m\mu$  at various dilutions of the surfactant. This allowed the determination of the absorbance of a constant concentration of dye as a function of the surfactant concentration. The titration was performed in a nitrogen atmosphere to minimize  $CO_2$  absorption which changes the pH and therefore the absorbance (the  $pK_a$  of fluorescein is ca. 8). The solution was pumped from the titration vessel through capillary tubing to the sample compartment of the spectrophotometer. The pH was monitored in the titration vessel.

To obtain the CMC by surface tension measurements, the surfactant solution was prepared in water alone. The solution was then titrated with water and the surface tension determined as a function of surfactant concentration.

## RESULTS AND DISCUSSION

In the spectral dye method when the dye-micelle complex is formed, the spectral characteristics of the dye are changed. This may result visually in a pronounced change in the color or fluorescence of the solution. In the case of fluorescein above the CMC, the solution exhibits a green fluorescence and a very slight pink coloration. However, below the CMC the fluorescence is quenched and the solution is a light greenish-yellow.

The absorption spectra in the visible region of the dye and the dye-micelle complex were determined. When the complex is formed the overall spectrum shifts bathochromically

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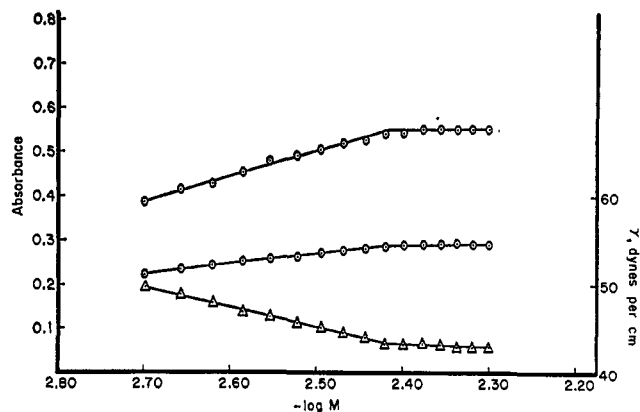


Figure 1. Critical micelle concentration plot for lauryldimethylbenzylammonium chloride

○ represents absorbance values for  $5.00 \times 10^{-6}M$  and  $1.00 \times 10^{-5}M$  dye, △ represents uncorrected surface tension values

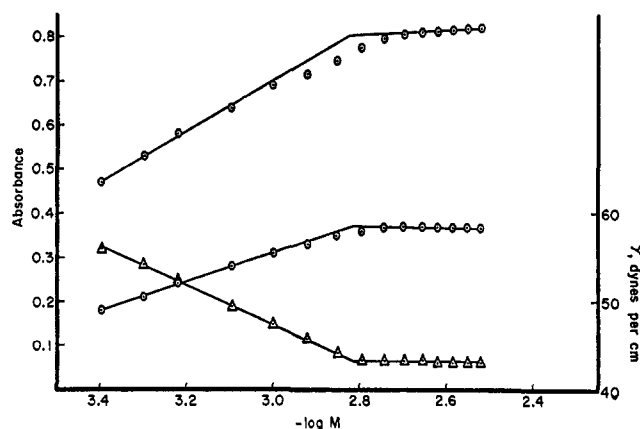


Figure 2. Critical micelle concentration plot for myristyldimethylbenzylammonium chloride

○ represents absorbance values for  $5.00 \times 10^{-6}M$  and  $1.00 \times 10^{-5}M$  dye, △ represents uncorrected surface tension values

and a new maximum occurs at  $503 m\mu$  which also represents a maximum difference in absorbance between the dye and the dye-micelle complex. Therefore, this wavelength was chosen for the spectral dye method of determining the CMC.

The results of determining the absorbance at this wavelength as a function of surfactant concentration at constant dye concentration are shown in Figures 1, 2, 3, and 4. Also shown in the figures are the results of the surface tension measurements for direct comparison. The normal plot of absorbance or surface tension against concentration does not give a sharp break at the CMC and therefore determining a value is difficult. The data plotted here is in terms of the negative logarithm of the concentration. This has been done

Table I. Critical Micelle Concentrations of Alkyldimethylbenzylammonium Chlorides

Method	CMC, $M \times 10^3$			
	Lauryl	Myristyl	Cetyl	Stearyl
Surface tension	3.8	1.5	0.34	0.093
Dye, $5.00 \times 10^{-6}M$	3.8	1.5	0.35	...
Dye, $1.00 \times 10^{-5}M$	3.8	1.5	0.34	...

