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Luminescence behaviour of 7-hydroxyflavone in aerosol OT reverse micelles: excited-state proton transfer and red-edge excitation effects

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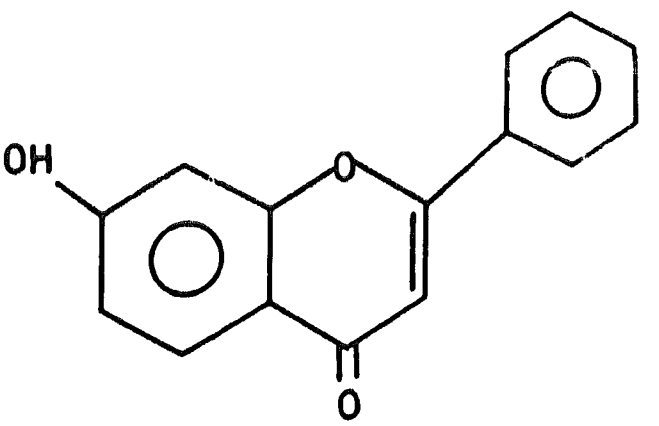
Abstract

The fluorescence emission properties of 7-hydroxyflavone (7HF) are examined in reverse micelles of aerosol-OT (AOT) in *n*-heptane. Excited-state proton transfer (ESPT) leading to dual-emission behaviour ($\lambda_{max} \approx 396$–$417$ nm and 545–550 nm which can be assigned to the normal and ESPT tautomer emission respectively) as well as red edge excitation shift (REES) of the normal fluorescence band are observed. Upon gradual addition of water to the 7HF–AOT–*n*-heptane solution, conspicuous enhancement of the ESPT tautomer emission intensity takes place together with a progressive red shift of the normal emission, that continues up to ($[H_2O]/[AOT]$) $= W_0 \approx 8$–10, beyond which no significant changes occur. Interestingly, with increasing value of W_0, the changes observed in the magnitude of the REES effect, $\Delta\lambda$ ($\Delta\lambda$ is the difference in λ_{max} of the normal fluorescence as λ_{exc} is shifted from midband ($\lambda = 310$ nm) to red edge ($\lambda = 350$ nm) of the absorption band) parallel to changes in the λ_{max} of normal emission, as well as that of the relative intensity I_T/I_N of the tautomer vs. normal emission bands. Even at high W_0 (e.g. $W_0 = 36$), these parameters do not reach the limiting values found in bulk water, indicating that 7HF is predominantly localized near the head groups of AOT, mostly in the bound water phase.

Keywords: Luminescence behaviour; 7-Hydroxyflavone; Aerosol OT reverse micelles; Excited states; Excitation effects

1. Introduction

7-Hydroxyflavone (7HF) (Scheme 1) is a simple model for naturally occurring biologically active flavones which are of immense therapeutic importance [1,2]. Excited-state proton transfer (ESPT) leading to dual-emission behaviour of 7HF has been extensively investigated [3–6]. In addition, solvent dipolar relaxation around the excited 7HF molecules has been shown by us to be an important deactivation pathway of the excited state of 7HF in both its anionic [7,8] and the neutral form [9]. For molecules where solvent dipolar relaxation is an important deactivation pathway of the excited electronic state, a shift in the fluorescence maximum towards a longer wavelength is caused by the shift in the excitation wavelength towards the "red edge" or longer-wavelength edge of the absorption band. This effect is known as "red-edge excitation shift" (REES). The REES effect is observed in motionally restricted viscous or condensed media, where the reorientation time τ_r of the solvent dipoles around the excited fluorophore is either comparable with or greater than the fluorescence lifetime τ_f. Under these conditions, excitation at the red edge of the absorption band selectively excites only those fluorophores which interact more strongly with the surrounding solvent, i.e. around which the solvent molecules are oriented in a way similar to that of the solvent relaxed state. Emissions from these molecules are shifted towards lower energies or longer wavelengths. The manifestation of the REES effect is a function of the motional restriction imposed on the solvent molecules around the fluorophore and hence can be used to probe the mobility of the environment itself using the fluorophore merely as a reporter group [10–13]. The present research explores the potential applications of these excited-state relaxation phenomena, as highly sensitive probes of the local environment of the 7HF



7 - HYDROXYFLAVONE

Scheme 1. Molecular structure of 7HF.

