Structural Studies of Thin AOT Films by Using the Polarity Fluorescent Probe Coumarin-153

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Received April 11, 1996. In Final Form: October 15, 1996[⊗]

The solvatochromic behavior of coumarin-153 (C153) is exploited for the characterization of thin surfactant films, which were made by dip-coating fused-silica slides in solutions of reverse micelles of bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT) in cyclohexane. The original reverse micellar structure is most probably conserved but becomes very rigid when transferred into the film. Evaporation of cyclohexane trapped in the film, as well as increasing the water content in the original reverse micellar solution, does not alter either the absorption and fluorescence maxima or the red-edge excitation shift (REES) of C153. When the film adsorbs a certain amount of water from the atmosphere, the C153 environment becomes more fluid and seems to get in contact with cyclohexane molecules. Furthermore, a blue-shift of 13 nm in absorption and of 27 nm in fluorescence indicates a transfer of C153 from a high- to a low-polarity environment. We suggest an extensive curvature change of the original structure as a possible explanation for the 'inversion' of the C-153 spectroscopic parameters, due to water adsorption.

Introduction

The study of surfactant films on solid substrates is of great importance, both from the fundamental and the applied points of view. Surfactant films are excellent transparent matrices for photophysical studies,[1] where molecular motion is possible, contrary to matrices made of other known materials. Thus they can serve as models of biological membranes, as hosts of functional species,[2−6] as layers for fine lubrication and coating,[3,7−9] as precursors of polymer films, etc. Surfactant self-assembled films have not attracted so much attention as the highly organized Langmuir−Blodgett films. However, the simplicity of their formation procedure gives them several advantages. In addition, there is increasing evidence that thin self-assembled surfactant films do form highly structured domains.[10,11] This finding may offer them roles so far exclusively assigned to their highly organized relatives.

We have previously found[11] that when a fused-silica (or glass) slide is dipped into solutions of AOT reverse micelles in cyclohexane, a thin, optically uniform, transparent film is formed. The optical properties of the film itself remain the same for days, indicating a high stability. We have also found[11] that the original reverse micellar structure is to a large extent transferred to the film itself. The object of the present work is to obtain additional information about the structure of such films and about the influence of some environmental factors on that structure. We have used coumarin-153 (see Figure 1 for the chemical structure) as a fluorescent probe and exploited the sensitivity of the absorption and fluorescence spectra of this dye to the polarity of its microenvironment to get indirect information about the films. As shown in the fundamental work by Fleming and Maroncelli,[12,13] both the absorption and the fluorescence maxima of C153 hold a linear correlation with the polarity of low-viscosity solvents. For more viscous systems the fluorescence maximum depends on the excitation wavelength. Since the absorption maximum keeps the linear correlation, the spectral behavior can yield information not only on micropolarity but also on the microviscosity of the C153 environment. In the present work, we first reviewed the behavior of the dye in some environments of different polarity and rigidity, including neat solvents, aqueous and reverse micellar solutions, and sol-gel matrices, and then extended the results to study film structure.

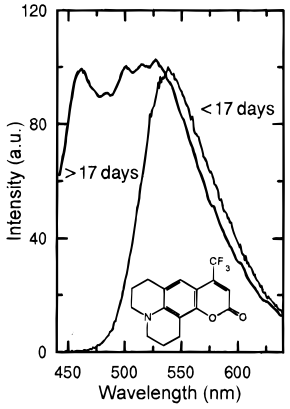

Figure 1. Fluorescence emission spectra of C153 (λ_{ex} = 400 nm) in a sol-gel matrix at an early drying stage (<17 days) and a late drying stage (>17 days). The concentration of C153 in the original sol was 50 μM. Inset: Chemical structure of C153.

Materials and Methods

Bis(2-ethylhexyl) sulfosuccinate sodium salt and cetyltrimethylammonium bromide (CTAB) were purchased from Fluka; methanol, ethanol, *n*-butyl acetate, dimethylformamide, dimethyl sulfoxide, and 37% hydrochloric acid were purchased from Merck; and finally, cyclohexane, tetramethoxysilane, Triton X-100, and coumarin-153 were purchased from Aldrich. All chemicals were used without further purification. Fused silica slides were

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[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1996.

