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Full Paper

Determination of the Critical Micelle Concentration of Triton X-100 Using the Compound 3-(benzoxazol-2-yl)-7-(*N*,*N*-diethylamino)chromen-2-one as Fluorescent Probe

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Abstract: In the present study we estimated the Critical Micelle Concentration (CMC) of Triton X-100 in an aqueous buffered medium (PBS buffer, pH 7.4), using 3-(benzoxazol-2-yl)-7-(*N*,*N*-diethylamino)chromen-2-one (BDC), a push-pull compound, as extrinsic fluorescence probe. The CMC value found, 0.33 mmol L^{-1}, shows good agreement with data from literature obtained using the fluorescence probe technique. Additionally, the polarity, in terms of the $E_T(30)$ scale, and the viscosity of the micelle microenvironment in which the probe is preferentially allocated, was estimated as being 46.9 kcal mol^{-1} and 70.3 cP, respectively, confirming that this microdomain is polar and highly viscous, with characteristics of polyethylene glycol groups consisting in the palisade layer at the micelle-water interface.

Keywords: critical micelle concentration; Triton X-100; extrinsic fluorescence; 3-(benzoxazol-2-yl)-7-(*N*,*N*-diethylamino)chromen-2-one; polarity; viscosity

1. INTRODUCTION

Surfactants are hierarchical materials that have gained increasing interest because of their applications, for example as templates to host and guide the growth of aligned and ordered materials with adjustable pore size, high surface areas, large pore volumes, alternative pore shapes, and controllable framework compositions [1-9]. Triton X-100 alone or combined with another drive agent has been extensively used as templates in the synthesis of mesoporous materials with catalytic activity, aiming different applications [9-10].

The Critical Micelle Concentration (CMC) is probably the simplest way of describing the colloid and surface behavior of a surfactant solution, being an important parameter for the description of this class of materials. When the concentration of surfactant exceeds the CMC, a micellar system is formed [9, 11], which generally has several applications [1, 2, 9]. The CMC can be determined by measuring changes in physical properties [12-15]. A simple and efficient approach for CMC determination consists in the use of an appropriate spectroscopic probe or a noninvasive spectroscopic methodology, using the intrinsic fluorescence naturally present within the micellar systems, to study the changes in the electronic absorption or emission energies and/or intensities [14-17].

In studies involving the characterization of 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one (BDC) [18, 19], we noted that this compound presents low tendency to formation of aggregates and good spectral sensibility to the solvent polarity due to its push-pull characteristics [19]. Additionally, it has proven to be useful as fluorescent probe to determine the CMC of self-assembling molecules in water [20]. This compound presents similar characteristics found in other fluorescent probes, as for example the

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compound C-153 (8-Trifluoromethyl-2,3,5,6-4*H*-1,*H*-11-oxa-3a-aza-benzo[de]anthracen-10-one) [21].

In the present study, the compound 3-(benzoxazol-2-yl)-7-(*N*,*N*-diethylamino)chromen-2-one (BDC), Figure 1, was used as extrinsic fluorescence probe to determine, by steady-state fluorescence, the Critical Micelle Concentration of Triton X-100 (TX-100) in phosphate-buffered saline (PBS buffer, pH 7.4) aqueous medium. Additionally, the polarity, expressed in terms of the empirical solvatochromic scale $E_T(30)$ [22] and the viscosity of the microenvironment in which the probe is preferentially allocated, was also estimated.

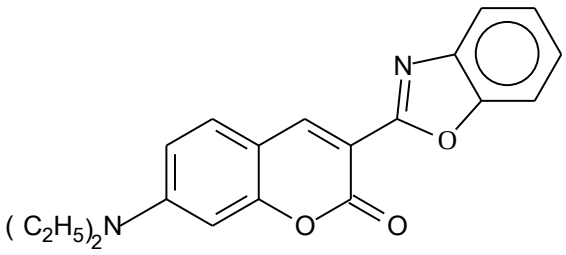

Figure 1. Representation of the compound 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one (BDC).

2. MATERIAL AND METHODS

Triton X-100 (polyethylene glycol tert-octylphenyl ether; n = 9–10, MW ≈ 625.0 g mol^{-1}, Union Carbide), a nonionic surfactant, Figure 2, was used without any treatment.