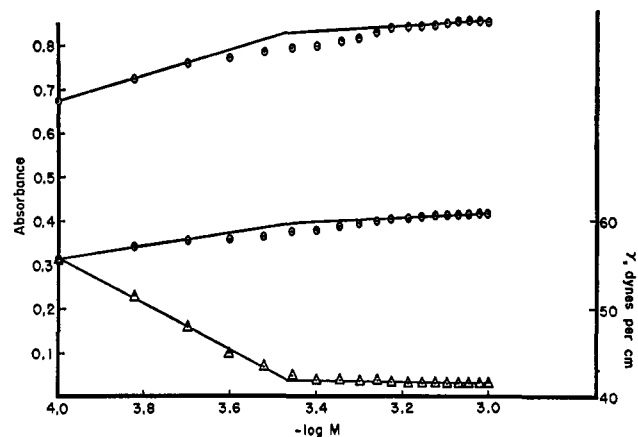


Figure 3. Critical micelle concentration plot for cetyldimethylbenzylammonium chloride

○ represents absorbance values for  $5.00 \times 10^{-6}M$  and  $1.00 \times 10^{-5}M$ , △ represents uncorrected surface tension values

before for the surface tension (12). This gives intersecting straight lines from which one can determine a value. The CMC's from the plots are tabulated in Table I. It can be seen how the experimental points differ from linearity as the carbon chain length increases. The accuracy of the method therefore decreases accordingly. Values for the CMC for the stearyl derivative were unobtainable using the spectral dye method. Because the surface tension gave consistently good results, this problem with the spectral dye method is attributed to a decreasing of the stability of the dye-micelle complex with increasing chain length. This is probable for the micelle fraction of charge decreases with increasing chain length for the trimethylbromide analogs (12). The agreement between the three determinations is good. The concentration of the dye has not noticeably affected the CMC. Undoubtedly, the dye does affect micelle formation; but, at  $10^{-5}M$  concentration and considering only two significant figures in the results, the effect is not observable.

Because the absorbance of the fluorescein is dependent on the pH and buffers were not desirable for this determination,

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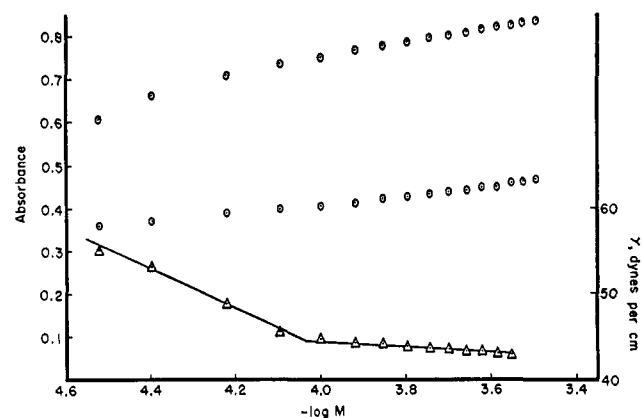


Figure 4. Critical micelle concentration plot for stearyl-dimethylbenzylammonium chloride

○ represents absorbance values for  $5.00 \times 10^{-6}M$  and  $1.00 \times 10^{-5}M$  dye, △ represents uncorrected surface tension values

controlling the pH was important. It was found that absorption of CO<sub>2</sub> was the main problem. This was minimized by titrating in a nitrogen atmosphere and pumping the solution through a flow cell. This procedure reduced pH fluctuations. The pH at the beginning of the titrations was about 5.5 to 6.0 and constant and slowly increased, but less than one pH unit, during the course of the titration. This increase was due to the titrant being at a higher pH, *ca.* 8. The exact pH at the beginning of the titration is not critical; but, having it relatively constant initially is necessary for good results. The more acidic fluoresceins, dichloro- and tetrabromo-, would probably give more stable absorbance values at these pH's for their pK<sub>a</sub>'s are lower. Results show that the CMC obtained