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S0743-7463(96)00344-7 CCC: $14.00

Table 1. Absorption and Fluorescence Maxima (in nm) of C153 in Different Solvents (5 μM; 25 °C) and Dielectric Constants of the Solvents (25 °C)

solvent	λ_{ex}(max)[a]	λ_{em}(max)[a]	dielectric constant
	Nonprotic		
cyclohexane	395	454	2.02
triethylamine	(396)	(466)	2.44
n-butyl acetate	409	490	5.01
dimethylformamide	422	523	36.71
dimethyl sulfoxide	426	530	46.45
	Protic		
methanol	422	536	32.63
ethanol	421	530	24.30
n-propanol	(422)	(528)	20.10
n-butanol	(421)	(525)	17.10
	Micellar Environment		
CTAB	435	532	
Triton X-100	427	525	

[a] Values in parentheses are from ref 13.

purchased from ETS Thuet-Biechelin, 120, rue du General de Gaulle, 68740 Blodelsheim, France. Millipore water was used in all experiments.

Films were deposited on fused-silica slides by dip-coating in cyclohexane solutions containing reverse micelles of bis(2-ethylhexyl) sulfosuccinate sodium salt (AOT). Slides were cleaned in sulfochromic solution. After a few minutes of drying in air, films were put in a desiccator containing dried silica gel, or they were dried in vacuum at 50 °C for 50 min. For providing a reproducible humid atmosphere, when needed, silica gel was taken out and the bottom of the desiccator was filled with water, 1 cm apart from the films.

All measurements were performed at ambient temperature. Absorption measurements were made with a Perkin-Elmer Lambda 15 spectrophotometer, and fluorescence measurements, with a home-assembled spectrofluorometer using Oriel parts.

Results and Discussion

Solvatochromic Behavior of C153. When coumarin-153 (C153) is electronically excited, the dipole moment increases from 6.6 D in the ground state to a value of between 14.2 and 16.0 D in the S_1 state, depending on the solvent[12] (with no obvious trend related to solvent polarity). Therefore, after excitation, reorganization of the solvent molecules leads to a relaxed state of free energy. The larger the solvent polarity, the lower the energy of the relaxed state and the larger the red shift in both absorption and emission spectra. It has been previously shown that the frequencies of the C153 emission and absorption maxima are in a linear relationship with the solvent polarity[12,13] (solvent polarity expressed according to Kamlet et al.[14]). Thus, the fluorescence emission maxima λ_{em}(max) in a given solvent can be estimated from the experimentally determined absorption maxima λ_{ex}(max).[12,13]

In order to illustrate the solvatochromic effect of C153, we determined the maxima in the solvents cyclohexane (CH), *n*-butyl acetate (BA), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), so as to span the polarity scale of nonprotic solvents (Table 1). Protic solvents do not follow the same correlation. The observed fluorescence emission maximum wavelength, in most protic solvents, exceeds the value expected for nonprotic solvents at a given absorption maximum. A comparison of the emission and absorption maxima in ethanol and methanol with those in DMF may illustrate this behavior (Table 1). The reason for the different behavior of protic solvents is an additional solvation mechanism provided by their hydrogen-bond-donating abilities.[13] In Table 1 we have also included values obtained with aqueous cetyltrimethylamonium bromide (CTAB) and Triton X-100 micelles, for comparison. The role played by protic vs nonprotic solvents is repeated in the case of the quaternary ammonium group carrying surfactant as compared with Triton X-100. Thus a greater shift is observed with CTAB. This large shift, especially, in the absorption spectrum is not just due to polarity but probably stems from a nonspecified bond created between the cationic surfactant and C153 and providing an additional solvation mechanism.

