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Fluorescence polarization anisotropy as a novel tool for the determination of critical micellar concentrations

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Abstract

A new technique is proposed for the estimation of critical micellar concentrations (cmcs) based on fluorescence polarization anisotropy (r) measurements as a function of surfactant concentration ([surf]). Representative results are presented for Triton X 100 (TX 100), sodium dodecyl sulphate (SDS) and cetyl trimethyl ammonium bromide (CTAB). The first and second cmc values (cmc1 and cmc2) obtained from break points in r vs. [surf] plots are in reasonable agreement with existing literature data.

Keywords: Critical micellar concentration; Fluorescence polarization anisotropy; 7-Hydroxyflavone; Phenosafranin; 1-Pyrene carboxaldehyde

1. Introduction

A general characteristic of surfactants is the formation of micelles above a certain concentration in aqueous solution. This concentration is called the critical micellar concentration (cmc). In addition to this cmc (first cmc), a change in the properties of surfactant assemblies occurs at a concentration above the first cmc, which is usually called the second cmc. Certain physical properties, such as conductivity, surface tension, osmotic pressure, light scattering and chemical shift, when plotted against the surfactant concentration, show break points at the first and second cmcs [1]. Another line of approach for cmc determination is based on the use of appropriate spectroscopic probes by studying the variations in their electronic absorption or emission energies and/or intensities [2].

In this study, we have used fluorescence polarization anisotropy (FPA) [3] as a novel tool to estimate micellar cmcs. For fluorophores associated with micelles, the population of the oriented dipoles will be guided by the location of the fluorophores in the surfactant assembly. Since the magnitude of polarization depends on the competition between the rates of emission and rotational diffusion, polarization of the embedded probe molecule can reflect the nature and changes in the microenvironment in which it is located. Here, we present representative data using 7-hydroxyflavone (7HF) [4], 1-pyrene carboxaldehyde (1-PyCHO) [5–7] and phenosafranin (PSF) [8] as probes. Their photophysical properties have been shown to be sensitive to variations in their local environment.

In this work, we selected three commonly used micellar systems, namely Triton X 100 (TX 100) (neutral), sodium dodecyl sulphate (SDS) (anionic) and cetyl trimethyl ammonium bromide (CTAB) (cationic). We demonstrate that, with an appropriate choice of fluorophore–surfactant combination, FPA measurements can be used to obtain reliable estimates of the first cmc (cmc1) and the second cmc (cmc2).

2. Experimental details

The fluorophores 7HF, 1-PyCHO and PSF were purchased from Aldrich Chemical Co., Sigma and Molecular Probes Inc. respectively. Their purities were confirmed by checking their absorption and emission spectra in standard solvents, and they were used without further purification. The surfactants TX 100, SDS and CTAB were of the best grades available from Sigma and were used as received. Ethanol was of Uvasol grade, obtained from Merck. All fluorophore–surfactant solutions were freshly prepared using water from a Millipore Milli Q water purification system, and were allowed to stabilize for 1 h prior to experimental analysis.

The following protocol was used to prepare the solutions containing the fluorophores and surfactants. Concentrated stock solutions of the fluorophores were prepared in appro-

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priate solvents, i.e. in water in the case of PSF and in ethanol in the case of 7HF and 1-PyCHO. Measured aliquots of aqueous PSF solution were placed in sample tubes to which stock surfactant solution and water were added in the required amounts to obtain different surfactant concentrations, keeping the fluorophore concentration constant. For 7HF and 1-PyCHO, aliquots of ethanolic solutions were placed in sample tubes. The ethanol was evaporated using a current of dry air and appropriate amounts of stock surfactant solutions and water were then added. The final concentrations of the fluorophores in the surfactant solutions were as follows: PSF, 2×10^{-5} M; 7HF, 2×10^{-5} M; 1-PyCHO, 1×10^{-6} M.

Steady state absorption spectra were recorded with a Hitachi model U 2000 spectrophotometer. Fluorescence emission and polarization measurements were carried out using a Hitachi model F 4010 spectrofluorometer equipped with polarization accessories and a thermostatically controlled cell holder. For polarization measurements, the sample temperature was allowed to stabilize at 25 ± 0.5 °C before each measurement.