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fluorophore. To date, except for a few investigations [9,14], not much work has been done on the photophysical properties of flavones in different organized assemblies such as micelles and reverse micelles. These systems are useful as models for biological membranes and at the same time serve as excellent testing grounds for examining probe fluorescence response to changes in the microenvironment.

Amphiphilic molecules, e.g. aerosol OT (AOT) (sodium bis[2-ethylhexyl]sulphosuccinate), when dissolved in organic solvents such as *n*-heptane form spheroidal aggregates called "reverse micelles" [15–18]. These have an external shell made up of hydrocarbon chains of the amphiphilic molecules and an inner core consisting of the polar or charged head groups and the counterions. The water encased in reverse micelles is a peculiar solvent, exhibiting properties differing markedly from those of free bulk water [15,17] but similar to the water intimately associated with biological macromolecules. The average size of the reverse micelles is dependent on the amount of solubilized water, which is expressed by the water-to-surfactant molar ratio $W_0 = [H_2O]/[AOT]$. The properties of encased water change continuously with W_0, as well as with the distance from the polar heads to the centre of the reversed micelles. The "water pool" consists of at least two populations: "bound" water molecules which are associated with the polar head groups and "free" water, the latter developing at the centre of the water pool as the hydration of the surfactant polar heads becomes complete [15,17]. With increasing W_0, bound and free water coexist and exchange rapidly.

In this paper we present a report on the fluorescence emission properties of 7HF encased in AOT reverse micelles in *n*-heptane, whose local environment is modulated by varying W_0. For the first time, the extremely sensitive REES effect and the dual-fluorescence behaviour of 7HF have been used to detect the changes in the local environment in reverse micelles.

2. Experimental details

7HF was obtained from Aldrich Chemical Company. The purity of the sample was checked by comparing the electronic absorption and emission spectra with published data [1,4]. AOT was purchased from Sigma Chemicals and used without further purification. 7HF was directly dissolved in freshly prepared AOT solutions (55–60 mM) in spectrograde *n*-heptane (Merck). The same concentration of 7HF (20 μM) in AOT reverse micelles was maintained for all experiments. Assuming that a given fluorophore species is totally solubilized in the micellar phase and distributes among the micelles according to Poisson statistics, the average number of fluorophore molecules per micelle is given by $\langle n \rangle = [F]/[M_T]$ where [F] is the macroscopic fluorophore concentration and $[M_T]$ is the total micellar concentration [19]. Using literature data for the aggregation number of AOT in *n*-heptane [16], the typical micellar concentration $[M_T]$ is estimated to be about 0.0025 M. The average number $\langle n \rangle$ of 7HF molecules per micelle would then be typically 0.008. This ensures that, in general, not more than one molecule of 7HF occupies a given micelle. Under such conditions, solubilization of 7HF molecules should cause negligible perturbation of the structure and related properties of the micelles.

Steady-state absorption and fluorescence spectra were recorded at room temperature (298 K) with a Hitachi model U-2000 spectrophotometer and model F-4010 spectrofluorometer respectively. All fluorescence spectra were in general corrected for the wavelength dependence of the sensitivity of the apparatus. $W_0 = 0$ represents that condition when no water is added into the AOT–*n*-heptane solutions.

