Determination of the critical micelle concentration of surfactants and amphiphilic block copolymers using coumarin 153

Telmo J.V. Prazeres, Mariana Beija ¹, Fábio V. Fernandes, Paulo G.A. Marcelino, José Paulo S. Farinha *, J.M.G. Martinho *

Centro de Química-Física Molecular and IN – Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal

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Abstract
We describe a method to determine the critical micelle concentration (CMC) of low molecular weight surfactants and amphiphilic diblock copolymers in water based on the use of the fluorescent dye coumarin 153 (C153). The method is based on the measurement of the fluorescence intensity, solvatochromic shift and fluorescence anisotropy of C153 and was tested with the low molecular weight surfactants SDS, CTAB and Triton X-405 in water, for which we obtained, within the experimental error, CMC values identical to those previously published. The method was further used to determine the CMC of a family of poly(N-decylacrylamide)-b-poly(NN-diethylacrylamide) amphiphilic block copolymers synthesized by RAFT polymerization, with an equal poly(N-decylacrylamide) hydrophobic block length and increasing poly(N,NN-diethylacrylamide) hydrophilic block lengths. By measuring the fluorescence anisotropy of C153 in block copolymer aqueous solutions, it is also possible to detect the formation of pre-micellar aggregates at concentrations below the CMC. The dye C153 is more appropriate than pyrene to study self-assembly in water, where pyrene forms ground and excited state aggregates at very low concentrations. Furthermore, C153 is an excellent anisotropy probe with a very high limiting anisotropy (0.375) that can be used to determine the CMC, detect the presence of pre-micellar aggregates, and evaluate the fluidity of the hydrophobic core of block copolymer micelles.

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1. Introduction

Above a critical concentration, amphiphilic molecules spontaneously self-assemble in aqueous solutions to form aggregates of various morphologies [1–4]. Small surface active amphiphiles (surfactants), can be either ionic (cationic, anionic, and zwitterionic) or non-ionic, and usually form spherical micelles above a critical micelle concentration (CMC). The micellization results from the balance of the hydrophobic attractions (the hydrophobic effect leading to the association of the hydrocarbon chains to minimize the interactions with water) [4–6], and the ionic or steric repulsions between the ionic or hydrophilic head groups. The micelles are stabilized in water by the favorable interactions between the hydrophilic groups and the water molecules forming a stable hydrated shell between the hydrophobic core and the bulk water. For ionic surfactants, the shell surrounding the core is a charged diffuse Guoy–Chapman layer, while for the non-ionic surfactants it is a hydrated “hairy” palisade layer [3,7].

Linear amphiphilic diblock copolymers also self-assemble at a critical concentration in selective solvents (i.e. good for one block and poor for the other) [8,9]. In water, amphiphilic block copolymers often self-assemble into spherical micelles with a core–shell (or core–corona) structure in which the hydrophobic block forms the core and the hydrophilic block the outer spherical shell or corona [10–18]. Depending on the relative lengths of the hydrophobic and hydrophilic blocks, micelles are dubbed star micelles if the core is small compare to the corona, and crew cut micelles if the water soluble block is small compare to the hydrophobic block [19]. Amphiphilic copolymers can also self-assemble into other morphologies (wormlike micelles, flower-like micelles, etc.), depending on a number of structural factors and experimental conditions [20–24].

The CMC is an important parameter to characterize micelle formation, both for small surfactant molecules and for amphiphilic block copolymers. The determination of the CMC involves the measurement of a physical quantity that changes upon micellization, such as surface tension, solution enthalpy, or light scattering intensity [3]. A very useful method to determine the CMC is based on fluorescent probes added to the solution or covalently linked to the hydrophobic moiety of the amphiphilic molecule [25,26]. In

* Corresponding authors.
E-mail addresses: farinha@ist.utl.pt (José Paulo S. Farinha), jgmartinho@ist.utl.pt (J.M.G. Martinho).
1 Current address: Centre for Advanced Macromolecular Design (CAMD), School of Chemical Sciences and Engineering, University of New South Wales, NSW 2052, Sydney, Australia.

