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3-Benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one as a fluorescence probe for the investigation of micellar microenvironments

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Abstract

It was shown that 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one is suitable to perform as a fluorescent probe, to analyse physical parameters related to organised systems. Micropolarity, microviscosity, critical aggregation and micelle concentration data, in SDS and CTAB micelles, using the proposed compound as fluorescent probe show the compound is allocated, in both micelles, in a strongly polar hydrophilic region (the parameter $E_T(30)$ is equal to 61.3 and 61.9 kcal/mol, respectively, in SDS and CTAB micelles, whereas Lippert's Δf parameter is equal to 0.35). The CMC of SDS in pure water (7.5×10^{-3} mol/dm^3) agrees with the reported values. For SDS and CTAB micelles in buffered medium (PBS buffer, pH 7.4, NaCl 0.9%, w/w), the estimated critical aggregation concentrations (4.6×10^{-3} and 9.2×10^{-4} mol/dm^3, respectively, for SDS and CTAB) also agree with the results obtained using equivalent conditions.

Keywords: Coumarin derivative; Fluorescent probe; Micropolarity; Microviscosity; CMC; CAC

1. Introduction

Fluorescent probes have been used with success in the sensing of organised media [1–4]. These media has been a particular focus of scientific research, principally considering the capability of some of them to efficiently accommodate and transport drugs [5–7]. Also, some of these systems have been used as models to simulate particularities of biological environments, or even to investigate why the course of photosensitised reactions tend to suffer significant changes when they occur in organised systems [6,7].

In recent studies involving the characterisation of some coumarin derivatives [8–10], Machado et al. have observed that 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one shows a considerable solvatochromism, besides dual fluorescence strongly dependent on solvent polarity [9]. These characteristics show to be useful parameters for its use as fluorescent probe to study organised environments.

In this paper, different physical properties of two organised systems (SDS and CTAB micelles) were evaluated using the compound 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one.

2. Experimental

The compound under study was synthesised by Oliveira-Campos and co-workers [11] (Fig. 1).

Sodium dodecyl sulphate, SDS (VETEC), cetyltrimethyl ammonium bromide, CTAB (MERCK), and glycerol (VETEC), were used as received. SDS and CTAB solutions were prepared in phosphate buffer (PBS, pH 7.4) using deionised water, based on a procedure described previously [12–14]. Sodium chloride was added at a concentration of 0.9% (w/w), to make the salinity of the solution equivalent to that presented by a physiological fluid, and to control the ionic strength of the medium.

The essays using micelles were done at a concentration of 30.0×10^{-3} mol/dm^3 for SDS, and 20.0×10^{-3} mol/dm^3 for CTAB.

The absorption spectra were recorded using a Shimadzu UV-RECORDING model 2501PC spectrophotometer. The fluorescence measurements were done using a HITACHI F-4500 spectrofluorimeter. The fluorescence quantum yields were estimated from the corrected fluorescence spectra using 9,10-diphenylantracene in cyclohexane ($\Phi_F = 0.90$ at 293 K) as standard [15]. Solutions were prepared with absorbance values lower than 0.100 at the excitation wavelength aiming to minimise self-absorption effects.

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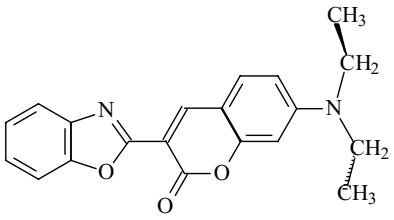

Fig. 1. Structure of 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one.

The viscosity measurements were performed at 298 K using a RHEOCALC V1.3 model RV (Brookfield Engineering Labs) viscometer. For these measurements, mixtures having different glycerol/water (v/v) ratios were used to prepare a calibration curve.

3. Results and discussion

Fig. 2 presents the fluorescence spectra of 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one in both micelles.

Both fluorescence bands present an emission profile typical of the probe maintaining intense polar interactions with the solvent. In this situation, the observation of only one fluorescence peak is usual, and is attributed to the emission of the S_2 (TICT) state [9]. This behaviour is more evident if the compound under study is solvated in protic solvents, mainly alcohols. The measured fluorescence quantum yields (Table 1) are comparable, lower than the value in methanol, but higher than that verified for pure water [9], although the medium is predominantly aqueous. The rate of non-radiative deactivation of the excited state in SDS/PBS is only two times the fluorescence rate, differently from what occurs in water, where $k_{nr} \approx 11k_f$ [9]. This is only justifiable if the microenvironment in which the probe is allocated is considerably viscous to reduce its mobility, partially favouring fluorescence as deactivation route. A similar behaviour should be observed in CTAB/PBS micelles.