Since the dipolar relaxation of these solvents at room temperature (e.g. 12 ps in ethanol)[12] is much faster than the fluorescence lifetime (e.g. 3.9 ns in ethanol),[15] the observed emission maxima do not vary with different excitation wavelengths. If C153 is dissolved in a medium of higher viscosity and the dipolar relaxation of the solvent molecules around the fluorophore occurs on the same time scale as the fluorescence lifetime, the emission maximum λ_{em}(max) shows an excitation wavelength dependence.[16] Such a shift in λ_{em}(max) toward higher wavelength, caused by a shift in the excitation wavelength toward the red edge of the absorption band, is called the red-edge excitation shift (REES). As a physical quantity, the REES is defined as the difference between the emission maxima for excitation at the red edge and at the maximum of the absorption spectrum. Starting from a system of low viscosity and high polarity the magnitude of the REES increases with increasing viscosity. Thus, the REES can be used as an indicator of the fluorophore environment. On the contrary, when viscosity increases to a point where solvent relaxation is far too slow to compete with the intrinsic fluorescence, the REES is zero and emission arises from a state close to the Franck−Condon state, as in the case of a nonpolar medium. This phenomenon is demonstrated in the next paragraph.

C153 in a SiO_2 Matrix. Metal oxide sol-gel matrices were made by hydrolysis of tetramethoxysilane followed by condensation−polymerization of the ensuing hydroxide, leading to the oxide, as previously described.[17] During the drying process the silicate network becomes more rigid and finally provides a matrix where encapsulated dyes may find themselves in an environment of high rigidity. When C153 (50 μM) is introduced in the solution of the reactants, the absorption and fluorescence maxima at an early stage of the sol-gel process are 433 ± 1 and 540 ± 1 nm, respectively (cf. Figure 1). Moreover, the emission spectrum is not shifted by changes in the excitation wavelength. We conclude that at this early stage of gelation C153 is embedded in an environment of high polarity and relatively low viscosity. This picture does not change during a drying period (20 °C) of about 2 weeks. The increasing polymerization and the decreasing content of methanol at this stage of the drying process do not drastically affect the microenvironment of C153.

After about a period of 17 days an emission peak at λ_{em}(max) = 460 ± 3 nm appeared in the fluorescence spectrum (Figure 1). Its importance increased with further drying. The red emitting maximum became dependent on the excitation wavelength. Its emission maximum, when excited at the red edge of the absorption band (λ_{ex} = 485 nm) appeared at 540 ± 2 nm, i.e. at the same position as that observed at an earlier stage of drying (Figure 1). Apparently, C153 is not located in an unique environment, but in environments of different viscosities. The emission band at 460 nm represents a highly restricted

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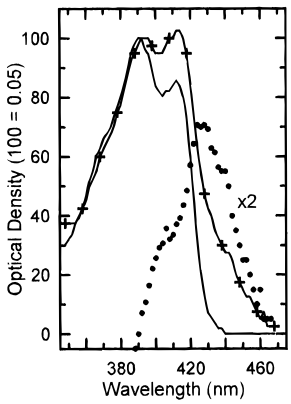

Figure 2. Absorption spectra of C153 (10 μM) in cyclohexane containing 0.2 M AOT and 0.4 M water (+) and in cyclohexane and enlarged (2×) difference spectrum (···). The cyclohexane spectrum is intensity-normalized at its emission maximum.

Table 2. Absorption Maxima, Fluorescence Maxima (Red-Edge Excitation), and Red-Edge Excitation Shifts of C153 in AOT Systems at 25 °C (in nm)

AOT	λ_{ex}(max)	λ_{em}(max)	REES
reverse micelles	424 ± 3	524 ± 2	≥8
LC phase	423 ± 1	532 ± 1	13
fresh film	422 ± 1	503 ± 1	5
dried film	421 ± 1	501 ± 1	3
fresh film/water ads	409 ± 2	492 ± 1	11
dried film/water ads	409 ± 2	492 ± 1	21

environment. Since the high rigidity prevents the stabilization of the C153 excited state by the polar surrounding, the fluorescence arises from a state close to the Franck−Condon state.