by both methods agree, that a change in concentration of the dye yields no observable change in the CMC for a given surfactant, and that fluorescein is generally a useful dye to determine the CMC of quaternary ammonium salts spectrophotometrically. It was found, however, that the accuracy of the method decreased with increasing alkyl chain length. Excellent results were obtained for the lauryl and myristyl derivatives, good results for the cetyl derivative, and poor results for the stearyl derivative. Surface tension measurements gave consistently good results.

RECEIVED for review April 11, 1969. Accepted May 21, 1969.

## Spectrophotometric Method for Rapid Determination of Oak Leaf Condensed Tannin

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THE VEGETABLE TANNINS are receiving increasing attention due primarily to their influence on food palatability, their inhibitory effects on the growth of micro-organisms, fungi and insects, and their toxicity to chicks and mammals. In several fields of investigation, therefore, a rapid method for the determination of tannins in plant tissues has become an urgent necessity.

Traditional methods for the quantitative analysis of tannins, such as the hide powder method (1) and Löwenthal method (2), suffer from several disadvantages, including extreme tediousness, lack of specificity, and dependence on aqueous extraction of plant tissue, which does not achieve quantitative extraction of tannins (3,4). Rapid chromatographic and spectrophotometric methods have been successfully applied to the determination of simple phenols in plant material (3,5,6), but it has proved impossible to elute more than insignificant amounts of oak leaf (*Quercus robur* L.) condensed tannin from homogenized paper or thin-layer chromatogram spots (7). Though hydrolysable tannins can be extracted quantitatively from chromatograms by continued strip elution, condensed tannin appears to be irreversibly bound to the cellulose, much of it remaining at the origin. Analytical methods depending on elution of phenolic material followed by spectrophotometric determination with or without the aid of a color-developing reagent are thus not applicable to the condensed tannins.

Bradfield and Flood (8) found a quantitative relationship between the amount of simple phenol in a chromatogram spot

and the direct light absorption reading obtained by placing excised spots in the sample cell of a spectrophotometer. Such a method was found to be inapplicable for oak leaf condensed tannin, because it forms a streak of uneven coloration, rather than a concise spot, with developing reagents, due to concentration of the tannin at the origin. Photoelectric scanning of excised spots, as used by Reid (9) for tobacco leaf extracts, suffers from the same drawbacks. The azo-dye method of Pridham (5) was attempted but it was found that the orange dyes obtained from oak tannin with diazotized *p*-nitroaniline could not be eluted with methanolic potash. Column chromatography was found to be unsuitable for the quantitative estimation of condensed tannins, which tend to be irreversibly absorbed onto cellulose, perlon, silica gel, alumina, and Sephadex. Although Sephadex columns were used for the qualitative preparation of individual tannins, following the method of Somers (10), yields of condensed tannin were far from quantitative, owing to some losses on the column. The gravimetric ether-precipitation method adopted by Feeny and Bostock (7) for determination of tannins in oak leaf extracts is tedious and involves considerable volumes of ether. It is thus unsuitable for the rapid determination of tannin content in leaf extracts.

In the method described below, the difficulty of eluting condensed tannin from paper chromatograms is by-passed by dissolving the entire excised streak in sulfuric acid. Simultaneous degradation of the tannin by the acid gives rise to colored solutions, the intensity of which can be measured spectrophotometrically and used to determine the original tannin concentration.

### EXPERIMENTAL

Samples of aqueous acetone extracts of fresh plant material are applied to the origins of two-way paper chromatograms (Whatman No. 1), which are then developed in 2% acetic acid and *n*-butanol/acetic acid/water (60:15:25) solvents to separate hydrolysable tannins and monomeric phenolic material from the condensed tannin (7). After drying the chromatograms, one is sprayed with FeCl<sub>3</sub>/K<sub>3</sub>Fe(CN)<sub>6</sub>

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