Table 1
Spectroscopic data for the probe in SDS and CTAB micelles: absorption and emission wavelength, Stokes' shift and fluorescence quantum yields

Medium	λ_{abs}^{max} (nm)	λ_{exc}^{max} (nm)	λ_{em}^{max} (nm)	$\Delta\bar{\upsilon}$[a] (cm^{-1})	Φ_F[b]	k_{nr}/k_f[b]
SDS/PBS buffer	463	463	495	1396	0,33	2.030
CTAB/PBS buffer	462	462	496	1476	0.32	–
Methanol[b]	442		490	2216	0.42	1.381
Water[b]	452		497	2003	0.08	11.11

[a] Calculated from emission and excitation maxima, expressed in wavenumber.
[b] Ref. [9].

3.1. Polarity in the micelle environment

The polarity of the microenvironment in which the probe is allocated in both micellar systems was estimated using the $E_T(30)$ empirical solvatochromic scale [16] and the spectroscopic data available for this probe [9]. As can be seen, the data present a good linear correlation between the emission quantum yields and $E_T(30)$ (Fig. 3).

The values found, respectively, 61.3 and 61.9 kcal/mol, for SDS and CTAB, agree with the expected, confirming the allocation of the probe in a highly polar medium, as suggested by Almgren et al. [17], which showed that aromatic probes and aromatic or ionic quenchers reside preferentially in the polar shell of ionic micellar systems. The values are quite similar, indicating that the probe is in an equivalent neighbourhood in both micelles.

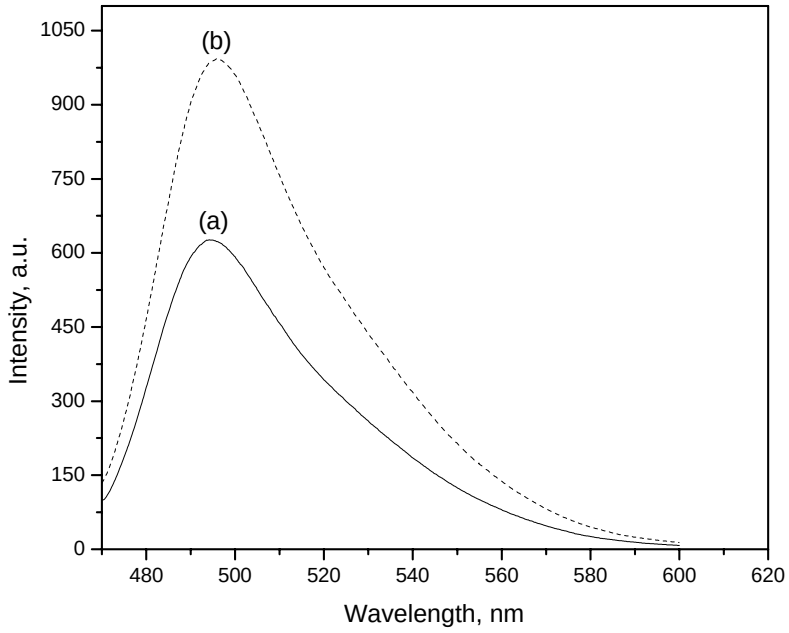

Fig. 2. Fluorescence spectra of the probe (10^{-6} mol/dm^3) in SDS/PBS buffer (30×10^{-3} mol/dm^3) (a), and CTAB/PBS buffer (20×10^{-3} mol/dm^3) (b).