The fluorescence anisotropy (r) values were obtained from the expression

$$r=(I_{\parallel}-GI_{\perp})/(I_{\parallel}+2GI_{\perp}) \quad (1)$$

where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities of the vertically and horizontally polarized emission respectively when the sample is excited with vertically polarized light. The G factor denotes the ratio of the sensitivities of the detection system for vertically and horizontally polarized light [3]. Each surfactant was studied over a range of surfactant concentrations, keeping the fluorophore concentration constant in each case. For all anisotropy studies, the longer wavelength absorption maximum of each fluorophore was chosen as the excitation wavelength.

3. Results and discussion

Our preliminary findings suggest that, with the appropriate choice of optical probe, FPA measurements offer a promising new approach for the reliable estimation of cmc1 and cmc2 and of changes in the morphology of the probe and phase transitions in the micelles at and above the cmc values. According to Mukherjee [9], cmcs are defined as the intersection of two straight lines joining the points below and above the cmc.

As an important preliminary step in the present work, we first ascertained the suitability of each fluorophore for use as an FPA probe in the surfactant systems studied. The results of initial screening work are summarized in Table 1. In this context, two considerations are especially important. First, the fluorophore should not show complications due to photodegradation, coulombic interactions or related effects, which should be evident from the absorption, fluorescence emission and excitation spectra in the surfactant solution concerned. Secondly, in order to obtain reliable results based on FPA measurements, the FPA should show a significant difference (0.1 or more) from the baseline value (i.e. in aqueous solution with no surfactant added) at higher ranges of surfactant concentration. This latter consideration was chosen to be the guiding factor in determining the suitability of the fluorophore as a probe for cmc determination via FPA measurements. For example, in the 1-PyCHO–SDS system, the change in anisotropy on going from a pure aqueous solution of the optical probe to a surfactant solution of [surf] $\sim10^{-2}$ M is about 0.02 only. This suggests that the probe molecules are present in a comparatively labile environment and are not sufficiently associated with the host surfactant micelles in order to be capable of reflecting the structural changes associated with the surfactant micelles. The site selection of the fluorophore is important for the determination of the cmc values.

Table 1
Suitability of the fluorophores as FPA probes in the surfactant systems studied

Fluorophore	TX 100	CTAB	SDS
7HF	−[a]	−[a]	+
PSF	+	−[a]	−[a]
1-PyCHO	+	+	−[b]

+, Suitable; −, unsuitable. [a]Complications due to photodegradation and/or coulombic interactions. [b]Intrinsically low changes in FPA value on solubilization in micelles at high surfactant concentration.

Table 2
Spectral characteristics of the fluorophores in the micellar solutions (at the highest surfactant concentrations used) and in water

Fluorophore	Concentration (M)	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)
7HF	2×10^{-5}	312 (water)	410, 531 (water)
		313 (SDS)	408, 528 (SDS)
1-PyCHO	1×10^{-6}	368 (water)	467 (water)
		362 (CTAB)	441 (CTAB)
		362 (TX 100)	439 (TX 100)
PSF	2×10^{-5}	520 (water)	584 (water)
		535 (TX 100)	568 (TX 100)

The spectral characteristics of the fluorophores in the surfactants studied, together with the corresponding values in water, are presented in Table 2. Plots of r vs. [surf] are shown in Fig. 1. Sharp breaks are obtained at [surf] values corresponding to cmc1. A less pronounced but clearly perceptible break is also obtained at values corresponding to cmc2. It is well known that, at cmc1, the changes in the physical constants associated with the change in surfactant aggregation are quite sharp. It is also noteworthy that the changes in the physical constants with surfactant aggregation at cmc2 are much less pronounced than at cmc1 [10]. This is because, at cmc2, the morphological changes in micelles occur over a wider range of surfactant concentration. The cmc values thus obtained are presented in Table 3. These are generally in

