7-ALKOXY COUMARINS AS FLUORESCENCE PROBES FOR MICROENVIRONMENTS

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Summary

7-Alkoxy and 4-methyl-7-alkoxy coumarins show solvent-dependent fluorescence emission. The monomeric fluorescence emission of these alkoxy coumarins was exploited as a probe to measure the surface polarity of the micelles formed by ionic (sodium dodecylsulphate and cetyltrimethylammonium bromide) and non-ionic (Triton X-100) detergents. By comparing the solvent-dependent fluorescence of these alkoxy coumarins in various homogeneous solvents, the polarity of the micelles was determined qualitatively. All three micelles are more polar than hydrocarbon solvents but are less polar than water.

1. Introduction

Micelles have received much attention from workers in a wide variety of areas, who approach the subject with various interests and points of view. It need not be amplified that a knowledge of the structure of the micelle, of the local microenvironment, of the local concentration and of the relative orientation of the solubilized molecules is of fundamental importance. Fluorescence probes have been elegantly and effectively employed to study the structure of organized assemblies. The general idea behind a fluorescence probe is that a molecule, whose fluorescence emission can serve as a sensor of microenvironments, will display distinct fluorescence properties which uniquely characterize each environment. In earlier studies, ionic and zwitterionic aromatic molecules were commonly employed because of the extreme sensitivity of their fluorescence yields to solvent polarity. Recently, however, objections have been raised regarding the validity of using highly polar probes for hydrophobic regions such as micellar interiors. Most recent studies have utilized aromatic hydrocarbons as probes that are less likely to perturb the properties of the host system. Studies on the photochemical reactivity in micellar media consist of molecules carrying carbonyl chromophores [1 - 3]. Therefore, it is important to provide information regarding the microenvironment of such molecules in micelles. It is in this connection

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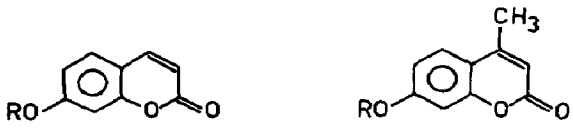

(a) (b)

Fig. 1. (a) 7-*O*-alkoxy coumarins (**1**, R = CH_3; **3**, R = $CH_2(CH_2)_2CH_3$; **5**, R = $CH_2(CH_2)_4CH_3$) and (b) 4-methyl-7-*O*-alkoxy coumarins (**2**, R = CH_3; **4**, R = $CH_2(CH_2)_2CH_3$; **6**, R = $CH_2(CH_2)_4CH_3$) used as probes.

that we have utilized the solvent-dependent fluorescence intensity of 7-*O*-alkyl coumarins (**1** - **6**) (Fig. 1) to gain some knowledge of the solubilization sites of carbonyl compounds in various micelles and the properties of their microenvironments.

2. Results

Excitation (300 - 330 nm) of dilute solutions (about 10^{-4} M) of alkoxy coumarins **1** - **6** leads to strong solvent-dependent fluorescence in the range 340 - 460 nm. This is attributed to the monomeric excited singlet state. The fluorescence emission maximum is slightly dependent on the solvent polarity and the emission maximum is gradually shifted, although only by a small amount, to longer wavelengths when the solvent is changed from benzene to water (Table 1). The relative fluorescence intensity measured in several

TABLE 1

Fluorescence emission maximum of alkoxy coumarins **1** - **6** in various solvents

Medium	*Fluorescence emission maximum* (nm)[a] *of the following compounds*					
	1	*2*	*3*	*4*	*5*	*6*
Benzene	378	372	—	—	—	—
Acetone:water (1:1)	386	378	388	380	389	382
Acetonitrile	382	377	381	377	386	378
Acetonitrile:water (1:1)	386	379	386	381	390	382
Methanol	384	376	384	379	388	380
Methanol:water (1:1)	388	380	390	385	392	384
Methanol:water (1:19)	392	384	396	388	396	386
Water	392	384	—	—	—	—

[a]The excitation wavelength was 330 nm for **1** and **2** and 320 nm for **3** - **6**.

