Micelle effect on ground and excited state proton transfer reactions involving the 4-methyl-7-hydroxyflavylium cation

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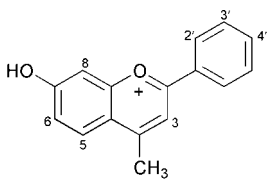
In aqueous solution, the 4-methyl-7-hydroxyflavylium ion ($\mathbf{AH}^+$) undergoes a ground state dissociation reaction to form the quinoidal base **A** and, when excited, an efficient excited state proton transfer (ESPT) leading to ***A**. The effect of micelles on the ground and excited state proton transfer reactions has been investigated. As far as the acid–base properties of the ground state system are concerned, negatively charged SDS micelles stabilize the acidic form $\mathbf{AH}^+$, while the positively charged CTAB and, to a lesser extent, the neutral Triton X 100 micelles destabilize $\mathbf{AH}^+$. Moreover, the neutral form **A** exhibits a strong decrease in molar absorption coefficient that cannot be explained by electrostatic reasons only, suggesting that specific interactions of **A** with the hydrophobic part of the surfactants may play some role. Even the excited state dissociation constant (K_a^*) is affected by the micelles, and the pK_a^* is positively or negatively shifted with respect to that in water by negatively or positively charged micelles, respectively. It was also observed that the excited states can be intrinsically affected by the micelles. In particular, the excited $\mathbf{AH}^+$ is not only stabilized by the negative SDS micelle, but also exhibits a luminescence lifetime longer than that in water, most probably because of the electrostatic interaction exerted on it by the negative charge of the micelles.

Aqueous solutions of cetyltrimethylammonium bromide (CTAB), polyoxyethylene(10)isooctylphenylether (Triton X-100) and sodium dodecyl sulfate (SDS) give rise to micelles when their concentration is greater than their respective critical micellar concentration (CMC).[1] The effect of micelles on the acid–base, ground state equilibrium of dyes is well documented. Indeed, in an elegant set of experiments on the fluorescence of aminocoumarin and hydroxycoumarin located at the surface of micelles, Fernández and Fromherz[2] discovered that the acid dissociation constants are profoundly affected (shifted) in the presence of micelles. The magnitude and direction of ΔpK_a were related to the charge of the micelle (negative and positive shifts, respectively, for positively and negatively charged micelles). In the case of neutral micelles, the observed negative shift was attributed to a decrease in the polarity at the micelle surface. Similar shifts of pK_a (positive or negative depending on the surface charge of the micelle) were recently observed in the acid dissociation of the 4′-hydroxyflavylium cation in CTAB, Triton X and SDS micelles.[3] Such similar behavior suggests that even the flavylium species tends to be located at the micelle interface.

Maçanita *et al.*[4] recently observed that in SDS micelles, the pH domain of the red color of the cationic form of Malvin, the natural anthocyanin, is shifted to higher pH values.

Ground state pK_a's are commonly obtained either by UV-visible absorption spectroscopy or luminescence measurements. The luminescence technique has certain advantages over absorption spectroscopy but does require some care, since it leads to results identical to those obtained by absorption spectroscopy only if the excited state proton transfer (ESPT) process does not occur. ESPT is a well known phenomenon[5,6] that consists of reversible, adiabatic transfer of a proton involving an excited acid and its excited conjugate base. Being new chemical species, excited states may exhibit, among other properties, acidity constants (largely) different from those of the parent ground state compounds.[5–11]

We have shown recently that synthetic flavylium salts undergo excited state proton transfer processes;[11] this process is particularly efficient in the case of the 4-methyl-7-hydroxyflavylium[11] cation, being one of the most efficient among those reported in the literature.[5–11] The strong ESPT effect makes this compound a useful tool in investigating the effect of micelles on the acid–base properties of excited states, reported in this study along with the properties of the ground state. Our findings are discussed in the framework of the theoretical model developed by Weller.[6,9]