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this case the CMC can be determined from a change in the fluorescence spectra intensity or shape, a solvatochromic shift, a change in fluorescence lifetime or in the polarization occurring upon micellization. Among those probes, pyrene has been the most used because the intensity and shape of its fluorescence spectrum and lifetime are sensitive to the local environment [27–33]. Indeed, the pyrene lifetime increases from ca. 200 ns in water [31,32] to ca. 450 ns in cyclohexane with the corresponding increase of quantum yield [34], due to the suppression of non-radiative pathways of de-excitation [34,35]. Besides, the vibronic structure of the emission spectrum also changes [36], without significant variation of their spectral position [31,37,38]. The variation of the vibronic fluorescence intensity ratio of the first to the third monomer peaks, \( r_1/r_3 \), is often used to probe the polarity of the medium (defining the so-called Py scale) [35,33]. This ratio can be used to study self-assembly of amphiphilic species in water because, while pyrene in water has \( r_1/r_3 \approx 1.9 \), above the CMC pyrene migrates to the micelles hydrophobic core and in an apolar medium this ratio changes to \( r_1/r_3 \approx 1 \).

Coumarin 153 (C153), a derivative of 7-aminocoumarin with a trifluoromethyl group at position 4 (structural properties summarized in Table 1), is structurally rigid because the rotation of the 7-trifluoromethyl group at position 4 (structural properties summarized in Table 1) is close to the theoretical value of 0.4 (predicted for collinear transition dipole moments in the electronic absorption and emission transitions) [57]. Indeed, the anisotropy decay in homogeneous media is a single exponential (in water the rotation correlation time is 85 ps [58]) with limiting anisotropy values as high as 0.7 [59,60], that generally decrease in heterogeneous media [61–66]. Since C153 is a hydrophobic dye, it preferentially locates at the hydrophobic core of the micelles [67]. This property, along with its interesting photophysical characteristics, led us to evaluate C153 as a probe to determine the CMC of surfactants and amphiphilic block copolymers. We believe that C153 can advantageously replace pyrene in this application, which not only tends to aggregate at very low concentrations because of its very low solubility in water (<10^{-7} M), but also strongly adsorbs to the walls of glass/quartz cells. This causes frequent irreproducible results since the fluorescence decay times, and the vibronic structure and intensity of the pyrene fluorescence spectrum are influenced by emissive aggregates directly excited from the ground state or produced by excited state reactions.

In this work, we show that using C153 we can calculate reliable CMC values for several surfactants and linear diblock copolymers in water, and also obtain information about the presence of pre-micellar aggregates and the polarity of the micelle core microenvironment. Moreover, the use of C153 is easier, less time consuming, more accurate and more reproducible than the use of pyrene.

### 2. Experimental

#### 2.1. Materials

THF (Aldrich, 99%) was distilled over CaH₂, sodium dodecyl sulfate (SDS, Fluka, 99%), Triton X-405 (70% in H₂O, Aldrich) and cytosyltrimethylammonium bromide (CTAB, Fluka, 98%), and Coumarin 153 (C153, Fluka, 98%), were used as received.

Phenanthrene-α-end-labeled poly(N-decylacrylamide)-b-poly(N, N-diethylacrylamide) copolymers (Phe-PDAm-b-PDEA, Scheme 1) with different PDEA block lengths and a PDEA block with the same length (Table 3) were synthesized using a sequential RAFT polymerization strategy, as reported elsewhere [25,68–70]. The phenanthrene-α-end-labeled poly(N-decylacrylamide) (Phe-PDAmacroCTA) with an average-number molecular weight of \( M_n = 2720 \) g/mol, corresponding to a polymerization degree of 11 and \( M_n/M_0 = 1.13 \) (determined by MALDI-TOF MS) was synthesized using \( N -(4-(9-phenanthrenyl)butyl)-2-[(2-phenyl-1-thioxo)thio]propanamide (PBTP) as a chain transfer agent [69]. The average molecular weight and dispersity of the copolymers were obtained by size exclusion chromatography using a multi-angle light scattering detector (GPC-MALS) [25,69].

