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# THE EFFECT OF ALIPHATIC ALCOHOLS ON FLUORESCENCE QUENCHING AND FLUORESCENCE POLARIZATION OF LUMINESCENCE PROBES IN HEXADECYLTRIMETHYLAMMONIUM BROMIDE MICELLES

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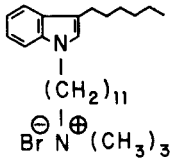
**Abstract**—The fluorescence quenching of the indole chromophore by $NO_2^-$ and the fluorescence depolarization of several luminescence probes in aqueous solutions containing hexadecyltrimethylammonium bromide (HDTBr) were measured as a function of added $C_2$–$C_4$ aliphatic alcohol concentration. The fluorescence decay profiles of pyrene in the micellar solutions were also measured to estimate the aggregation number of the micelles. The addition of *n*-butyl alcohol significantly reduces the fluorescence quenching rate and the aggregation number and increases the extent of fluorescence depolarization in HDTBr micellar systems. The addition of ethyl alcohol shows a similar but smaller effect.

## INTRODUCTION

The addition of alcohols to aqueous solutions of detergents containing micellar aggregates is known to influence the properties of micelles (Fendler and Fendler, 1975). Although the effect of added alcohol upon the CMC of surfactant solutions has been studied extensively, (Miyagishi, 1974, and references therein), relatively little effort has been directed toward measurement of the effect of added alcohol upon dynamic properties of micelles and of solutes that are sequestered by micelles. Of the pertinent recent studies, one employed fluorescence polarization (Shinitzky *et al.*, 1971) to evaluate the microviscosity of hexadecyltrimethylammonium bromide (HDTBr)* micelles as a function of added alcohol. Perylene was employed as the fluorescence probe. The results implied that the microviscosity of HDTBr micelles increased dramatically from ~20 to ~200 cP upon the addition of cetyl alcohol. In another investigation (Robinson *et al.*, 1975), the kinetics of dye absorption onto sodium dodecyl sulfate (SDS) micelles was determined by stopped-flow techniques. The addition of methanol leads to an increase in the absorption rate, while the addition of dodecanol did not significantly influence the absorption rate. The rate of exchange of long chain alcohols between the micellar and aqueous phase has been examined by pulse radiolysis (Almgren *et al.*, 1979), and it was found that the entrance rates of alcohols were all close to diffusion controlled but that the exit rates depended on the alcohol structure. Very recently, pyrene excimer formation has been used to detect the effect of alcohol on the variation of the micelle aggregation number (Lianos and Zana, 1980). The aggregation number of 0.01 *M* HDTBr solution changes from 82 to 18 with the addition of *n*-butanol (0.8 *M*). With the addition of alcohols, the aggregation number of 0.05 *M* tetradecyltrimethylammonium bromide solution also is reduced from 68 to 36 (0.4 *M* *n*-pentanol), 27 (0.7 *M* *n*-butanol), and 47 (0.1 *M* *n*-hexanol), respectively.

In the present study, we have investigated the effect of added alcohol on the fluorescence quenching of 11-(3-hexyl-1-indolyl)undecyltrimethylammonium bromide (6-In-11$^+$) on the microviscosity, on the CMC, and on the aggregation number of ion micelles.



6-In-11$^+$

## MATERIALS AND METHODS

*Chemicals.* 11-(3-hexyl-1-indolyl)undecyltrimethylammonium bromide (6-In-11$^+$), hexadecyltrimethylammonium bromide (HDTBr), and sodium nitrite were available from previous studies (Turro *et al.*, 1980). The purest grade of available ethanol (EA), *n*-propanol (NPA), *n*-butanol (NBA), *iso*-butanol (IBA), *t*-butanol (TBA), and polypropyrene glycol were used as supplied. Perylene (Aldrich, Gold label) and pyrene (Tokyo Kasei, UP grade) were recrystallized from ethanol. α,α′-1,3-Di-naphthylpropane (DNP) was synthesized by a literature procedure (Chandross and Dempster, 1970).