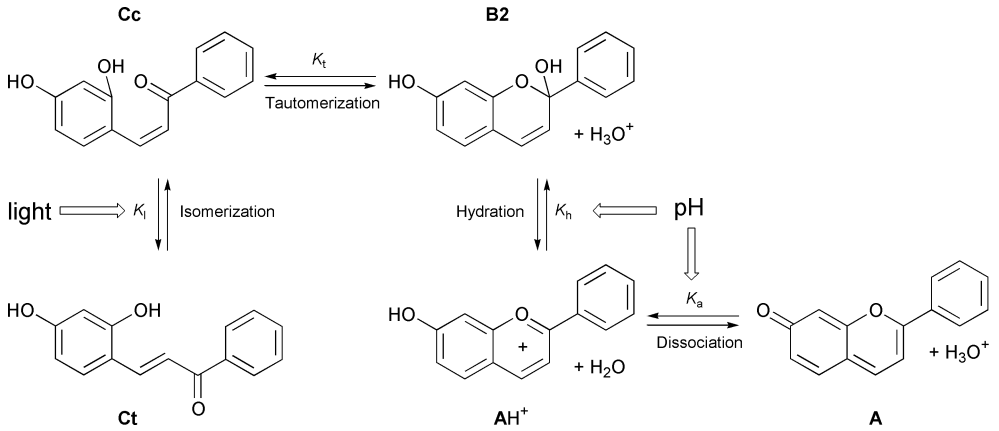


4-Methyl-7-hydroxyflavylium cation

Results and discussion

Aqueous solutions

Before discussing the micelle effect on the ground and excited state behavior of the 4-methyl-7-hydroxyflavylium cation, it is relevant and instructive to recall the principal properties of this system[9] starting from the properties of flavylium compounds. Synthetic flavylium ions can undergo structural transformations that involve several species connected by a set of



Scheme 1 Structural transformations of flavylium-type compounds, exemplified with the 7-hydroxyflavylium cation.

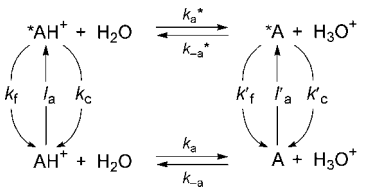
equilibria that can be displaced by pH changes and by light excitation[12–15] as illustrated in Scheme 1 for the 7-hydroxyflavylium cation. These species include the flavylium cation $\mathbf{AH}^+$, the quinoidal base **A** formed subsequent to a proton transfer reaction from the flavylium cation to a water molecule, the hemiacetal form **B2** obtained by hydration of the flavylium cation in the 2 position, the *cis*-chalcone species **Cc** formed from the hemiacetal **B2** through a tautomeric process and the *trans*-chalcone form **Ct** resulting from the isomerization of *cis*-chalcone.

In neutral and acidic media, the hydration reaction of the 4-methyl-7-hydroxyflavylium cation, which leads to the hemiacetal form, **B2**, does not take place, thereby simplifying the reaction scheme that is now restricted to the sole proton transfer reaction leading to the quinoidal base [eqn. (1)].[16]

$$\mathbf{AH}^+ + H_2O \rightleftharpoons \mathbf{A} + H_3O^+ \quad (1)$$

The pK_a of this reaction in water at 25 °C was found to be 4.4 by absorption spectroscopy.[11] The acidic form displayed an emission only at pH's well below 4.4. That is, the emission from the basic form is obtained even when the acidic form is selectively excited, as it is the unique species present. This is typical behavior of systems in which an ESPT process occurs.

A theoretical model,[9] developed in the framework of the Weller theory[6] and based on Scheme 2, can be used for the treatment of ESPT phenomena. In the scheme, I_a is the light absorbed by the acidic species, k_f is the rate constant of the fluorescence emission of the acidic species, k_c is the sum of the rate constants for the remaining excited state processes that



Scheme 2 Schematic representation of the ground and excited state processes taking place in the 4-methyl-7-hydroxyflavylium system.

deactivate the $*\mathbf{AH}^+$ excited state. For the basic species, the equivalent symbols are labelled with a ′.

According to this approach, an ESPT can be explained on the basis of two parameters, namely η_A^* and pK_{ap}^* [eqn. (2) and (3)].

$$\eta_A^* = \frac{k_a^*}{k_a^* + k_c + k_f} \quad (2)$$

$$K_{ap}^* = \frac{k_a^* \tau_{*AH^+}}{k_{-a}^* \tau_{*A}} \frac{1}{\eta_A^*} = \frac{1 + k_a^* \tau_{*AH^+}}{k_{-a}^* \tau_{*A}} \quad (3)$$

where τ_{*A} [$\tau_{*A} = 1/(k_c' + k_f')$] and τ_{*AH} [$\tau_{*AH^+} = 1/(k_c + k_f)$] are the lifetimes of the excited basic and acidic species, respectively. η_A^* (the proton transfer efficiency) represents the yield of formation of the excited base from the excited acid, and K_{ap}^* is the apparent (experimentally measurable) acidity constant of $*\mathbf{AH}^+$, which depends on the acidity constants (K_a^*) of the excited state and on the ratio of the lifetimes of the acidic and basic excited species. These quantities can be experimentally determined by means of titrations plots as shown previously.[9] The pattern of the emission intensities of the acidic and basic species *vs.* pH displays two inflection points separated by a plateau. In our case, the first inflection point is related to pK_{ap}^*, the plateau to η_A^* and the second inflection point to pK_a. For more details see ref. 9. In the case of the 4-methyl-7-hydroxyflavylium cation, $\eta_A^* = 0.93$ and $pK_{ap}^* = ca.\ -1$.[11]