It has to be stressed that this work is not focused on the investigation of the sol-gel process. We include these results to demonstrate that the lack of solvent relaxation shifts the fluorescence of C153 in a polar medium almost toward the emission band in cyclohexane (Table 1).

Cyclohexane Solution Containing AOT Reverse Micelles. The light absorption of C153 (10 μM) in a 0.2 M AOT/CH solution containing water (0.4 M) is characterized by a very broad band with two maxima at 394 and 414 nm (Figure 2). Supposing that C153 is either dissolved in cyclohexane or associated with the reverse micelles, the absorption spectrum of the latter case can be calculated by subtracting the C153 spectrum in cyclohexane from the overall absorption spectrum. Figure 2 shows that C153 associated with the reverse micellar structure has its absorption maximum at 424 ± 3 nm (cf. Table 2).

In further experiments, the influence of the water content on the absorption characteristic of C153 has been investigated. Keeping the concentration of AOT constant (0.2 M), the water content has been varied from 0.2 M up to 6.0 M. No significant differences in the absorption spectrum of C153 associated with AOT micelles have been found. In contradiction to dyes located in the core of the reverse micelles, C153 does not make the difference between 'structured' and 'free' water.[18] By 'structured' water we mean the water forming the hydration layer, and by 'free' water we mean water forming a water pool.

Excitation at the red shoulder of the absorption spectrum (λ_{ex} > 460 nm) should exclusively excite C153 associated with reverse AOT micelles. Upon excitation at 460 nm, the maximum of the fluorescence emission occurs at 516 ± 1 nm. Changing the excitation wavelength to the very red end (λ_{ex} = 485 nm) causes a progressive shift, with a final maximum emission value of 524 ± 2 nm (Table 2) . Due to the limitations imposed by the interfering fluorescence of 'free' C153 in CH, only a minimum value of 8 nm ($\lambda_{ex} \geq$ 460 nm) for the magnitude of the REES could be determined. The observation of a REES indicates that C153 finds itself in a considerably restricted environment. Considering the correlation between absorption and fluorescence maxima (Table 1), the fluorescence emission maximum at 524 nm, obtained by excitation at the very red edge of the absorption spectrum, is well correlated with the absorption maximum of 424 nm. Thus we consider the obtained fluorescence of 524nm as the fluorescence characteristic of the fully relaxed state. We then conclude that C153 reports a reverse micellar domain with a solvent polarity between those of DMF and DMSO (Table 1). This conclusion is supported by the excitation maxima for the above solvents, which are around 424 nm (Table 1). Since the observed maxima are in line with the correlation obtained for nonprotic solvents and since changes in the water content do not alter the spectroscopic parameters, C153 seems not to be in contact with the water molecules located in the core of the reverse micelles but is located in a polar environment. We then suggest that a large percentage of C153 is located between the hydrophobic alkyl chains close to the polar AOT headgroups. The corresponding values obtained with aqueous micelles (Table 1) and experiments carried out in our laboratory with the same system and other probes[11] come in support of such conclusions.

AOT−Water−Cyclohexane Mixture in Its Liquid-Crystalline Phase. In the presence of a small amount of hydrocarbon but high concentrations of AOT and water we get a liquid-crystalline (LC) phase, according to the phase diagrams for AOT−water−isooctane mixtures[19] and for AOT-water-heptane mixtures.[20] We have prepared a solution of 1.4 M AOT and 8.4 M water in cyclohexane. Though this solution does not organize into a LC phase instantaneously, if left at room temperature for 2 days, a system with the optical properties of LC phases is formed. This solution is characterized by a high macroscopic viscosity, low light transparency, and light-scattering properties. Despite its low transparency, it is possible to determine a C153 (50 μM) absorption maximum of 423 ± 1 nm. The fluorescence emission wavelength, when excited at the red edge of the absorption band, is 532 ± 1 nm. The fluorescence band is dependent on the excitation wavelength. We observed a total REES of 13 nm (Table 2). The observed fluorescence and absorption maxima do not follow the correlation for nonprotic solvents (see Table 1, DMF) and rather match the spectroscopic parameters of ethanol (Table 1). We conclude that C153 is located between the hydrophobic alkyl chains close to the polar AOT headgroups, similarly to the case of reverse micelles. However, the high water content in the LC phase permits C153 to form hydrogen bonds in the ground state.[13] As in the reverse micellar case, the observation of the REES indicates a C153 environment of restricted mobility.