The elimination of the α-end thiocarbonylthio group from Phe-PDAm-b-PDEA block copolymers by aminolysis was previously determined in Table 1: Structural Properties of C153:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>( l_{\text{ex}}^{\text{max}} ) (nm)</th>
<th>( l_{\text{em}}^{\text{max}} ) (nm)</th>
<th>( \phi )</th>
<th>( \chi ) [ns]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>543</td>
<td>549</td>
<td>0.12</td>
<td>[43]</td>
</tr>
<tr>
<td>MeOH</td>
<td>548</td>
<td>548</td>
<td>0.10</td>
<td>0.5</td>
</tr>
<tr>
<td>50% ETOH</td>
<td>542</td>
<td>542</td>
<td>0.38</td>
<td>4.7</td>
</tr>
<tr>
<td>ETOH</td>
<td>531</td>
<td>531</td>
<td>0.38</td>
<td>3.4</td>
</tr>
<tr>
<td>PyOH</td>
<td>530</td>
<td>530</td>
<td>0.38</td>
<td>3.4</td>
</tr>
<tr>
<td>MeCN</td>
<td>418</td>
<td>521</td>
<td>0.59</td>
<td>5.03</td>
</tr>
<tr>
<td>CH₃CN</td>
<td>409</td>
<td>501</td>
<td>0.56</td>
<td>5.6</td>
</tr>
<tr>
<td>EtOAc</td>
<td>393</td>
<td>455</td>
<td>0.89</td>
<td>4.31</td>
</tr>
</tbody>
</table>

\( * \) \% v/v; MeOH = methanol, ETOH = ethanol, PrOH = propanol, MeCN = acetonitrile, EtOAc = ethylacetate, CH₃CN = Cyclohexane.
reported [25,69]. It was also shown that the fluorescence lifetime of the phenanthrene derivative in THF was ca. 47 ns, indicating that by removing the α-end thiocarbonylthio group, fluorescence quenching was effectively eliminated. This conclusion is supported by a previous observation in which the thiol group (−SH) does not quench the fluorescence of C343 [70].

### 2.2. Low molecular weight surfactant solutions in water

The samples for the fluorescence experiments were prepared by using a solvent-assisted solubilisation method in order to uniformly distribute the C153 molecules in the surfactant aqueous solutions. A concentrated solution of C153 was prepared in THF (6.6 × 10^{-5} M). To approximately 15 μL of this solution we slowly added ~1 mL of aqueous surfactant solution. A set of surfactant solutions (concentrations ranging from ~5 × 10^{-5} to ~0.01, 0.1 and 0.4 M for CTAB, Triton X-405 and SDS, respectively), containing the same amount of C153 (1.0 × 10^{-6} M) were prepared. The quantity of THF was always lower than 1.5 v/v of the total micellar aqueous solution (the samples were subjected to a gentle flow of N2(g) for approximately 15 min and the final volume was adjusted again by adding a required volume of water). It was assumed that the presence of this small quantity of THF in water does not affect the micellar system properties [8,71].

### 2.3. Phe-PDcAm-b-PDEA block copolymer solutions in water

The block copolymer solutions in water were also prepared by using a solvent-assisted solubilisation method, in order to obtain monodisperse micelles, prevent the presence of large aggregates, and uniformly disperse the C153 in the micelle cores. THF was used because it is a good solvent for both blocks and the dye. We first prepared concentrated solutions (~75 g/L) of copolymers CP2–CP5 (Table 3) in THF. A small volume of these concentrated solutions were transferred into small vials already containing the required amount of C153, and immersed in an ice bath at ~2°C. Then, cold water was added dropwise under gentle agitation until reaching the required volume. The final solutions (always containing less than 2% v/v of THF) were equilibrated for at least 24 h (and stored) at 4°C.