To complete the previously reported data in aqueous solutions, we have here measured the lifetimes of the two species (0.11 and 0.18 ns for the acidic and basic species, respectively). These lifetimes, used in eqn. (2) and (3), permitted the evaluation of k_a^*, k_{-a}^* and K_a^*. The measured and calculated parameters are reported in Table 1.

Effect of micelles on the ground state molecule

The absorption spectra of the 4-methyl-7-hydroxyflavylium cation recorded at various pH's in the presence of the CTAB, Triton X100 and SDS micelles are illustrated in Fig. 1. From the decrease in the values of the absorbance maxima of the acidic species reported in the three plots of this figure it is

Table 1 Ground and excited state parameters of the acid–base equilibrium of 4-methyl-7-hydroxyflavylium cation[a]

Medium	pK_a[b]	η_{*A}[c]	pK_{ap}^*[d]	k_a^*/s^{-1}[e]	k_{-a}^*/s^{-1}[f]	pK_a^*[g]
CTAB	2.25	0.95	0.05			
Triton X	3.3	0.7	−1.2	2×10^{10}	*ca.* 10^9	*ca.* −1.3
SDS	6.5	0.54	0.3	2×10^9	2×10^{10}	1.0
Water	4.4	0.93	*ca.* −1	1×10^{11}	8×10^9	−1.14

[a] Aqueous solution at 25 °C. [b] Ground state pK_a of eqn. (1). [c] Yield of formation of the excited base, ***A**, from the excited acid, $*\mathbf{AH}^+$ (proton transfer efficiency, see Scheme 2). [d] Apparent pK_a for the dissociation equilibrium of the excited $*\mathbf{AH}^+$. [e] Reaction rate constant for the deprotonation of the excited acidic form. [f] Reaction rate constant for the protonation of the excited basic form. [g] pK_a of the dissociation equilibrium of $*\mathbf{AH}^+$.

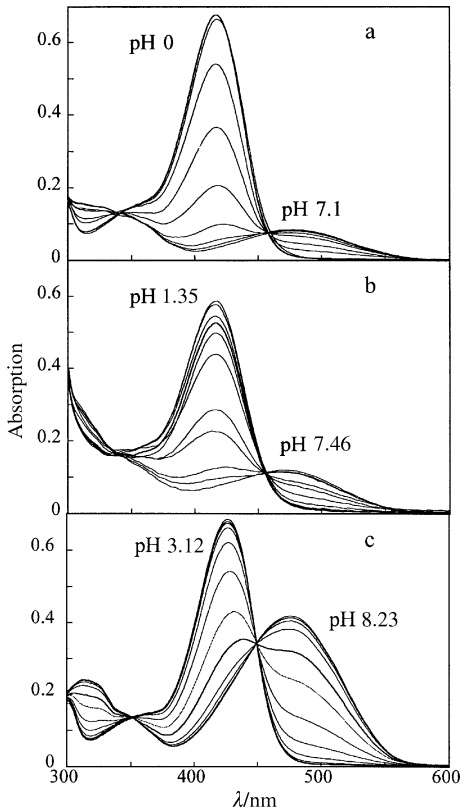


Fig. 1 Absorption spectra of the 4-methyl-7-hydroxyflavylium cation 2.5×10^{-5} M as a function of pH in (a) CTAB, pH: 7.1, 5.0, 4.5, 4.0, 3.5, 3,0, 2.5, 1.5, 0.0; (b) Triton X 100, pH: 7.5, 4.9, 4.4, 3.8, 3.5, 3.0, 2.5, 2.0, 1.6, 1.0, 0.5; (c) SDS, pH: 8.2, 7.9, 7.5, 7.1, 6.6, 6.2, 5.7, 5.3, 4.8, 4.2, 3.6, 3.1.

possible to obtain Fig. 2, which shows the change in the molar fraction distribution of $\mathbf{AH}^+$ as a function of pH in the three different environments. The best fit of these curves gave the pK_a values for eqn. (1) in the three different micelles collected in Table 1.