AOT Films. For AOT films, which have been made by dip-coating of fused-silica slides in cyclohexane solution containing AOT reverse micelles, we obtained an absorption maximum of 422 ± 1 nm (Figure 3). Apparently, in the AOT films C153 faces a surrounding of polarity identical to the reverse micellar situation. Supported by recently obtained fluorescence quenching and energy

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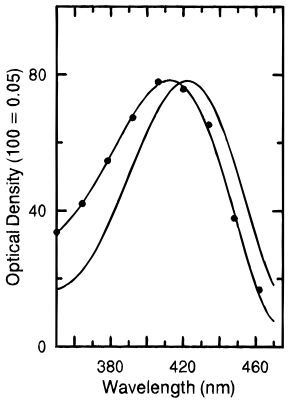

Figure 3. Absorption spectrum of C153 in fresh AOT films and after water adsorption (●).

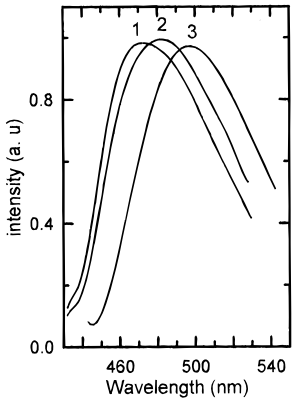

Figure 4. Normalized fluorescence emission spectra of C153 in AOT films ($\lambda_{ex} = 410$ nm): dried film after water adsorption (1); fresh film after water adsorption (2); and fresh film (3).

transfer results,[11] it appears obvious that the original micellar structure is to a large extent transferred to the film itself.

Supposing that the correlation between the emission and absorption maxima over the entire solvent polarity range can be applied to AOT films as well, one would expect a fluorescence maximum > 520 nm, when excited at the very red edge of the absorption spectrum. On the contrary, we found $\lambda_{em}(max) = 503 \pm 1$ nm for $\lambda_{ex} = 485$ nm. The magnitude of the REES was determined as 5 nm (Table 2). Even exciting C153 molecules, whose surroundings are already oriented to some extent toward the relaxed state of minimum free energy, solvent relaxation does not complete within the lifetime of the S_1 state. We conclude that the reverse micellar environment becomes extremely rigid when adsorbing onto the glass slide.

In order to investigate the influence of the water content on the C153 parameters in the film, we have increased the quantity of water in the original micellar solutions up to 6.0 M. C153 did not detect any differences in the molecular structure of the AOT films prepared from stock solutions of different water content.

Dried AOT Films. In other experiments we have heated (50 °C; 50 min) and dried films under vacuum in order to evaporate CH entrapped between surfactant. Both the determined absorption maxima (421 ± 1 nm) and the fluorescence emission behavior ($\lambda_{em}(max) = 501 \pm 1$ nm for $\lambda_{ex} = 485$ nm; REES = 3 nm; Table 2) do not show any important differences. The remaining CH molecules in AOT films appear not to influence the domains which host C153.

Summarizing these results, C153 seems to be, in all AOT systems presented so far, located between the hydrophobic alkyl chains close to the polar AOT headgroups. Except in the LC phase, C153 is in none of the systems investigated in contact with water molecules. AOT films contain probably to a high extent reverse AOT micelles. The cyclohexane molecules appear not to be in contact with C153, and thus, a possible CH penetration between the alkyl chains is not extended to the C153 hosting region. The micellar domain reported by C153 becomes extremely rigid when transferred on the fused silica slides.