*Spectroscopy.* Fluorescence spectra were measured on a Hitachi–Perkin–Elmer MPF-2A or on a SPEX Fluorolog

*Abbreviations: DNP, α,α′-1,3-Di-naphthylpropane; EA, ethanol; HDTBr, hexadecyltrimethylammonium; IBA, isobutanol; 6-In-11$^+$, 11-(3-hexyl-1-indolyl)undecyltrimethylammonium bromide; NBA, *n*-butanol; NPA, *n*-propanol; SDS, sodium dodecyl sulfate; TBA, *t*-butanol.

spectrophotometer. Fluorescence decay curves were obtained using a single photon counting technique. Observed decay curves were analyzed via a computer deconvolution method (Turro *et al.*, 1980).

*Microviscosity measurements.* (a) *Fluorescence depolarization method.* Fluorescence depolarization was determined on a SPEX Fluorolog provided with a polarization accessory. To minimize the polarization due to the instrument, a scrambler was installed in front of the entrance slit of the emission monochromator. Fluorescence intensities were corrected in a standard manner (Azumi and McGlynn, 1962).

The relation between molecular anisotropy, $r$, and microviscosity, $\eta$, is given in the following Eq. (Shinitzky *et al.*, 1971)

$$\frac{1}{r} = \frac{1}{r_0}\left(1 + \frac{k\tau T}{\eta V}\right) \quad (1)$$

where $r_0$ is the limiting molecular anisotropy when $T/\eta \to 0$, $k$ is the Boltzmann constant, $T$ is the temperature (K), $\tau$ is the fluorescence lifetime (ns), and V is the volume of the fluorescence probe molecule.

Perylene and 6-In-11$^+$ were employed in the fluorescence depolarization studies. The viscosities of solvents and solvent mixtures employed for calibration were obtained from the literature (Curme and Johnston, 1953). As a calibration, the parameters of Eq. 1 were determined from the temperature dependence of fluorescence depolarization and from fluorescence lifetimes in propylene glycol (perylene) or 90% propylene glycol/10% water (6-In-11$^+$): perylene, $\lambda_{ex}$ 413 nm, $\lambda_{em}$ 470 nm, $r_0 = 0.25$ and $V = 9.0 \times 10^{-25}$ cm$^3$/molecule. 6-In-11$^+$, $\lambda_{ex}$ 290 nm, $\lambda_{em}$ 350 nm, $r_0 = 0.1$ and $V = 3.0 \times 10^{-24}$ cm$^3$/molecule.

(b) *Intramolecular excimer method.* Microviscosities were computed from the fluorescence spectra of DNP by the comparison of the intramolecular excimer/monomer ratio observed in micellar solutions for DNP with the ratio obtained in homogeneous solvents of known viscosity (Turro *et al.*, 1979).

*Micelle aggregation number.* The micelle aggregation number was estimated from the decay profile of pyrene fluorescence (Atik *et al.*, 1979). The time dependence of pyrene monomer fluorescence at large $t$ after pulsed excitation is

$$\ln (I_M/I_M^0) \sim -(n + k_1 t) \quad (2)$$

where $I_M^0$ and $I_M$ are the fluorescence intensities of pyrene monomer at $t = 0$ and $t$, respectively, $n$ is the mean occupancy number of pyrene in the micelle, and $k_1$ is the decay rate constant of the excited pyrene monomer. The micelle aggregation number, $N$, was calculated from the following Eq.

$$N = ([\text{Det}] - \text{CMC})/n \quad (3)$$

where [Det] and CMC are the total concentration of the detergent and the critical micellar concentration, respectively.

Fluorescence decay profiles of pyrene were obtained at 380 nm, with a nitrogen laser excitation (~10 ns pulse, 337 nm) and graphically analyzed.