TABLE 2

Relative fluorescence intensity of alkoxy coumarins 3 - 6 in various organic solvents and solvent–water mixtures

Medium[a]	*Relative fluorescence intensity*[b] *of the following compounds*			
	3	*4*	*5*	*6*
Benzene	10^{-3}	10^{-3}	10^{-4}	10^{-3}
Acetone–water				
100	0.003	0.003	8×10^{-4}	0.002
70	0.06	0.06	0.04	0.07
50	0.24	0.31	0.15	0.27
Acetonitrile–water				
100	0.02	0.05	0.02	0.06
90	0.06	0.15	0.07	0.15
80	0.11	0.23	0.13	0.24
70	0.17	0.33	0.20	0.29
Methanol–water				
100	0.07	0.32	0.22	0.32
90	0.11	0.49	0.35	0.50
80	0.18	0.63	0.47	0.59
70	0.36	0.80	0.65	0.73
60	0.77	0.90	0.82	—
50	1.00[c]	1.00[c]	1.00[c]	1.00[c]
5	1.69	1.34	1.91	1.73

[a] Values denote the percentage by volume of the first-named compound.
[b] Excitation wavelength, 320 nm.
[c] Taken as the reference.

protic and aprotic solvents and solvent–water mixtures was found to be solvent dependent. It is interesting to note that these alkoxy coumarins exhibit intense fluorescence in water and are weakly fluorescent in aprotic organic solvents such as benzene, acetone or acetonitrile. The results are summarized in Table 2.

The relative fluorescence intensities measured in various solvents and solvent–water mixtures showed a poor correlation with the macroscopic properties (the polarity parameters) of the medium such as the dielectric constants and the Z, $E_T{}^{30}$ and Y values. Consequently, our discussions and interpretations are only of a qualitative nature. To check the effect of ions, the emission spectra of 1 - 6 were recorded in 1:1 methanol:water mixtures containing various amounts of NaCl and KBr. The variation in the fluorescence intensities of 1 - 6 in the presence of NaCl was negligible. A small but significant decrease (about 20% at 0.025 M KBr) in the fluorescence intensities of 1 - 6 was observed in KBr solutions. This is most probably caused by intersystem crossing of the excited singlet state of alkoxy coumarins 1 - 6 [4] induced by the bromide ions (heavy atoms).

TABLE 3

Relative fluorescence intensity of alkoxy coumarins 1 - 6 in various surfactant solutions

Medium	*Relative fluorescence intensity*[a]					
	1	*2*	*3*	*4*	*5*	*6*
Methanol–water[b]						
0	0.13	0.23	0.07	0.32	0.22	0.32
30	1.45	1.35	—	—	—	—
50	1.00[c]	1.00[c]	1.00[c]	1.00[c]	1.00[c]	1.00[c]
70	—	—	0.36	0.80	0.65	0.73
SDS						
0.005 M	—	—	1.23	0.70	0.84	0.73
0.01 M	—	—	1.24	1.09	0.91	1.13
0.02 M	1.52	1.18	1.23	1.12	0.86	1.10
0.03 M	1.45	1.15	1.26	1.13	0.86	1.09
0.04 M	1.43	1.13	1.19	1.09	0.84	1.07
CTAB						
0.005 M	—	—	—	0.26	0.16	0.19
0.01 M	—	—	0.24	0.21	0.16	0.18
0.02 M	0.88	0.42	0.18	0.19	0.13	0.17
0.03 M	0.78	0.37	0.17	0.18	0.12	0.17
0.04 M	0.63	0.32	0.15	0.14	0.11	0.16
Triton X-100						
0.005 M	—	—	0.68	0.38	0.13	0.13
0.01 M	—	—	0.44	0.26	0.09	0.12
0.02 M	1.66	0.83	0.30	0.20	0.08	0.11
0.03 M	1.54	0.69	0.27	0.19	0.07	0.11
0.04 M	1.50	0.61	0.26	0.17	0.07	0.11

[a]The excitation wavelength was 330 nm for 1 and 2 and 320 nm for 3 - 6.
[b]Values denote the percentage by volume of methanol.
[c]Taken as the reference.

Fluorescence spectra of 1 - 6 were recorded in detergent solutions of concentrations both below and above the critical micelle concentration (CMC). The results obtained show that the fluorescence spectra in detergent solutions are identical with those in organic and aqueous solutions. However, the intensity of emission in detergent solutions is lower than that in water and much higher than that in non-polar organic solvents. The fluorescence spectra of 1 - 6 in micellar media are similar to those already reported for the parent coumarin [2]. The results are summarized in Table 3.