3. Results and discussion

3.1. Excited-state proton transfer

7HF is insoluble in *n*-heptane but soluble in H_2O containing 5% methanol [4]. However, it can be solubilized in AOT–*n*-heptane reverse micelles. Fig. 1 shows typical emission spectra of 7HF in AOT–*n*-heptane reverse micelles with increasing water-to-surfactant molar ratio $W_0 = [H_2O]/[AOT]$. The excitation wavelength is 310 nm which is the maximum of the lowest energy absorption band of 7HF [3,4]. At $W_0 = 0$, i.e. with no added water, the emission spectrum shows a single blue–violet emission band with $\lambda_{max} \approx 396$ nm. Increase in the size of the water pool by increasing W_0 causes the development of a second green emission band ($\lambda_{max} \approx 545$–550 nm), with a concomitant red shift of the blue–violet band maximum which reaches a value of 417 nm at $W_0 \geq 10$. The fact that the emission spectrum shows such

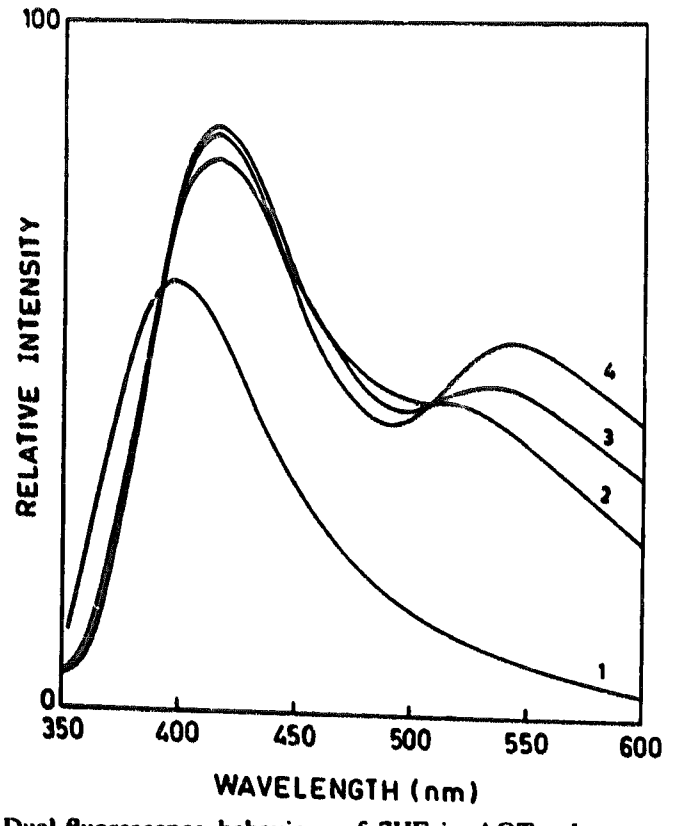

Fig. 1. Dual-fluorescence behaviour of 7HF in AOT–*n*-heptane reverse micelles at different W_0 ([AOT] = 57 mM; $\lambda_{exc} = 310$ nm): curve 1, $W_0 = 0$; curve 2, $W_0 = 4$; curve 3, $W_0 = 10$; curve 4, $W_0 = 36$.

pronounced sensitivity to the changes in W_0 values is consistent with the localization of 7HF molecules predominantly in the "water pool". From its spectral position the blue–violet fluorescence can be assigned to the $S_1(\pi\pi^*) \rightarrow S_0$ fluorescence of the normal, non-proton transferred form of 7HF [3–6]. Recently the large Stokes-shifted green emission has been attributed to a solvent-mediated ESPT process where the excited-state tautomer ($\lambda_{max} \approx 530$–$540$ nm) as well as anionic form ($\lambda_{max} \approx 515$–$520$ nm) of 7HF are generated in methanol [6]. Wolfbeis et al. [4] have also shown that, in alkaline pH, the green emission arises from the anionic form with $\lambda_{max} \approx 528$ nm.