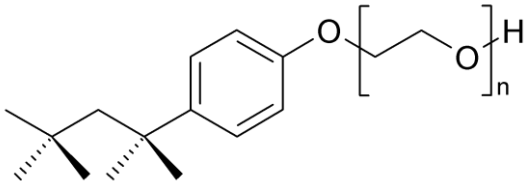

Figure 2. Representation of the surfactant Triton-X-100 [23].

The compound 3-(benzoxazol-2-yl)-7-(*N*,*N*-diethylamino) chromen-2-one (BDC), used as fluorescent probe in this study, was kindly provided by Dr. Ana M. F. Oliveira-Campos. A stock solution containing this compound was prepared in ethyl alcohol (UV/HPLC grade, VETEC Química). Similarly, a stock solution of Triton X-100 (36 mmol dm^{-3}) was prepared using buffered deionized water (phosphate buffer, PBS, pH 7.4, with a NaCl content of 0.9% (w/w)) [24-26]. Aliquots of this solution were used to prepare solutions containing 5.0 x 10^{-6} mol dm^{-3} of BDC and different concentrations of Triton X-100, ranging between 0 and 3.6 mmol L^{-1}. The solutions were prepared in such a way to keep the absorbance of the probe below 0.100 at the excitation wavelength of the probe (442 nm) in order to avoid light reabsorption effects in the fluorescence measurements.

The UV-Vis absorption and emission spectra were recorded using, respectively, a Shimadzu-2501 PC spectrophotometer and a Hitachi F-4500 spectrofluorimeter. For the fluorescence measurements the slits (excitation/emission) were both fixed in 2.5 nm and the photomultiplier (PMT) voltage set at 700 V. All fluorescence measurements were done at 293 K using a 10 mm square cuvette in a right-angle configuration.

The fluorescence quantum yields of BDC in different media were estimated from the corrected fluorescence spectra, using methodology proposed by Eaton [27]. The compound 9,10-diphenylanthracene in cyclohexane (Φ_F = 0.90 at 293 K) was employed as fluorescence standard.

3. RESULTS AND DISCUSSION

Figure 3 presents the fluorescence spectra of BDC in TX-100 micelles, *N*,*N*-Dimethylformamide (DMF) and chloroform.

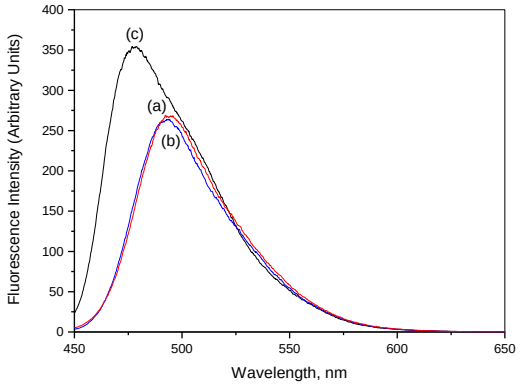

Figure 3. Emission spectra of the compound 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one (BDC) in: (a) Micelles of Triton X-100 (in a PBF buffer, pH 7.4); (b) *N*,*N*-Dimethylformamide (DMF) and (c) Chloroform. Excitation wavelength: 442 nm.

The profiles of the fluorescence spectra in DMF and in TX-100 micelles, and their respective quantum yields, are very similar, Table 1. This

suggests that BDC maintains intense polar interactions in the microdomains in which is preferentially allocated in the TX-100 micelles. This is corroborated by the E_T (30) [22] of these microdomains, estimated as being 46.89 kcal mol^{-1}. It should be emphasized that this value was estimated from the Φ_F estimated for BDC allocated in TX100 micelles, using the linear correlation between Φ_F and E_T (30) proposed in [20]. This result shows a good agreement with the reported in a previous study involving this same probe in two other surfactants (SDS and CTAB), where it was concluded that BDC tends to be allocated in a viscous, polar and hydrophilic region [20]. Despite the presence of water in this microdomain, the high viscosity of the medium should compensate at least partially the suppression of fluorescence caused by this solvent. Similar to the observed in CTAB and SDS, the microenvironment in which BDC is allocated in TX-100 possesses a high viscosity, estimated as being 70.26 ± 0.02 cP, a value little smaller than the viscosity of PEG 400 [28]. This value was estimated from a plot of the dependence of Φ_F with the viscosity of glycerol/water mixtures, proposed in [20]. Thus, this environment is sufficiently viscous to minimize the mobility of BDC, making difficult the decay of this probe in the excited state by other routes than the fluorescence. According to Kumbhakar et al., one of the reasons for the high viscosity in this microenvironment, also known as palisade layer, is the hydration of ions, especially cations, since water molecules in this region tends to undergo a kind of clustering [29**]**.