Fluorescence spectra of 1 - 6 were recorded in sodium dodecylsulphate (SDS), cetyltrimethylammonium bromide (CTAB) and Triton X-100 with respect to the detergent concentration. Figures 2 - 4 illustrate the typical behaviour of coumarins in various detergents. In all cases an abrupt variation in the intensity of emission was noticeable at around the CMC. The CMC values measured using the fluorescence intensity of 1 - 6 agree well with the

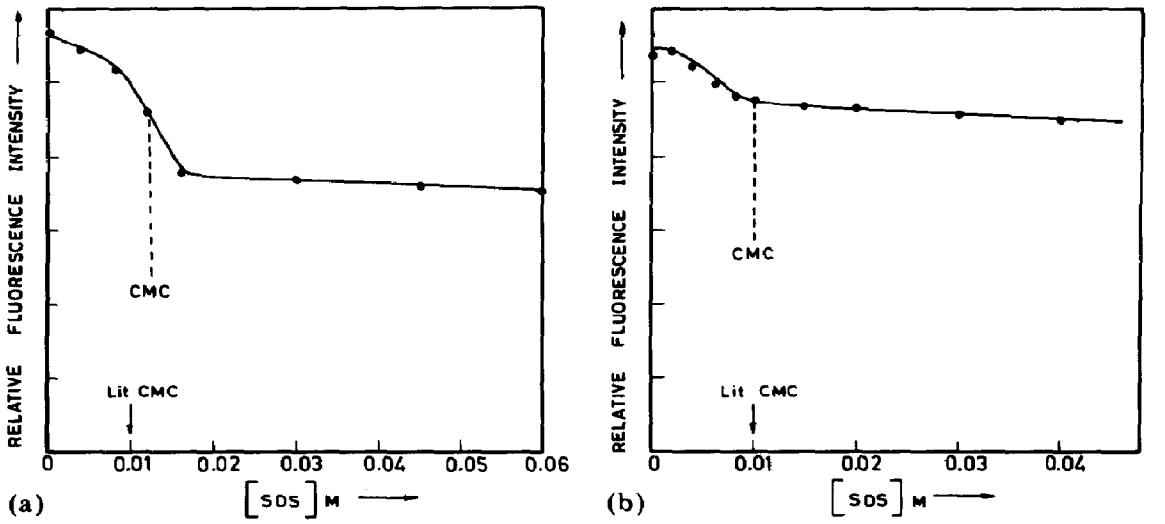

Fig. 2. Variation in the relative fluorescence intensity of (a) **1** (concentration, 5.5×10^{-4} M) (CMC, 1.25×10^{-2} M) and (b) **2** (concentration, 1.3×10^{-4} M) (CMC, 1.0×10^{-2} M) with respect to the SDS concentration.

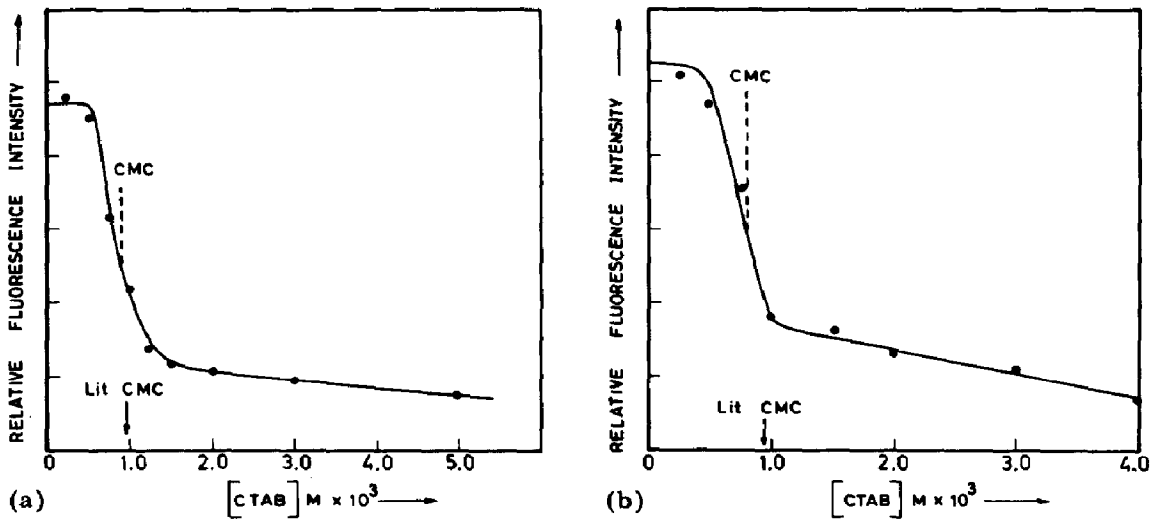

Fig. 3. Variation in the relative fluorescence intensity of (a) **5** (concentration, 1.5×10^{-4} M) (CMC, 9×10^{-4} M) and (b) **6** (concentration, 1.1×10^{-4} M) (CMC, 8×10^{-4} M) with respect to the CTAB concentration.