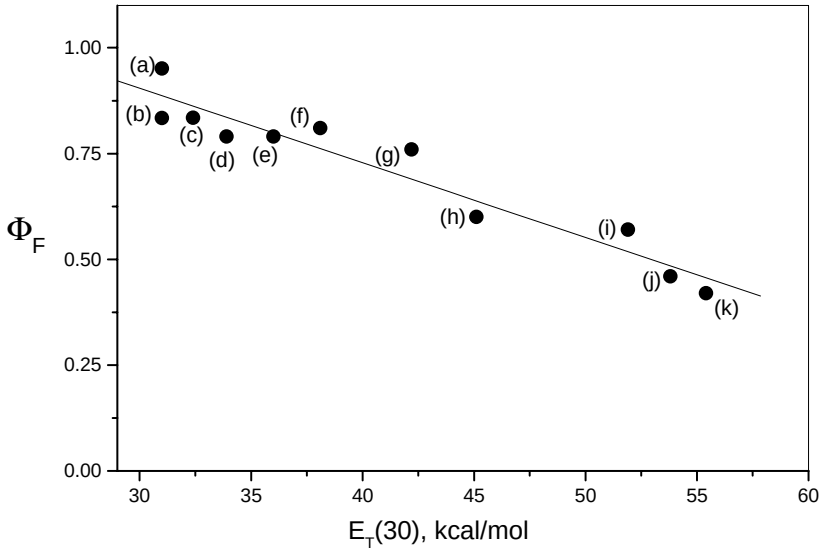

Fig. 3. Ratio between fluorescence quantum yield and $E_T(30)$ parameter: (a) *n*-decane, (b) *n*-hexane, (c) carbon tetrachloride, (d) toluene, (e) 1,4-dioxane, (f) ethyl acetate, (g) acetone, (h) dimethylsulfoxide, (i) ethanol, (j) ethyleneglycol, (k) methanol. $\Phi_F = 1.434 - 0.018E_T(30)$ ($R = -0.96$).

A good quantitative description of the medium by an empirical polarity scale needs to take into account all non-specific and specific solute–solvent, solvent–solvent and, if at higher concentrations, even solute–solute interactions. A simple definition of solvent polarity is, in reality, a very difficult task. Reichardt has proposed that solvent polarity can be defined as the overall solvation capability (or solvation power) of solvents, which in turn depends on the action of all possible, non-specific and specific, intermolecular interactions between solute ions or molecules and solvent molecules, excluding, however, those interactions leading to definite chemical alterations of the ions or molecules of the solute (such as protonation, oxidation, reduction, chemical complex formation, etc.) [16].

Although $E_T(30)$ cannot be considered a complete solvatochromic scale to describe polarity, it was capable to give a good description of the studied systems, probably due to the large number of solvated charged species in the medium. The characteristics of the probe (in which an ICT process occurs, resulting in a charge separation between the diethylamino group and the rest of the molecule) [9] make it useful to describe the $E_T(30)$ scale.

In Fig. 3, the Φ_F data in pure water was avoided considering that it could falsify the final result, since this value is considerably low, probably due to the aggregation phenomena. The estimated $E_T(30)$ value for water is quite different from the tabulated [16].

Although limited for the description of specific solute–solvent interactions [16,18], the Lippert's Δf scale has been used to evaluate microenvironments of organised media. Krishanamoorthy and Dogra [3,4] obtained a value close to 0.28 in SDS micelle, using an imidazol derivative with a diethyl amino group in its structure. Kalyanasundaram and Thomas [19] reported a Δf close to 0.30 using pyrene as probe, in a system composed by SDS micelles in water. Our estimate using this scale resulted in a value equal to 0.35, for buffered SDS and CTAB [20], compatible with a probe positioned in a polar neighbourhood [18].

The interaction of the probe with ions, especially anions, in a highly hydrophilic region, formed by the surfactant head groups, counter ions, other ions and water molecules, justifies its behaviour. A strong polar interaction between anions and the diethyl amino group will favour the displacement of the equilibrium [9].

$$S_1(LE) \rightleftarrows S_2(TICT)$$

Toward the S_2 (TICT) state, which explains the shape of the fluorescence spectrum of the probe in both micelle media.

3.2. Microviscosity in the micelle environment

The plot of the Φ_F data versus viscosity (expressed in terms of $\ln \eta$) for the probe in glycerol/water mixtures shows that it is sensitive to viscosity (Fig. 4), with a good linear correlation.

This binary mixture was selected considering that besides being a mixture of protic solvents, the $E_T(30)$ parameter for both solvents [16] is near the value estimated for the microenvironment under study.

From the plot Φ_F versus $\ln \eta$ it was possible to estimate the viscosity of the microenvironment in which the probe is allocated. The values are 7.40 ± 0.03 and 6.78 ± 0.03 cP, respectively, in SDS and in CTAB. They are close values and, as expected, represent sufficiently high viscosities to justify the photophysical behaviour of the probe.

Fig. 5 presents for these mixtures, as expected for the probe in protic solvents, a fluorescence profile showing preponderantly the band attributed to the S_2 (TICT) state.