It is noteworthy that the E_T(30) of different glycols decreases as the size of the chain increases [22]. Based on this information, the E_T(30) of the palisade layer of TX-100 should be typical of oligomers based on glycol. In other words, and considering the shape proposed for TX-100 micelles [12], BDC tends to occupy polar microdomains consisting of polyethylene glycol groups (n = 9-10), near the micelle-water interface.

Table 1. Spectroscopic data for BDC in micelles and some different solvents: absorption and emission wavelengths, Stokes' shifts, fluorescence quantum yields, and the empirical solvatochromic parameter E_T(30) estimated for the different media.

Medium	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)	$\Delta\bar{\upsilon}$ [a] (cm^{-1})	Φ_F	E_T(30) (kcal mol^{-1})
TX-100/PBS buffer	452	494	1881	0.59	46.9[d]
SDS/PBS buffer [c]	463	495	1396	0.33	61.3
CTAB/PBS buffer [c]	462	496	1476	0.32	61.9
N,*N*-DMF	439	494	2536	0.56	43.2
Chloroform	441	479	1799	0.84	39.1
Methanol [b]	442	490	2216	0.42	55.4
Water [b]	452	497	2003	0.08	63.1

[a]Calculated using the absorption and emission maxima, expressed in wavenumber; [b]Ref. [19]; [c]Ref. [20]; [d]Considering the linear correlation between Φ_F and E_T(30), as shown in Ref. [20].

Figure 4 presents the dependence between the fluorescence spectra of BDC and the concentration of TX-100.

As shown in Figure 4a, the increasing presence of TX-100 aggregates tends to magnify the BDC fluorescence, when compared to what occurs in water, Table 1. As the concentration of TX-100 increases, the rise in the population of pre-aggregates, aggregates and micelles favors the incorporation of BDC in the palisade layers, intensifying the fluorescence. Figure 4b evidences this: the rise of the ratio I/I_0 as the concentration of TX-100 increases, with a change in the rate when the concentration of micelles predominates, followed by the stabilization of this ratio. Above the CMC, where BDC is predominantly associated to the micelles, the fluorescence is relatively intense. The very small blue shift of the emission maxima (about 113 cm^{-1}) that occurs as BDC fluorescence intensity increases from its solvation only in PBS buffer until TX-100 micelles, reinforces the proposition that the probe tends to migrate to the palisade layers of TX-100, as previously discussed.

The CMC of TX-100 was estimated from the analysis of the graph of the second derivative of the ratio between fluorescence intensity and concentration of the probe, $\Delta^2(I/I_0)/\Delta C^2$, versus concentration of TX-100, Figure 5.