## RESULTS AND DISCUSSION

### *The CMC of 6-In-11$^+$*

The fluorescence of 6-In-11$^+$ has been shown to be sensitive to the environment (Turro *et al.*, 1980). In water, the fluorescence band maximum ($\lambda_{max}$) for 6-In-11$^+$ appears at 370 nm (fluorescence lifetime, $\tau$, is ~17 ns), and shifts to shorter wavelength for 6-In-11$^+$ in HDTBr micelles ($\lambda_{max}$ 350 nm, $\tau \sim 8$ ns). Thus, the CMC of HDTBr solutions was determined from the fluorescence band shift of 6-In-11$^+$. Table 1 shows the effect of alcohols upon the CMC of HDTBr micellar phase is insensitive to the nature of alcohol. The CMC of HDTBr in pure water is 0.78 m$M$. With the addition of 0.3 $M$ of EA, NPA, and TBA, the CMC slightly decreases, while the same effect was observed by the addition of only 0.06 $M$ of NBA and IBA. These trends seem to be correlated to the solubility of alcohol in water. Since EA, BPA and TBA are completely miscible with water, the partitioning of these alcohols between water and HDTBr micellar phases is in favor of the former. The partitioning of NBA and IBA between the two phases is in favor of HDTBr micellar phase because of their low solubility in water phase. The effect upon the CMC of HDTBr shown in Table 1 is parallel with the results in the literature (Corrin and Harkins, 1946).

### *Effect of added alcohol on the fluorescence quenching of 6-In-11$^+$ by $NO_2^-$*

Figures 1 and 2 show the effect of EA and NBA on the fluorescence quenching of 6-In-11$^+$ by $NO_2^-$. The quenching rate constants were computed from fluorescence lifetime measurements. For the water phase, plots of the decay rate vs quencher concentration did not obey the Stern–Volmer relationship at NBA concentrations greater than about 0.5 $M$. Thus, the quenching rate constant at the NBA concentrations

Table 1. The CMC of HDTBr and fluorescence lifetime of 6-In-11$^+$

| Alcohol | Solubility in water* | [Alcohol], $M$ | $\tau$, ns [HDTBr] 0.5 m$M$ | $\tau$, ns [HDTBr] 1.7 m$M$ | CMC†, 1 m$M$ |
|---|---|---|---|---|---|
| None | — | — | 16.7 | 7.7 | 0.78 |
| Ethanol | Infinite | 0.32 | 17.2 | 8.0 | 0.75 |
| *n*-Propanol | Infinite | 0.33 | 16.1 | 8.2 | 0.61 |
| *n*-Butanol | 7.45% | 0.33 | — | — | <0.5 |
| | | 0.06 | 16.6 | 8.0 | 0.68 |
| *iso*-Butanol | 10% | 0.32 | — | — | <0.5 |
| | | 0.06 | 15.9 | 8.5 | 0.55 |
| *t*-Butanol | Infinite | 0.37 | 15.6 | 8.5 | 0.54 |

*Riddick and Bunger, 1970.

†CMC determined from the 6-In-11$^+$ fluorescence $\lambda_{max}$ shift ([6-In-11$^+$], 16 $\mu M$).

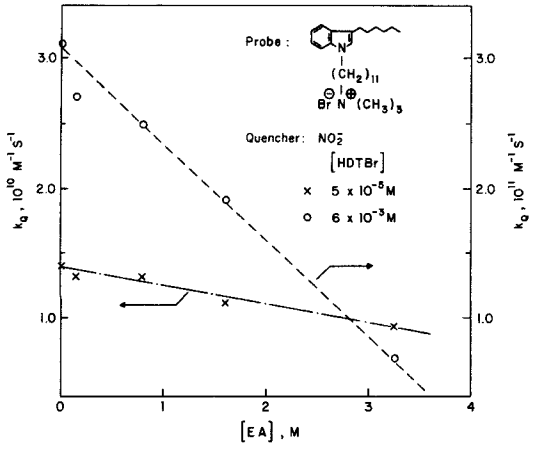



Figure 1. Effect of ethanol (EA) upon the fluorescence quenching rate of 6-In-11$^+$ by $NO_2^-$ in HDTBr micellar solution at room temperature. The rate constant was determined from fluorescence lifetime. The concentration of 6-In-11$^+$ is 60 $\mu M$.

over 0.5 $M$ is not shown in Fig. 2. The rate constant for quenching of 6-In-11$^+$ fluorescence by $NO_2^-$ in aqueous solution in the absence of micelles is $1.4 \times 10^{10}\ M^{-1}\ s^{-1}$, i.e. close to diffusion controlled. However, in solutions containing HDTBr micelles, the quenching constant is computed to be $\sim 3 \times 10^{11}\ M^{-1}\ s^{-1}$ i.e. about one order of magnitude greater than the value expected for diffusional quenching in water. This anomolously large quenching constant is attributed to the high local concentration of $NO_2^-$ associated with the micelle surface (Turro *et al.*, 1980).