published values [5]. The surfactant concentration also has an influence on the measured intensity which decreases with increasing detergent concentration even above the CMC. This is especially well pronounced for cationic (CTAB) and non-ionic (Triton X-100) micelles. For anionic (SDS) micelles a limiting value was reached at or above the CMC.

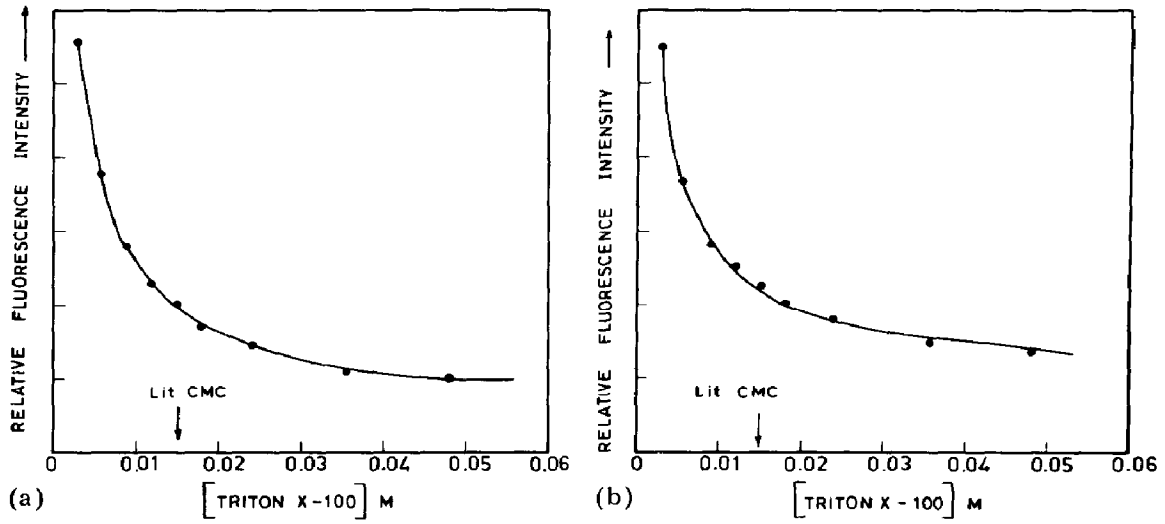

Fig. 4. Variation in the relative fluorescence intensity of (a) 3 (concentration, 1.2×10^{-4} M) and (b) 4 (concentration, 1.2×10^{-4} M) with respect to the Triton X-100 concentration.

3. Discussion

We postulate that for 1 - 6 the fluorescence originates from the lowest excited $\pi\pi^*$ singlet state and competes with a radiationless process consisting mainly of intersystem crossing to the triplet state [6]. According to the energy level diagram proposed by Song and coworkers [6] it can be visualized that the $n\pi^*$ singlet and triplet states are respectively just above and just below the S_1 ($\pi\pi^*$) state in non-polar solvents. Under these conditions intersystem crossing from the S_1 ($\pi\pi^*$) state to the closely lying $n\pi^*$ triplet state is facilitated and therefore the fluorescence yield is poor. However, in polar solvents the enhanced intensity of the emission can be interpreted as being due to a decreased efficiency of the intersystem crossing from the S_1 ($\pi\pi^*$) state to the T_1 ($\pi\pi^*$) state as a consequence of a lowering in the energy of the $^1\pi\pi^*$ state below that of the $^3n\pi^*$ state. In summary, the variation in the fluorescence intensity can be attributed to the change in energy levels ($n\pi^*$ and $\pi\pi^*$) by the solvent.