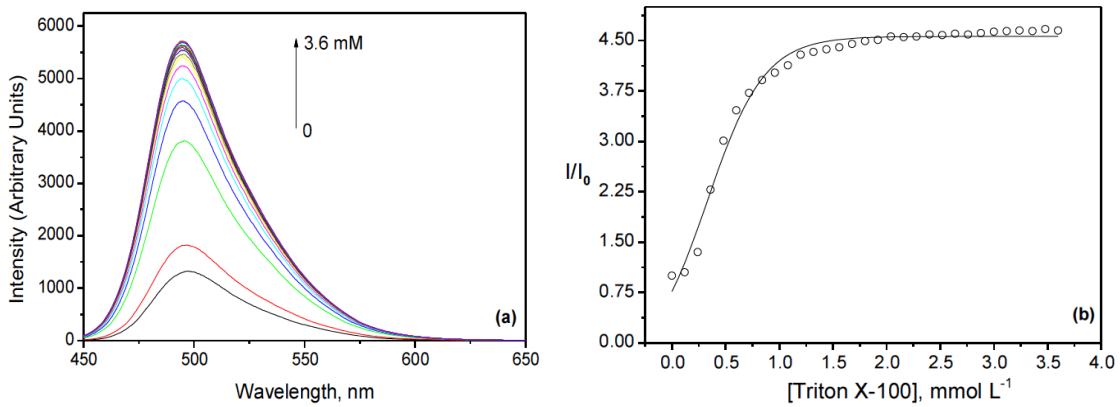

Figure 4. a) Fluorescence spectra of the compound 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one (5.0×10^{-6} mol L^{-1}), for different concentrations of Triton X-100, in the range between 0 and 3.6 mM; λ_{exc}= 442 nm. b) Ratio I/I_0 vs. concentration of Triton X-100. The fluorescence intensities correspond to maximum values of emission of BDC in micelles dispersed in the buffered aqueous medium.

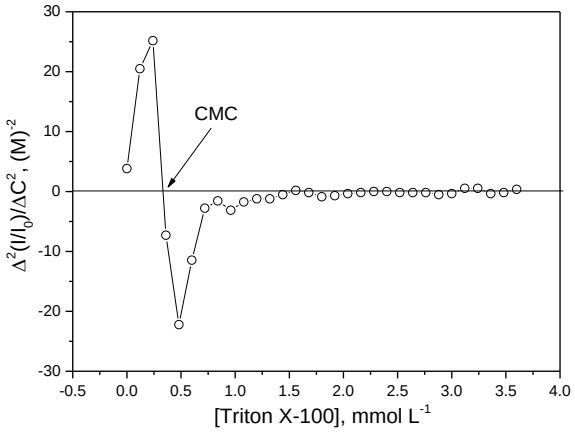

Figure 5. Graph of $\Delta^2(I/I_0)/\Delta C^2$ versus concentration of TX-100 in buffered water at pH 7.4, for solutions of BDC (5.0×10^{-6} mol L^{-1}).

The value found (0.33 mmol L^{-1}) shows a very good agreement with the reported in the literature, especially in determinations using the fluorescence probe technique. For example, Ruiz and Aguiar also found 0.33 mmol dm^{-3}, using this technique [12]. Karimi et al., demonstrating the potential application of surface plasmon resonance, estimated a CMC of 0.27 mmol L^{-1} for TX-100 in aqueous medium, using silver nanoparticles as probe [14]. Jaiswal et al., exploring the intrinsic fluorescence of TX-100 in water, found a CMC of 0.29 mmol L^{-1}, while using two different extrinsic fluorescent probes (C-153, or 8-Trifluoromethyl-2,3,5,6-4*H*-1,*H*-11-oxa-3a-aza-benzo[de]anthracen-10-one**,** and ANS, or 8-anilinonaphthalene-1-sulfonic acid), found respectively, 0.24 and 0.27 mmol L^{-1} [15]. The same authors, using Isothermal Titration Calorimetry, estimated a CMC of 0.21 mmol L^{-1} [15].

4. CONCLUSION

In the present study the Critical Micelle Concentration (CMC) of the surfactant Triton X-100 was estimated using the extrinsic fluorescence probe 3-(benzoxazol-2-yl)-7-(*N*,*N*-diethylamino) chromen-2-one (BDC), in aqueous and buffered (PBS buffer, pH 7.4) medium. The value found, 0.33 mmol L^{-1} agrees well with the reported in the literature, principally in determinations using the fluorescence probe technique. The observed behavior by BDC confirms the usefulness of this compound as fluorescent probe. The steady-state fluorescence measurements confirms that the microdomain in which BDC is preferentially allocated in Triton X-100 micelles (the palisade layer) is considerably polar (46.9 kcal mol^{-1}) and highly viscous (70.3 cP). This high viscosity is probably a consequence of the hydration of the ions from the buffered medium in the palisade layer, since water molecules in this microdomain tend to be as clusters.

5. ACKNOWLEDMENTS

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