The quenching constant tends to gradually decrease with increasing EA concentration, the decrease with added EA in solutions containing HDTBr micelles is much sharper (Fig. 1). A similar trend is qualitatively observed with added NBA (Fig. 2). The quantitative

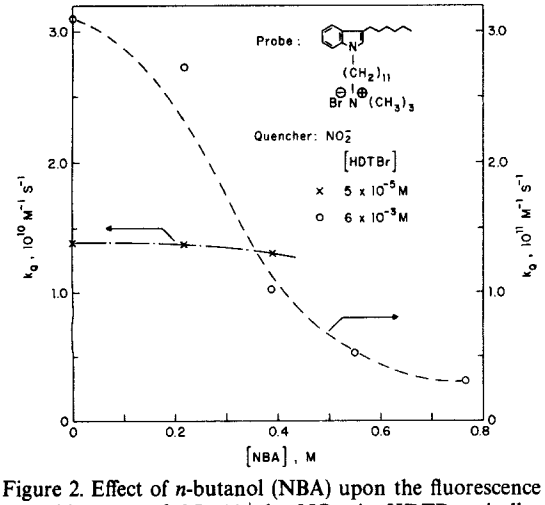



Figure 2. Effect of *n*-butanol (NBA) upon the fluorescence quenching rate of 6-In-11$^+$ by $NO_2^-$ in HDTBr micellar solution at room temperature. The rate constant was determined from fluorescence lifetime.

difference in the effects of EA and NBA on the quenching constant may be expressed in terms of the decrease ($\Delta k_q$) in quenching rate constant per mole of added alcohol:

| | |
|---|---|
| $\Delta k_q$ ($H_2O$, EA) | $0.15 \times 10^{10}\ m^{-1}\ s^{-1}$/ mol of alcohol |
| $\Delta k_q$ ($H_2O$, NBA) | $0.25 \times 10^{10}$ |
| $\Delta k_q$ (HDTBr micelle, EA) | $0.73 \times 10^{11}$ |
| $\Delta k_q$ (HDTBr micelle, NBA) | $5.2 \times 10^{11}$ |

In water, $\Delta k_q$ of NBA is 1.7 times larger than that of EA, while $\Delta k_q$ of NBA in the HDTBr micelle is about 7 times larger than that of EA in the micelle. Thus, it is clear that NBA significantly decreases the fluorescence quenching rate of $NO_2^-$ in the HDTBr micelle. The difference between the two alcohols is attributed to the difference of their solubility in the micelle.

*Microviscosities in micelles*

The microviscosity of HDTBr micelles was evaluated by two distinct, independent methods. The first method involves the measurement of fluorescence depolarization of probes in micelles and correlation of the results with comparable data in homogeneous solvents of known viscosities via Eq. 1. Perylene was selected as a calibrating fluorescence probe because its use in this regard has been well established (Shinitzky *et al.*, 1971). The functionalized detergent 6-In-11$^+$ was used as a probe whose indole chromophore in HDTBr micelles has been established, by previous studies, to be located in the micellar interior (Turro *et al.*, 1980). A second method, employing the excimer/monomer intensity of DNP as a reported probe of microviscosity (Turro *et al.*, 1979) was also employed. It was felt that the use of two methods and three probes would provide checks of the admittedly risky procedures of employing probes to monitor microviscosities.