The results show that the equilibrium shown in eqn. (1) is strongly affected by the micelle type in the sense that the positively charged CTAB, and to a lesser extent the neutral Triton X micelles, destabilize $\mathbf{AH}^+$, decreasing its pK_a with respect to that exhibited in water, whereas the negatively charged SDS micelles stabilize $\mathbf{AH}^+$, displacing its pK_a to higher pH compared to H_2O. The observed effects are consistent with the results reported earlier for similar systems[2,3] and indicate that the $\mathbf{AH}^+$ form tends to be located in the bulk water in the case of positively charged or neutral micelles and close to the surface when the micelles are negatively charged. Moreover, the absorption spectrum of the basic species **A** is strongly influenced by the presence of the micelle; besides a small red shift, a decrease in the molar extinction coefficient of the lowest energy band was observed in all cases.[17] The effect on the molar extinction coefficient (decrease) increases on going from the negatively charged SDS to the neutral Triton X to the positively charged CTAB (see Table 2). In contrast, the absorption spectrum of the acidic form ($\mathbf{AH}^+$) is slightly affected by the nature of the micelle, showing only a small red shift of the absorption maximum in all cases (see Table 2). The strong decrease in the molar extinction coefficient of the basic species in the positively charged CTAB, and to a minor extent in the neutral Triton X micelle, suggests a strong interaction between the basic species and the CTAB and Triton X micelles; on the contrary, the interaction with the negatively charged SDS micelles is less important (Table 2). These results seem to indicate that electrostatic interactions are important in the stabilization or destabilization of charged species and their position with respect to the charged suface of the micelles, but cannot explain the effect on the spectroscopic properties, such as the absorption spectrum, of the uncharged species **A**. Thus, the decrease observed in the absorption spectrum of **A** seems also to be due to specific interactions between the uncharged species and the hydrophobic chains of the surfactants, suggesting that the basic species **A** can penetrate the hydrophobic part of the micelles. This conclusion is supported by the observation that the shape of the absorption spectrum of a solution of **A** in *n*-octane, obtained by extraction from a water solution at pH 6.0, closely resembles that of **A** in 0.05 M CTAB at pH 6.0.

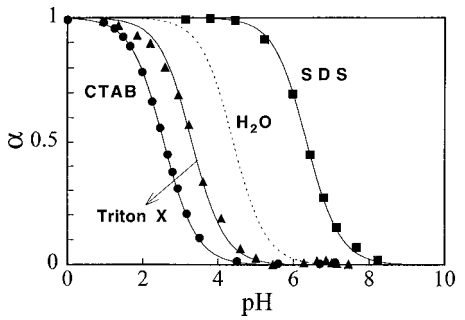


Fig. 2 Molar fraction distribution (α) of the acidic form of the 4-methyl-7-hydroxyflavylium ion at 25 °C in CTAB ($pK_a = 2.25$), Triton X100 ($pK_a = 3.3$), SDS ($pK_a = 6.5$) and water ($pK_a = 4.4$, dashed line).

Effect of micelles on the excited state properties

CTAB micelles. As noted earlier, the absorption spectrum of the acidic form of the 4-methyl-7-hydroxyflavylium salt (pH 1.0) is unaffected by the presence of the CTAB micelles, whereas the absorption spectrum of the basic form (pH 6.0) in 0.05 M CTAB shows a slight red shift and a large decrease of the molar absorption coefficient of the lowest energy band [Fig. 1(a)], compared with that in water.[11] In parallel with the UV-visible absorption spectrum, the emission spectrum recorded at pH 6.0 also appears to be affected by the presence of CTAB micelles (Fig. 3), showing a slight red shift and a strong decrease in intensity in comparison with the same spectrum in water.

Measurement of the relative emission quantum yields in the absence and presence of CTAB micelles at pH 6.0 shows that they are identical within experimental error. The large difference in intensity between the two fluorescence spectra is due only to a decrease in the molar extinction coefficient of the

Table 2 Ground state parameters of the species involved in the acid–base equilibrium of 4-methyl-7-hydroxyflavylium cation at 25 °C

	Acidic species		Basic species		
Medium	λ^a/nm	ε/M^{-1} cm^{-1}	λ^a/nm	ε/M^{-1} cm^{-1}	$K_a{}^b$
CTAB	419	27000	477	3200	5.6×10^{-3}
Triton X	417	27000	473	6000	5.0×10^{-4}
SDS	426	27000	476	17300	3.2×10^{-7}
Water	416	27000	470	19500	4.0×10^{-5}

[a] Wavelength of the lowest energy absorption maximum. [b] Equilibrium constant of eqn. (1).