### 3. Results and discussion

#### 3.1. Low molecular weight surfactants

The fluorescence quantum yield of C153 is higher in apolar solvents and its dipole moment increases upon electronic excitation to the first excited singlet state (Tables 1 and 2). Consequently, the fluorescence spectra changes with the local polarity (the intensity changes and there is a spectral shift in the wavelength of the maximum intensity) and this can be used to determine the critical micelle concentration (CMC) of self-assembling molecules in water. Fig. 1A–C shows the fluorescence spectra of C153 in aqueous solutions of different surfactants (Triton X-405, SDS and CTAB – Scheme 2), while Fig. 2A–C shows the spectra normalized at the wavenumber of the maximum intensity. The spectra show that by increasing the surfactant concentration above a critical value, the C153 fluorescence quantum yield increases and the fluorescence band maximum is blue-shifted. Figs. 1D–F and 2D–F show the plot of the fluorescence intensity (F.I.) and wavenumber (ν_{max}) at the maximum of the fluorescence band as a function of the molar concentration of surfactant. The fluorescence intensity of C153 in water is very low, due to its low quantum yield in water, φ = 0.10–0.12 (Table 2). For low concentrations of surfactant, the dye is mostly in water and so the fluorescence intensity is very low and practically invariant (Fig. 1A–C). In the case of Triton X-405, the fluorescence intensity slightly increases, probably due to some association between coumarin C153 and the surfactant molecules. Above the CMC a steep increase in the fluorescence intensity of C153 is observed because at concentrations larger than the CMC, C153 partitions into the hydrophobic core of the micelles. This also explains the blue shift observed for C153 at high surfactant concentrations (cf. Fig. 2A–C): since the dipole moment of the dye is higher in the first electronic excited state than in the

#### 2.4. Absorption and fluorescence measurements

The UV–Vis absorption spectra were recorded in a Shimadzu UV-3101PC. The steady-state fluorescence spectra were acquired in a SLM-AMINCO 8100 Series 2 spectrofluorometer. The fluorescence spectra were corrected for the background and for the response of the detection system. The steady-state fluorescence-polarized components relatively to the direction of the excitation light were acquired using Glan-Thompson polarizers and corrected for the background. The excitation wavelength was 410 nm (8 nm bandwidth) and the spectrum was recorded from 420 to 800 nm (8 nm bandwidth). The temperature was controlled with a water circulating bath (±0.2°C, Julabo-model F25).

<table>
<thead>
<tr>
<th>Copolymers</th>
<th>GPC-MALS</th>
<th>X_m</th>
<th>X_n</th>
<th>M_w/gmol⁻¹</th>
<th>M_w/M_n</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP2</td>
<td>11</td>
<td>146</td>
<td>21200</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>CP3</td>
<td>11</td>
<td>227</td>
<td>31600</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>CP4</td>
<td>11</td>
<td>295</td>
<td>40300</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>CP5</td>
<td>11</td>
<td>468</td>
<td>62300</td>
<td>1.02</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Structure of the Phe-PDcAm-b-PDEA copolymers (n = 11, m = 146, 227, 295, and 468).
ground-state, the change to an apolar environment desestabilizes more the first excited state than the ground state, and thus decreases the wavelength of fluorescence emission [72].

Fig. 1D–F shows the plot of the fluorescence intensity and Fig. 2D–F the wavenumber of the maximum of the fluorescence band as a function of the surfactant concentration. The CMC values were determined for each plot from the intersection of two lines drawn through the experimental points for surfactant concentrations below and above the critical surfactant concentration. The CMC values determined from the variation of the fluorescence intensity and the solvatochromic shifts with the surfactants concentration are summarized in Table 4. The values obtained for cationic (CTAB), anionic (SDS) and nonionic (Triton X-405) surfactants agree with the values reported in the literature (Table 4), obtained using different experimental procedures. However, the uncertainty in the CMC values are usually large due to the formation of pre-micellar aggregates and the limitations of the experimental methodologies.