Figure 3 shows the effect of NBA on the microviscosity of HDTBr micelles determined by the three probe molecules. The microviscosities at [NBA] = 0 are in the range 18–25 cP; values in good agreement with literature reports (Shinitzky *et al.*, 1971; Emert *et al.*, 1979; Turro *et al.*, 1979). In all cases the microviscosity decreases with added NBA. At the highest concentrations of NBA employed ($\sim 0.6\ M$), the microviscosities computed by the fluorescence depolarization method are comparable ($\sim$8–12 cP), but are much higher than the microviscosity measured by the fluorescence excimer/monomer method ($\lesssim 1$ cP). Although a 6-In-11$^+$ molecule has an indole chromophore between the $C_{11}$ and $C_6$ chain, the calculated viscosities agree well with the ones obtained from a perylene probe molecule. The fact seems to suggest that the chromophore rotates almost freely in the observed time range ($< 10$ ns). The reason for the discrepancy between the two methods is not clear at this stage,

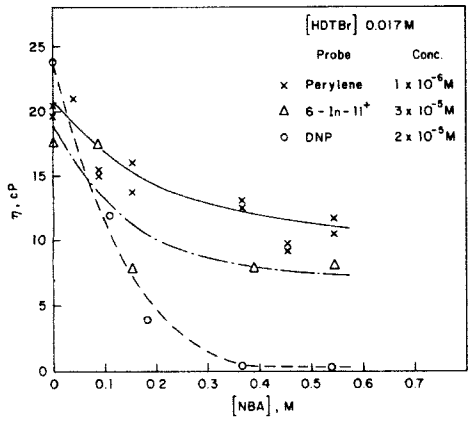



Figure 3. Effect of *n*-butanol (NBA) upon the microviscosity of HDTBr micelles at room temperature. The concentration of HDTBr is 0.017 *M*. The microviscosity was determined with different methods (see text).

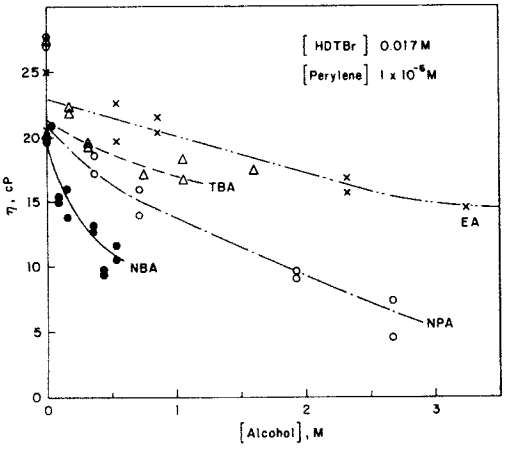



Figure 4. Effect of alcohols upon the microviscosity of HDTBr micelles at room temperature. The concentration of HDTBr is 0.017 *M*. Perylene (1 $\mu M$) was used as a fluorescence probe.

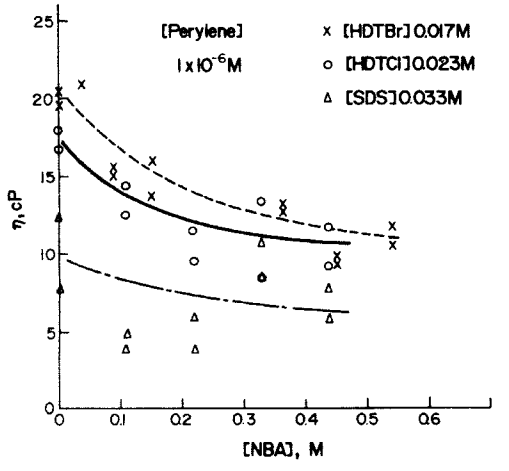



Figure 5. Effect of *n*-butanol (NBA) upon the microviscosity of ionic micelles at room temperature. Perylene (1 $\mu M$) was used as a fluorescence probe.

although it is possible to attribute this difference to the difference of the location of the probe molecules (Zachariasse, 1978). In any case, it is concluded that the effective microviscosity of HDTBr micelles clearly decreases with added NBA.

Figure 4 shows the effect of addition of several alcohols on the microviscosity of HDTBr micelles, as determined by the fluorescence polarization method with perylene as a probe. In each case the microviscosity is found to decrease with increasing alcohol concentration. The smallest effect is observed with EA, the smallest alcohol and the largest effect is observed with NBA.

A concentration of 0.1 *M* of NBA reduces the microviscosity to 80% of the value in the absence of alcohol, while about 2 *M* of EA must be added to achieve the same effect. Thus, on a molar basis, NBA is about 20 times more effective in reducing the microviscosity than EA. This efficiency is 2–3 times larger than that observed in the fluorescence quenching rate.