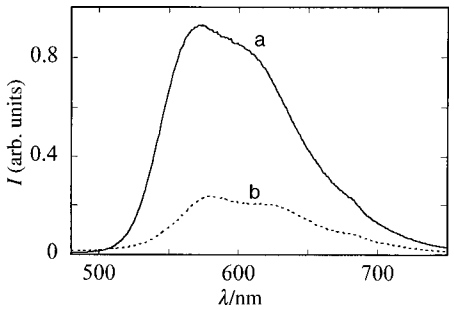


Fig. 3 Fluorescence emission spectra of the 4-methyl-7-hydroxyflavylium cation (pH 6.0, 2.5×10^{-5} M, $\lambda_{exc} = 460$ nm) in (a) water and (b) 0.05 M CTAB.

ground state basic species. Consequently, we conclude that the CTAB micelle does not have an appreciable effect on the excited state properties of the basic form at pH 6.0 of this flavylium compound, even though the microenvironment surrounding this species seems very different from that in pure water (see absorption results). A slightly different emission lifetime is also found in this medium relative to that in water (0.22 and 0.18 ns, respectively).

Even at pH 1.0, the fluorescence spectrum is not affected by the presence of the CTAB micelles and the same holds for the emission lifetime, which is almost identical to that in water (0.17 *vs.* 0.18 ns). This result implies that both the absorbing ($\mathbf{AH}^+$) and the emitting (**A**) species are located in the bulk water, far away from the micelles. This behavior can easily be explained by considering that: (i) the electrostatic repulsion keeps the excited $*\mathbf{AH}^+$ molecule far away from the positively charged surface of the micelles, (ii) the ***A** species is formed in the same place by a simple proton ejection, and (iii) the radiative deactivation of the ***A** species is so fast (hundreds of picoseconds) that it occurs before any diffusion toward the micelle takes place.

As in pure water, the emission spectrum of the acidic species $\mathbf{AH}^+$ in CTAB micelles can only be obtained in a highly acidic medium (*e.g.*, 2.4 M HCl, formal pH −0.38). The fluorescence lifetime of the $*\mathbf{AH}^+$ species at this pH is 0.12 ns. As displayed in Fig. 4, although the absorption spectra recorded in water and in highly acidic medium are practically identical (see Table 2), the intensity of the fluorescence band of the acidic form at *ca.* 500 nm is significantly greater than the emission band obtained in water. This behavior suggests a possible interaction between the $*\mathbf{AH}^+$ species and the micellar environment that stabilizes the excited acidic form. This effect resembles the one caused by negative SDS micelles on the ground state molecule and is probably due to the high concentration of Cl^- ions (2.4 M), which cover the positively charged surface of the CTAB micelle. The resulting negative layer likely exerts an electrostatic attraction on $*\mathbf{AH}^+$ with a consequent decrease in its excited state dissociation constant (positive shift of the pK_a^*). In agreement with this explanation

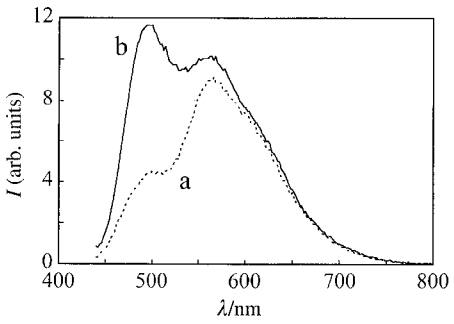


Fig. 4 Fluorescence emission spectra of the 4-methyl-7-hydroxyflavylium compound in acidic media (HCl = 2.4 M; pH −0.38, 2.5×10^{-5} M, $\lambda_{exc} = 460$ nm) in (a) water and (b) 0.05 M CTAB.

is the observation that in a highly concentrated salt medium (2.4 M NaCl), the chloride ions have the same effect on the emission spectrum of the 4-methyl-7-hydroxyflavylium cation.

Once the emission spectra of the basic and acidic forms in CTAB are known, it is possible, by means of the previously reported treatment,[9] to obtain the plot shown in Fig. 5, which reports the normalized emission intensity of the two species as a function of pH in 0.05 M CTAB. The best fit of the experimental points (full lines) gives $\eta_A^* = 0.95$ (defined by the plateau) and $pK_{ap}^* = 0.05$ (defined by the first inflection point from the left).