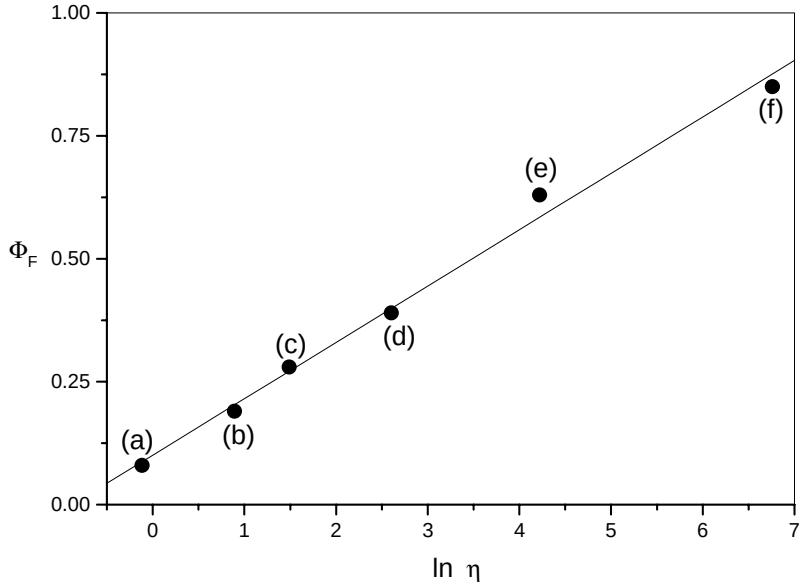

Fig. 4. Ratio Φ_F vs. $\ln \eta$ for glycerol/water mixtures, at 298 K: (a) pure water, (b) 80% water, (c) 60% water, (d) 40% water, (e) 20% water, (f) pure glycerol. $\Phi_F = 0.101 + 0.115 \ln \eta$.

Table 2 presents the spectroscopic data measured for the probe in glycerol/water mixtures.

An attempt to estimate the microviscosity using a binary mixture of two low polarity aprotic solvents (*n*-decane and Nujol®), was not well succeeded. This is probably due this solvent mixture is more suitable to reproduce hydrophobic microenvironments. In these microenvironments the probe presents dual fluorescence [9].

As the micelle medium is very unhomogeneous and highly dynamic, it is difficult to estimate absolute values for this property, unless using time resolved measurements or other elaborated techniques, such as electron spin resonance [22–24]. Evidently, the microviscosity values are related to the positioning of the probe into the aggregate. Ranganathan et al., using cetylpyridinium chloride as quencher of pyrene fluorescence, estimated a microviscosity of 7.2 cP for SDS micelles ([SDS] = 50×10^{-3} mol/dm^3) in water without NaCl. The authors observed that this parameter depends only on the micelle aggregation number and not on the particular surfactant and salt concentrations, and is related to the polar shell [23,24].

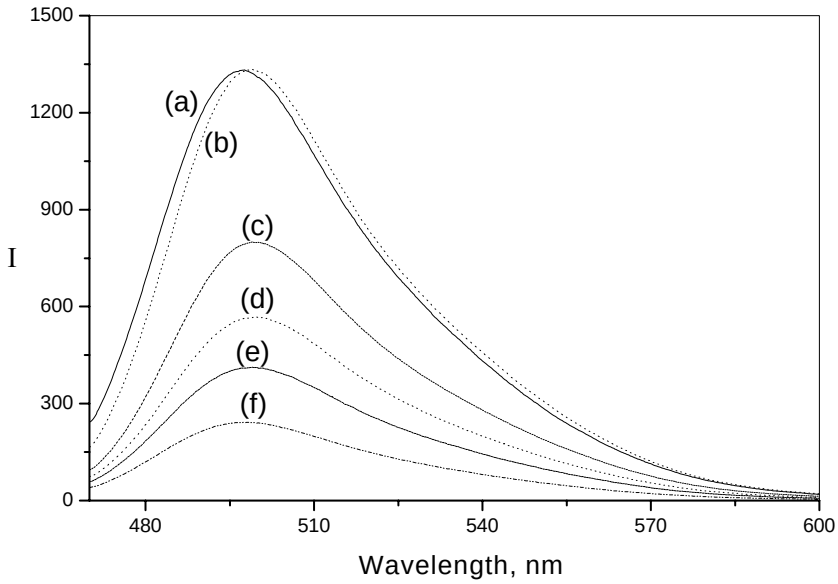

Fig. 5. Fluorescence spectra of 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one (10^{-6} mol/dm^3) in glycerol/water mixtures: (a) pure glycerol, (b) glycerol 80%, (c) glycerol 60%, (d) glycerol 40%, (e) glycerol 20%, (f) pure water.