Figure 5 shows the NBA effect upon the microviscosity of three ionic micelles, determined by the perylene fluorescence depolarization method. The microviscosities obtained in pure micellar solutions (without NBA) agree with the literature values (Turro *et al.*, 1979; Emert *et al.*, 1979). With the addition of NBA, the viscosity in three micelles (HDTBr, HDTCl and SDS) decreases. The effect upon the HDTBr, HDTCl and SDS micelles is comparable. Thus, the effect of NBA on the viscosity in ionic micelles does not depend significantly on the electric charge of the micelle.

The effect of long chain alcohols on micellar viscosity is very small. For example, *n*-octyl alcohol, *n*-undecanol and cetyl alcohol were found to produce no significant change in the microviscosity of HDTBr micelles. It should be noted that the solubility of these alcohols in micellar solution is substantially lower than that of the lower molecular weight alcohols investigated. The reported large increase in microviscosity produced by addition of cetyl alcohol (Shinitzky *et al.*, 1971) is not noted in our study. This may be due to our inability to prepare concentrations of cetyl alcohol in HDTBr solutions to the extent achieved by Shinitzky *et al.*

*Micelle aggregation number*

Table 2 shows the effect of NBA and EA on the aggregation number, *N*, of HDTBr, HDTCl, and SDS micellar solutions. The addition of NBA (0.4 *M*) significantly reduces the aggregation number, while that of EA to HDTBr solution gave little change on the *N* of the solution. This difference is most probably due to the difference of their solubility in the HDTBr micelle.

From the analysis of pyrene monomer fluorescence decay profiles, Lianos and Zana (1980) have determined the *N* of 0.01 *M* HDTBr solution without and with NBA (0.8 *M* at 25°C): *N* is 82 and 18, respectively. The present results are parallel to their results.

Table 2. The effect of alcohol on the micelle aggregation number, $N$

| Alcohol† | Aggregation number* HDTBr 0.017 $M$ | HDTCl 0.021 $M$ | SDS 0.031 $M$ |
|---|---|---|---|
| None | 96 | 78 | 75 |
| *n*-Butanol | | | |
| 0.1 $M$ | 93 | — | — |
| 0.4 $M$ | 52 | 47 | 20 |
| Ethanol | | | |
| 1 $M$ | 100 | — | — |
| 2 $M$ | 97 | — | — |

*The deviation of the value is estimated to be $\pm 10$.
†The temperature is 20°C.

Although $N$'s determined in the present work are a little larger than their values, this may be due to the difference of the temperature (present work, 20°C). Their determination of the $N$ of decyltrimethylammonium chloride solution on addition of butanol, pentanol, and hexanol showed a reduction of $N$ values. Almgren *et al.* (1979) determined the effect of *n*-pentanol upon the aggregation number of SDS micelles, $N$, from the luminescence quenching of $Ru(bipy)_3^{2+}$ by 9-methylanthracene; $N = 62, 45 \pm 5, 60 \pm 5$ for alcohol concentrations of 0, 0.01, 0.31 $M$, respectively. However, since the $N$ value without the alcohol was taken from the work done by others, it is uncertain whether or not the addition of the alcohol (0.3 $M$), influences the $N$ value. Present results seem to suggest that the reduction of the aggregation number is a common effect for the short-chain alcohols that are poorly soluble in water.

*Role of alcohols in the fluorescence quenching of 6-In-11*$^+$

As is discussed above, the addition of alcohols provides various effects on the structure and dynamics of micelles. The addition of alcohols reduces the aggregation number of HDTBr micelle, which in turn increases the micelle concentration and presumably the micelle surface. From the solubility measurements of NBA to the HDTBr micellar solutions, the mean number of alcohol molecules associated with the micelle is estimated to be in the range of 100–200 in the alcohol concentrations employed in this study (0.1–0.5 $M$), so that the micelles are swollen considerably. This effect could reduce the positive electrostatic potential on the micelle surface and the viscosity in the micelle. The increase of the micelle surface and micelle volume, along with reduction of electrostatic potential lead to a lower effective concentration of the $NO_2^-$ at the micelle surface upon addition of alcohol and results in a slower quenching rate.

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