Although the parent coumarin is moderately soluble in water (2×10^{-2} M), the alkoxy coumarins are only sparingly soluble in water (1, 2×10^{-3} M; 2, 1×10^{-3} M; 3 - 6, 10^{-5} M). Therefore, the relatively high concentration of 1 - 6 achieved in micelles is due to the solubilization of the probe molecules in the micelles. This is supported by the following observations: (a) the intensity of emission changes abruptly around the CMC of the micelles; (b) the relative fluorescence intensity of 1 - 6 decreases when the surfactant concentration is increased even above the CMC (CTAB and Triton X-100); (c) the measured fluorescence intensities in the three micelles are different. If the coumarin molecules are buried deeply inside the micelle or in the micellar core then a weak fluorescence for 1 - 6, corresponding to that

of hydrocarbon solvents, is expected, but the observed fluorescence intensities in the surfactant solutions are much greater than those in benzene and acetone solutions. However, this enhanced intensity is possible if the emission originates from the coumarin molecules that are dissolved in the aqueous phase. This is probable for **1** and **2** but not for **3 - 6** since **3 - 6** are sparingly soluble in water. Therefore, the observed high fluorescence intensity of **3 - 6** can only be due to coumarin molecules associated with micelles and must be a reflection of their microenvironment.

The relative fluorescence intensities of **1 - 6** measured in micelles of various surfactants (SDS, CTAB and Triton X-100) reveal that the micellar environment is more polar than protic and aprotic organic solvents but is less polar than water. Table 3 reveals that the microenvironment of **1 - 6** in the SDS micelle is more polar than that in CTAB and Triton X-100 micelles. Although this is consistent with data reported in the literature [7] that SDS is more porous than CTAB, the difference between the fluorescence intensity measured in SDS and that in CTAB micelles of approximately similar size but with different functional groups and counter-ions requires a careful evaluation. As mentioned earlier, bromide ions quench the fluorescence of **1 - 6** in homogeneous solutions. Therefore, the possibility that quenching of the fluorescence of **1 - 6** by the bromide counter-ions is the cause for the decreased fluorescence intensity in CTAB micelles cannot be ruled out [4]. Since the coumarin molecules are expected to be localized near this interfacial region, such quenching of the fluorescence intensity by bromide ions is possible. Thus the microenvironmental polarities of **1 - 6** are quite likely to be the same in CTAB and SDS micelles. However, the large difference between the fluorescence intensity of **3 - 6** in SDS micelles and that in Triton X-100 micelles probably reveals that the polarity of the coumarin environment and hence the extent of water penetration into these micelles is different. Thus micelles offer a site wherein organic reactions can be conducted in a more polar environment than that obtained in organic solvents.

4. Experimental section

The 7-alkoxy coumarins **1 - 6** were prepared as described earlier [3] and were purified by recrystallization (chloroform–carbon tetrachloride) and column chromatography (silica gel, hexane–methylene chloride). The solvents (AnalaR grade) were distilled once before use. Doubly distilled water was used for micellization. The surfactants SDS and CTAB (Sigma) were recrystallized twice from 95% ethanol and an ether–ethanol mixture respectively. Triton X-100 (Sigma) was used as received.

Stock solutions of **1** and **2** in water and in organic solvents (methanol, ethanol, acetonitrile, acetone and benzene) and **3 - 6** in organic solvents were prepared by dissolution of amounts of known weight. These stock solutions were diluted appropriately by water and organic solvents to obtain sample solutions of a fixed coumarin concentration but of various solvent:water

compositions. Stock solutions of surfactants (SDS, CTAB and Triton X-100) with known concentrations were prepared. From these solutions, micellar solutions containing **1** - **6** were prepared by stirring the surfactant solutions with known amounts of coumarin. Sample solutions with a specific surfactant concentration and a fixed coumarin concentration were prepared by diluting this solution appropriately with water and stock solutions of surfactants. After dilution the surfactant solutions were stirred overnight and kept aside for a few hours before the fluorescence spectra were recorded. Similarly, solutions containing coumarin and inorganic salts (NaCl and KBr) were prepared by mixing the stock solutions of coumarins and salts.

Uncorrected fluorescence spectra were recorded on a Perkin–Elmer spectrofluorometer (model MPF-44). The excitation of **1** - **6** was conducted between 300 and 330 nm. Since the relative fluorescence intensity I_F(rel) is used as the parameter to evaluate the micellar properties, all the measurements in a set were obtained under identical conditions. The spectral measurements were repeated at least three times by using independent solutions and the spectra thus obtained were utilized. Emission and excitation spectra were recorded in a routine manner.

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