Consideration of the results obtained in extremely acidic media (2.4 M HCl) suggest that the value of 0.95 for η_A^* is reasonable since it is determined by the plateau value in the pH range 1–3 where the HCl concentration is still too low to cause the observed Cl^- effect. On the contrary, $pK_{ap}^* = 0.05$ is determined by the inflection point below pH 1, a pH where the Cl^- ion concentration is relatively high, so high as to affect the acid–base equilibrium of the excited species. This means that the actual value of pK_{ap}^* is less than 0.05 and most probably also more negative than −1, the value determined in water.

The constancy of η_A^* indicates that the dissociation rate constant (k_a^*) and the lifetime (τ_{*AH^+}) of $*\mathbf{AH}^+$ are unchanged on going from water to CTAB micelles, in agreement with what was stated previously, that is $\mathbf{AH}^+$ absorbs and then its excited state dissociates to ***A** (95%) or emits (5%) in bulk water. Thus, in these systems, almost all the fluorescence emission observed results from the ***A** species and, depending on the proton concentration, the emitted light can be switched from ***A** in the bulk solution and ***A** at the surface of (or inside) the micelles. In fact, between pH 1 and 3 the emission comes from excited ***A** species in the bulk water obtained through deprotonation of the excited $*\mathbf{AH}^+$ in the bulk, while for pH > 3, light is absorbed and emitted exclusively by the basic species, which is located at the surface of (or inside) the micelles.

Triton X 100 micelles. As shown earlier [Fig. 1(b), Table 2] Triton X 100 affects the absorption spectra of the 4-methyl-7-hydroxyflavylium compound. Although the absorption spectra of the acidic form does not change, the basic form exhibits a decrease in the molar absorption coefficients of the lowest energy band that, even though to a lesser extent, resembles that seen in CTAB solutions. These results suggest that: (i) the acidic form $\mathbf{AH}^+$ is located in the bulk water, since it does not interact with the micelles and (ii) the basic form strongly interacts with the micelles and consequently must be located close to the surface (or inside the walls) of the Triton X 100 micelles.

The emission spectra of the acidic (pH 1.0) and basic (pH 6.0) forms of the 4-methyl-7-hydroxyflavylium salt in Triton X

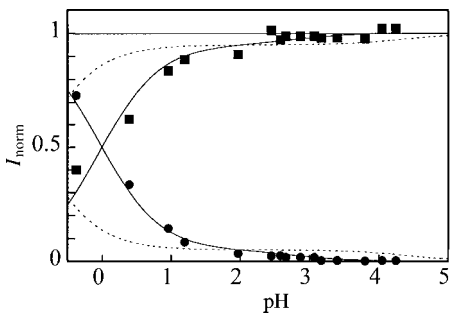


Fig. 5 Normalized fluorescence emission ($\lambda_{exc} = 456$ nm) of the acidic (●) and basic (■) forms of the 4-methyl-7-hydroxyflavylium compound in the presence of 0.05 M CTAB. The best fit was achieved for $\eta_A^* = 0.95$ and $pK_{ap}^* = 0.05$. The curves in water (dashed lines) are also reported for comparison purposes.

100 are very similar to those in CTAB media. The fluorescence lifetimes of the two species are 0.12 and 0.23 ns, respectively. The luminescence behavior can be summarized in Fig. 6, which reports the normalized fluorescence emission of the two species as a function of pH in 0.05 M Triton X 100. The best fits of the experimental points (full lines) give $\eta_A^* = 0.70$ and $pK_{ap}^* = -1.2$.

Inspection of Fig. 6 shows a decrease of η_A^* in Triton X micelles with respect to water, due to a less efficient ESPT phenomenon in the micelles than in water. This conclusion can be mathematically drawn by using in eqn. (2) and (3) the lifetime data measured in strongly acidic media in both Triton X micelles and water. In fact, a value of 2×10^{10} s^{-1} is obtained for k_a^*, which has to be compared with 1.1×10^{11} s^{-1} calculated in water. This indicates that some interaction of $*AH^+$ with the surfaces of the Triton X micelles must take place. This is not as difficult as with CTAB micelles, because in this case the surface of the micelle is not positively charged and does not repel the AH^+ and $*AH^+$ species. Concerning the position of pK_{ap}^*, the relatively high error in this measurement does not permit decisive conclusions or inferences to be made.