AOT Films Exposed to Highly Humid Atmosphere. When kept in a desiccator containing dried silica gel, the absorption and fluorescence characteristics of the fresh and dried AOT films (containing C153) remain constant for days. The situation changes dramatically when the films are exposed to an atmosphere exhibiting high humidity. Though the following observations were made under ambient conditions as well, we have kept the films in a chamber containing water in order to provide a reproducible humid atmosphere. After a wetting time of about 3 h, we observed a blue-shift in both fluorescence and absorption. The blue-shift is progressive, and the films reach their final state in about 15 min after the above delay time. For the fresh and the dried films the final absorption spectrum after adsorbing water has its maximum at about 409 ± 2 nm, which means a blue-shift of 13 nm (Figure 3). When excited at the very red edge of the absorption spectrum ($\lambda_{ex} = 470$ nm), the fluorescence emission maximum for 'humid' AOT films was around 492 ± 1 nm. Apparently, C153 finds itself in a much less polar environment after water adsorption. Both the observed fluorescence and absorption maxima (Table 2) indicate that C153 resides in an environment of low polarity, similar to *n*-butyl acetate (Table 1).

In contrast to the films prior to water adsorption, red-edge excitation populates the solvent relaxed state of minimum free energy, indicating a less rigid structure. Both 'humid' AOT films show a significant REES but differ in its magnitude (Table 2). The dried film contains no or only very little cyclohexane. It shows an emission maximum of 471 ± 1 nm after water adsorption, when excited at 410 nm (Figure 4). On the other hand, the CH-containing film reveals its emission maximum at 481 ± 1 nm, when excited at 410 nm (Figure 4). The differences in the magnitude of the REES (21 vs 11 nm) indicate that C153 is located in a more mobile environment, when a considerable amount of CH is present.

It must be stressed that a blue-shift of 13 nm in absorption and of 27 nm in fluorescence (dried films, excited at 410 nm; Figure 4) cannot be explained by gradual changes of polarity due to water adsorption. Since the fluorescence and absorption maxima for C153 in water are 548 and 432 nm,[15] respectively, an increasing water content in the C153 environment would lead to a red-shift. Moreover, the fact that the absorption and the red-edge excited emission maxima match those of the nonprotic solvent *n*-butyl acetate argues against the possibility of C153−water interaction.

Though the physical parameters of reverse AOT micelles adsorbed on fused silica are not comparable with those in cyclohexane solution, it is worth mentioning that, starting from the reverse micellar solution, an increase of water, together with a decrease in the hydrocarbon concentration, leads, finally, to the formation of a liquid-crystalline phase.[19,20] The spectral parameters of the AOT−water−cyclohexane liquid-crystalline phase (Table 2), however, argue against formation of a liquid-crystalline film in spite of water adsorption.

Apparently, C153 is no longer located at the same position between the hydrophobic alkyl chains close to

the polar AOT headgroup after water adsorption. It monitors a much less polar environment, indicating a larger distance from the hydrated headgroups. The extent of polarity decrease might be illustrated by the dielectric constants of the solvents DMF and BA (36.71 and 5.01, respectively), which yield identical absorption maxima as those observed in 'fresh' and 'humid' films. Moreover, C153 gets in contact with CH molecules, which are found to decrease the microviscosity of the dye environment. In our opinion, this C153 relocalization indicates an extensive curvature change and, possibly, a curvature inversion of the original structure, due to water adsorption. To what extent a state of normal micelles is reached has to be subject of further investigations.

Conclusions

When C153 is solubilized in AOT reverse micelles or in AOT thin films, it is located at the interface, i.e. between the AOT alkyl chains and close to the polar headgroup. Since it senses no variation, when transferred from a reverse micellar solution to a thin AOT film, other than a large increase in viscosity, we conclude that the original reverse micellar structure is preserved in the film, in accordance with previous findings.[11] The large changes in the C153 spectral behavior when the film is exposed to a humid atmoshere suggest an extensive curvature change and, possibly, curvature inversion by adsorption of water molecules.

Acknowledgment. This work was financed by the Human Capital and Mobility Project CHRX-CT-0273. M.H. is especially thankful to the European Commission that allowed him by the above project to visit the hosting laboratory in Patras.

LA960344W