The solvatochromic behavior of C153 also allows the determination of the relative polarity of the microenvironment where the dye is located (micelle core interior or core–shell interface), by the analysis of the relative variation of the hypsochromic shift ($\Delta \nu$) between the aqueous phase and the plateau value [85]

$$\Delta \nu = \nu_{\text{max(plateau)}} - \nu_{\text{max(H}_2\text{O)}} \text{cm}^{-1}$$

where $\nu_{\text{max(plateau)}}$ and $\nu_{\text{max(H}_2\text{O)}} = 18020 \text{ cm}^{-1}$ are the wavenumbers at the maximum of the fluorescence band (in the plateau region) and in water, respectively. The plateau is not well defined for Triton X-405, due to the low solubility of this surfactant in water. The values of $\Delta \nu$, obtained from the plots for these surfactants, are shown in Table 4. These values show that the polarity of the core of SDS and CTAB micelles is identical, while the core of Triton X-405 micelles is less polar.

3.2. Phe-PDcAm-b-PDEA block copolymer micelles

Micellar aggregates formed by block copolymers have various microenvironments: the micelle core is hydrophobic, the water-rich shell is hydrophilic and the core–shell interface has an intermediate polarity [67]. The Phe-PDcAm-b-PDEA copolymers CP2–CP5 form micelles in water, with a radius that depends on the length of the hydrophilic PDEA block, while the core size is almost constant and equal to $r_{\text{core}} = 4 \text{ nm}$ [25].

In order to prevent quenching of the C153 fluorescence by the thiocarbonylthio group, the copolymers were treated with an excess of hexylamine in order to remove this group. The critical micelle concentrations (CMC) of the copolymers were determined by the same procedure used for the low molecular weight surfactants. The samples were prepared by varying the copolymer concentration from $\sim 30$ to $\sim 1.1 \text{ g/L}$, and keeping the concentration of C153 constant ([C153] = 0.45 $\mu$M).

Fig. 3A and B shows the plots of the fluorescence spectra (A) and the fluorescence spectra normalized at the wavelength of the maximum of the fluorescence band (B), as a function of the concentration of CP3 in water. Similar plots were obtained for the other copolymers. The fluorescence intensity (F.I.) and the wavenumber

Scheme 2. Structures of Triton X-405 (n ~ 40), SDS and CTAB.
At the maximum of the fluorescence band are plotted as a function of the concentration of CP3 in Fig. 3C and D. As observed for low molecular weight surfactants, at a given onset concentration a pronounced increase in fluorescence intensity and \( m_{C22} \max \) was observed due to the increasing partition of the dye from the aqueous medium to the hydrophobic micelle cores.

Table 5 shows the CMC values for the copolymers obtained as the intercept of the two lines drawn for concentrations below and above the critical polymer concentration (Fig. 3).

The CMC values are similar for all copolymers and so almost independent of the length of the hydrophilic PDEA block. This is not surprising since the hydrophobic block is equal for all the copolymers, and the CMC values are much more sensitive to the length of the hydrophobic block than to the hydrophilic block size [86]. The discrepancies between the CMC values obtained from the fluorescence intensity and the solvatochromic shift can be attributed to the formation of pre-micellar aggregates that contribute diversely to the variation of the intensities and solvatochromic shifts for polymer concentrations below the CMC.