SDS micelles. As noted earlier [Fig. 1(c), Table 2], the absorption spectra of the acidic (pH 1.0) and basic (pH 8.0) forms of the 4-methyl-7-hydroxyflavylium salt are slightly dependent on the presence of the SDS micelles. This behavior suggests that both species (AH^+ and **A**) weakly interact with the negatively charged surfaces of the SDS micelles. While this interaction is understandable for the acidic species due to its positive charge, the interaction with the neutral basic form is unexpected, even though the changes in the absorption spectrum are the smallest observed in the series CTAB, Triton X and SDS micelles and may derive from specific interaction of **A** with the hydrophobic chain of the surfactant (see discussion of the ground state).

The fluorescence emission spectra of 4-methyl-7-hydroxyflavylium is strongly dependent on the concentration of SDS micelles, as shown in Fig. 7. As this figure shows, at pH 2.3, it is possible by simple addition of increasing amounts of SDS to switch from the pure emission spectrum of the basic form **A** to an emission spectrum mainly due to the acidic species AH^+. It is interesting to note that the switching occurs at about the same concentration as the critical micellar concentration (CMC) of the SDS micelle.[1] From this observation two points emerge: (i) the effect on the luminescence of the excited species is due to the micelle (actually to the charge of their surface) and not to the single SDS molecule and (ii) the luminescence intensity of the acidic species can be used to monitor the formation of micelles. In fact, by plotting the emission intensity of the acidic species *vs.* the SDS concentration (inset of Fig. 7) it is possible to obtain a measure of the critical micellar concentration of SDS.

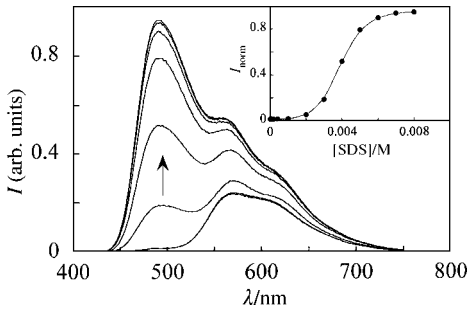


Fig. 7 Fluorescence emission spectra ($\lambda_{exc} = 415$ nm) of the 4-methyl-7-hydroxyflavylium (2.5×10^{-5} M) at pH 2.3 *vs.* SDS concentration: [SDS] = 0, 1, 3, 4, 5, 6, 7 and 8×10^{-3} M. Inset: Emission intensity at 495 nm as a function of SDS concentration.

The emission spectrum of 4-methyl-7-hydroxyflavylium ion is also pH dependent; the emission data have been treated by means of the previously reported model and the plot in Fig. 8, showing the normalized emission intensities of the two species (basic and acidic) as a function of pH in 0.05 M SDS, has been obtained. The best fit of the experimental points gives $\eta_A^* = 0.54$ and $pK_{ap}^* = 0.3$, which give, through eqn. (2) and (3), the k_a^*, k_{-a}^* and K_a^* values reported in Table 1.

All these values are different from those obtained in pure water and show that the negatively charged surfaces of SDS micelles stabilize the excited state of the positive acidic form as they do with the ground state molecule. In other words, as expected, both k_a and k_a^* decrease at the SDS micelle surface, explaining the changes in η_A^* and pK_{ap}^*.

Another point to highlight is the fact that, even in this medium, there is the theoretical possibility of changing the site of the emitting $*A$ species by changing the pH of the solution. The switching pH range (1–7) is greater in this case than in CTAB solution (pH 1–3). Thus, in these systems at a pH greater than 7, the light is absorbed and emitted exclusively by the basic species located in a region where it weakly interacts with the negative SDS micelle surfaces (micelle wall?). In the pH range 2–6 the light is almost exclusively absorbed by the acidic species AH^+ while 50% of the emission comes from the excited $*AH^+$ and 50% from the excited $*A$ species, obtained through deprotonation of the excited $*AH^+$, both at the surfaces of the micelles. When the pH is lower than 1, the light is absorbed and emitted exclusively by the acidic species located at the surface of the micelles.

The excited state lifetimes support this behavior. In fact, in SDS at pH 8.0 the luminescence decay is mono-exponential and its lifetime (0.25 ns) is only slightly different from that in pure water (0.17 ns). This small difference may reflect the different environments surrounding the emitting basic species in the two cases. This is also in agreement with the small interaction between **A** and the negative surface of the micelle suggested by the absorption experiments.