Table 2
Spectroscopic and other data for 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one

Water (%)	η[a] (cP) (at 298 K)	λ_{abs}^{max} (nm)	λ_{exc}^{max} (nm)	λ_{em}^{max} (nm)	$\Delta\bar{\upsilon}$[c] (cm^{-1})	Φ_F
100	0.89[b]	454	455	497	1857	0.08
80	1.92	456	456	499	1890	0.19
60	2.77	456	456	500	1914	0.28
40	13.39	457	460	499	1699	0.39
20	63.00	457	457	499	1842	0.63
0	663.81	456	459	498	1690	0.85

[a] Experimental data.
[b] Ref. [21].
[c] Calculated from excitation and emission wavenumbers.

3.3. Estimative of the critical micelle concentration for solutions containing SDS and CTAB in buffered media

Fig. 6 shows a typical graph relating the changes in the fluorescence intensity of the probe as function of the SDS concentration. In CTAB a similar behaviour is observed.

The critical micellar concentration (CMC) was estimated from the $\Delta^2 I/\Delta C^2$ versus surfactant concentration plot. A value of 4.6×10^{-3} mol/dm^3 was obtained for SDS, very close to the estimated by Benito et al. (5.0×10^{-3} mol/dm^3), using methylene blue as fluorescent probe, in buffered medium [25]. These values are lower than the usually stabilished CMC for SDS in water, around 8.0×10^{-3} mol/dm^3 [26,27]. The estimated CMC value of SDS using deionised water as solvent was 7.5×10^{-3} mol/dm^3, in good agreement with the value reported in the literature [26,27].

The values found for buffered solutions are not properly the CMC of SDS, but a critical aggregation concentration (CAC) for the experimental conditions used. This lower value can be taken as an indication of a cooperative effect due to the presence of electrolytes in solution, since the electrolytes tend to actuate reducing the repulsion between the hydrophilic groups of the micelle [28]. A similar effect is also observed when alcohols, principally long chain alcohols, are added to the solution containing the surfactant, which can form quasi-micelle aggregates with the surfactant [28]. It is known that certain molecules (long chain alcohols, emulsifying agents, salts, etc.) tend to interact cooperatively with monomer molecules that result in quasi-micelle aggregates.

For CTAB in PBS buffer, the estimated CAC was 9.2×10^{-4} mol/dm^3, similar to the values estimated under equivalent conditions [25,27,29]. Values between 0.7 and 1.5×10^{-3} mol/dm^3 have been reported [29], and are dependent on the degree of dissociation of the surfactant. Small anions tend to reduce the CMC of CTAB [29], which explains the measured value.

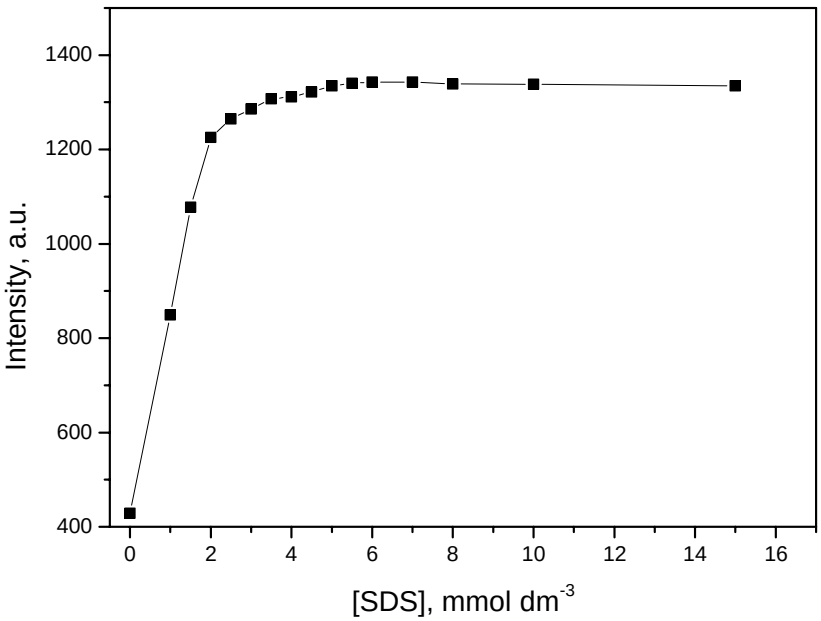

Fig. 6. Changes in the fluorescence intensity at 495 nm, for 3-benzoxazol-2-yl-7-(*N*,*N*-diethylamino)-chromen-2-one (λ_{exc} = 462 nm), with increasing SDS concentration, in a buffered medium with constant ionic strength. Probe concentration: 5.0×10^{-6} mol/dm^3.

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