In order to clarify this, we plot the steady-state fluorescence anisotropy \( r_{ss} \) as a function of CP3 and CP5 concentrations (Fig. 4). Similar curves (not shown) were obtained for the CP2 and CP4 copolymers. The inset in Fig. 4 shows the plot in terms of molar concentration for CP3. The steady-state fluorescence anisotropy \( r_{ss} \) values were calculated from

\[
\lambda_{\text{max}} = 410 \text{ nm; } T = 23 \degree C; [C153] = 1 \mu M
\]
where \( IVV \) and \( IVH \) are the fluorescence intensities obtained using vertically polarized excitation light and recording the horizontal (\( IVH \)) or the vertical (\( IVV \)) fluorescence polarization components.

The \( G \) factor is an instrumental parameter that corrects for polarization effects introduced by the fluorescence detecting system,

\[
G = \frac{I_{IV}}{I_{II}}
\]

where \( I_{IV} \) and \( I_{II} \) are the fluorescence intensities, obtained using horizontal polarized exciting light and recording the vertical (\( I_{II} \)) and horizontal (\( I_{IV} \)) polarized components, respectively.

When plotting the \( r_{ss} \) values for the copolymers CP2 to CP5 in terms of molar concentration, the curves are well superimposed. The \( r_{ss} \) values start to increase at \( \sim 40 \) nmol/L (as shown for CP3 in the inset of Fig. 4, grey region) well below the CMC values listed in Table 5. This increase in \( r_{ss} \) values below the CMC suggests the formation of pre-micellar aggregates. This is supported by the slight increase in the fluorescence intensity observed for C153 in Fig. 3C in this range of concentrations. These pre-micellar aggregates can be either formed spontaneously or to be induced by the presence of C153.

The \( r_{ss} \) anisotropy values can be fitted by two straight lines for concentrations below and above the CMC. The polymer concentration at the intersection of the two straight lines is close to the CMC values determined by both the fluorescence intensity and the solvatochromic shifts (Table 5).

Going back to the data in Fig. 3C and D we observe that the fluorescence intensity method is more sensitive to the presence of pre-micellar aggregates than the solvatochromic shift method. Indeed, the CMC values calculated from fluorescent intensity data are always lower than those calculated from the solvatochromic shift data, suggesting that the fluorescence quantum yield is more sensitive to changes in polarity than the solvatochromic shift.

Due to the large size of the block copolymer micelles, their rotational dynamics is too slow to be probed during the lifetime of C153, and therefore the rotational dynamics of the micelles do not contribute to fluorescence depolarization. Thus, one should expect the steady-state fluorescence anisotropy values (\( r_{ss} \)) to reach the limiting anisotropy of C153, \( r_0 = 0.375 \) [51,59]. Since the maximum anisotropy is lower than this limiting anisotropy, the PDcAm chains at the micelle core must have some fluidity, which contributes to the depolarization of C153. However, a distribution of C153 between the micelle core and the water (where the anisotropy of C153 is very low) might also contribute to the low maximum anisotropy [58].
4. Conclusions

The CMC values obtained for SDS, CTAB and Triton X-405 from the fluorescence intensity and solvatochromic shifts of coumarin C153 are in agreement with those reported in the literature. In addition, we extended its use for the determination of the critical aggregation concentration of block copolymers in aqueous solutions. This shows that C153 can be effectively used as a standard probe to determine the CMC of self-assembling surfactants and block copolymer micelles. Although pyrene has been the most commonly used fluorescence probe for the local polarity, the use of coumarin C153 has large advantages since its solubility in water is larger than that of pyrene, and it has a lower tendency to form fluorescent aggregates. Moreover, the change in the fluorescence intensity ratio $I_{340}/I_{375}$ of the vibronic peaks of pyrene when it passes from a polar to an apolar environment is relatively small, and the fluorescence vibronic peaks can be influenced by the formation of excimers or other fluorescent aggregates. On the other hand, the experiments with C153 are easier to perform, less time consuming, more accurate, and more reproducible than with pyrene. In addition, we found that the variation in steady state anisotropy of C153 can be used to detect the formation of pre-micellar aggregates and evaluate micelle core fluidity.

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References