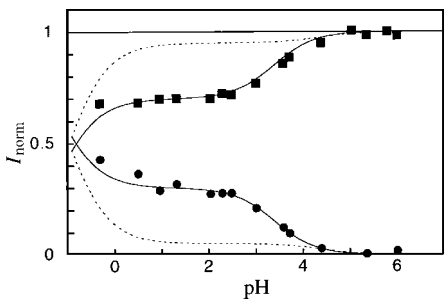


Fig. 6 Normalized fluorescence emission ($\lambda_{exc} = 455$ nm) of the acidic (●) and basic (■) forms of 4-methyl-7-hydroxyflavylium compound in the presence of 0.05 M Triton X 100. The best fit was achieved for $\eta_A^* = 0.70$ and $pK_{ap}^* = -1.2$. The curves in water (dashed lines) are also reported for comparison purposes.

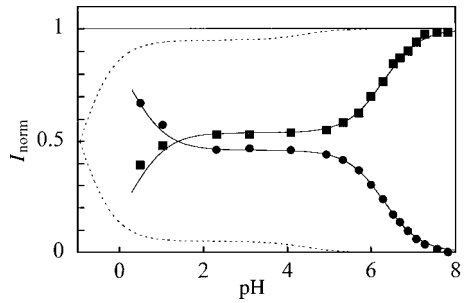


Fig. 8 Normalized fluorescence emission ($\lambda_{exc} = 450$ nm) of the acidic (●) and basic (■) forms of 4-methyl-7-hydroxyflavylium in the presence of 0.05 M SDS. The best fit was achieved for $\eta_A^* = 0.54$ and $pK_{ap}^* = 0.3$. The curves in water (dashed lines) are also reported for comparison purposes.

At pH − 0.3 the decay is also mono-exponential with a lifetime of 0.62 ns, attributed to the emission of the excited $*AH^+$ species at the surface of the micelle. The large increase in the lifetime of the acidic species in SDS with respect to that in water (0.62 *vs.* 0.11 ns) suggests a strong perturbation of the excited state properties of AH^+ due to the negatively charged cloud of the micellar surface.

At pH 4 the luminescence decay is no longer mono-exponential and a good fit of the data was achieved with two lifetimes of 0.62 and 0.28 ns, attributed to the emission of the excited $*AH^+$ and ***A** species, respectively, both at the micellar surface. The constancy (within experimental error) of the excited state lifetime of ***A** upon changing the pH (and consequently the emission site, see before) also suggests that the site does not change much, indicating that the basic species **A** is close to the surface (and weakly interacting with it, both in its ground and excited states), whatever the pH.

Conclusion

We have investigated the effect of micelles on the acid–base properties of ground and excited states of the 4-methyl-7-hydroxyflavylium cation. It has been shown that both the acid dissociation constants of the ground and excited states are affected by the presence of micelles. As far as the ground state system is concerned, negatively charged SDS micelles stabilize AH^+, while the positively charged CTAB and, to a lesser extent the neutral Triton X 100 micelles, destabilize AH^+. This effect is consistent with the results reported for similar systems.[2,3] Nevertheless, the electrostatic interaction alone cannot rationalize the whole behavior of the system and in particular cannot explain why the *uncharged* **A** species exhibits a molar absorption coefficient dependent on the micelle type. This further suggests that a more specific interaction between **A** and the hydrophobic chain of the micelles may take place.

An effect qualitatively similar to that found for the acid–base properties of the ground state is observed on the excited state dissociation constant (K_a^*); pK_a^* is positively or negatively shifted with respect to that in water for negatively or positively charged micelles, respectively. It was also observed that the excited state species can be intrinsically affected by the micelles. In particular, $*AH^+$ is not only stabilized by the SDS micelles but it also exhibits an excited state lifetime longer than that in water, most probably because of the electrostatic attraction exerted on it by the negative charge of the micelles.

Experimental

4-Methyl-7-hydroxyflavylium chloride was prepared according to a published procedure.[18] The micelles were purchased from Aldrich. All other chemicals used were of analytical grade. The experiments were carried out in water at 25 °C. The pH of the solutions was adjusted by addition of HCl (pH < 2) or Theorell-Stenhagen universal buffer and measured with a Metrohm 713 pH meter. Absorption spectra were recorded on a Perkin–Elmer Lambda 6 spectrophotometer. Emission spectra were recorded on a Perkin–Elmer LS50 spectrofluorimeter (10 nm for the excitation and emission bandwidths). Lifetimes were measured by means of a SPEX Fluorolog-3 spectrofluorometer equipped with a Tau 3 phase-modulated accessory (time resolution ±20 ps).

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16 In basic media, some hydration reaction takes place; at pH 11.0 *ca.* 40% of **A** is converted to **B2** and **Cc**. Some decomposition was also observed (*ca.* 20%).
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