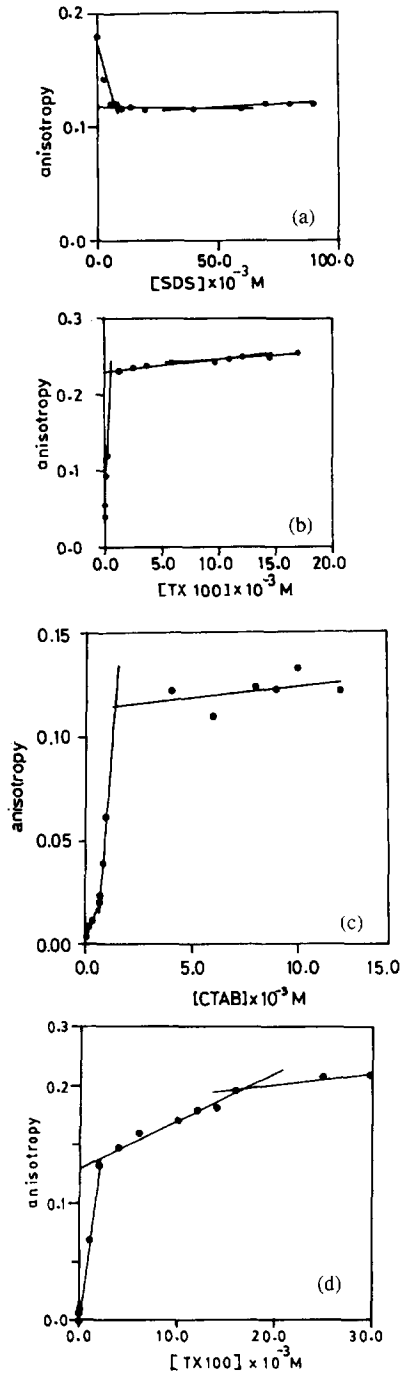

Fig. 1. Plot of fluorescence polarization anisotropy (FPA) (r) vs. surfactant concentration: (A) 7HF–SDS system; (B) PSF–TX 100 system; (C) 1-PyCHO–TX 100 system; (D) 1-PyCHO–CTAB system.

reasonable agreement with the corresponding literature data [10–16] which are also included in the table for comparison.

FPA measurements have been used previously for the estimation of the microviscosity in natural and model membranes [3]. To our knowledge, the present work shows for the first time the use of FPA for cmc determination. The occurrence of a second cmc at a higher surfactant concentration than the normal cmc has been reported for both SDS [10] and TX 100 [16,17]. However, the aggregation of surfactants in water increases only slightly with an increase in surfactant concentration above cmc2, and the change in micelle size is not very pronounced [10]. In the case of TX 100, for instance, Brown et al. [17] have observed the formation of aggregates of micelles at higher surfactant concentrations as a precursor to liquid–liquid crystal phase transitions. Chauvet et al. [16] have observed that the reaction kinetics in TX 100 micelles are strongly influenced by the surfactant concentration in the post-micellar range (below and above 10^{-2} M), which they have attributed to changes in the micellar structure and size. Since polarization anisotropy reflects the changes in the microenvironment of the fluorophore, our study can aptly reflect the changes in the fluorophore environment associated with micellar changes in shape and size. The reason for the discrepancy obtained between the estimated values of cmc for TX 100 using PSF and 1-PyCHO is not immediately apparent. This is probably related to the different site selection of the two fluorophores, which may lead to the fluorophores not sensing the morphological changes in the host micellar aggregates in an identical way. Such variations in the measurements with the use of different optical probes are not uncommon as is evident from the values reported by different workers in Table 3. Further work with other fluorophore–surfactant systems is in progress.

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Table 3
Critical micellar concentrations (cmcs) evaluated from FPA measurements with corresponding reported values

Surfactant	Estimated values (mM)			Reported values (mM)	
	Fluorophore	cmc1	cmc2	cmc1	cmc2
SDS	7HF	8.00 ± 0.50	40.00 ± 5.00	7.00 [11], 4.60 [12]	62.00 [10]
CTAB	1-PyCHO	0.65 ± 0.05	1.40 ± 0.20	0.71 [12], 1.00 [11], 0.30 [13]	0.75 [13]
TX 100	1-PyCHO	0.25 ± 0.05	11.00 ± 2.00	0.24 [14], 0.20 [11]	7.30 [15]
	PSF	0.80 ± 0.05	18.00 ± 2.00		

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