The presence of a clear isoemissive point in the emission spectra of 7HF with changing W_0 (Fig. 1) suggests that there are only two types of excited-state species and one appears at the cost of the other. In this case, from the spectral position of the green emission band ($\lambda_{max} \approx 545$–$550$ nm) we can assign this to the tautomer of 7HF generated by the ESPT process [6]. Complete overlap is found in the excitation spectra, monitored at the blue–violet and the green emission regions (data not shown). This indicates that the two emission bands of 7HF in AOT–*n*-heptane at $W_0 > 0$ arise from the same ground-state species.

The ratio of the intensity I_T of the green ESPT tautomer emission band to the intensity I_N of the blue–violet band of the normal form of 7HF, i.e. I_T/I_N, is an especially useful parameter for monitoring the enhancement in the relative yield of the ESPT tautomer emission with increasing W_0. Since the ESPT process in the case of 7HF is solvent mediated [5,6], it is expected that I_T/I_N should be sensitive to the immediate environment. Previous studies in different homogeneous solvents have indicated that I_T/I_N shows appreciable sensitivity to polarity, viscosity and H-bonding ability of the medium [9]. Fig. 2 is a plot of I_T/I_N with increasing W_0. The ratio I_T/I_N increases rapidly up to $W_0 = 8$–10 beyond which it shows very small changes even for large water content, e.g. $W_0 = 36$. Thus, it appears that beyond $W_0 \approx 10$ (Fig. 2) there is not much change in the immediate environment of 7HF molecules. Even at large W_0, e.g. $W_0 = 36$, the I_T/I_N ratio is 0.66 which is much less than observed in aqueous solution (containing 20% methanol) where $I_T/I_N \approx 18$.

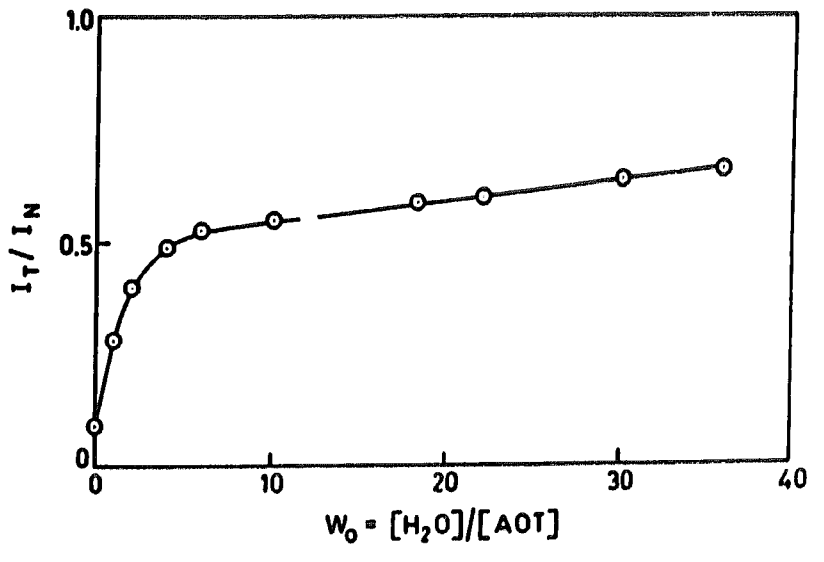

Fig. 2. I_T/I_N of 7HF as a function of W_0 in AOT–*n*-heptane reverse micelles ([AOT] = 55.6 mM; $\lambda_{exc} = 310$ nm).

3.2. Red-edge excitation shifts

A shift in the wavelength of fluorescence emission maximum towards longer wavelengths caused by a shift in the excitation wavelength towards the red edge of the absorption band is termed the "red-edge excitation shift" (REES) [10–13]. The origin of the red-edge effect lies in the altered fluorophore-solvent interactions in the ground and excited states. For polar molecules in which the dipole moment changes upon excitation, solvent dipolar reorientation around the excited fluorophore is an important deactivation pathway. Under conditions where the reorientation time τ_r of the solvent molecules around the fluorophore equals approximately τ_f, the fluorescence lifetime, the effect of solvent relaxation manifests in different ways [20]. Two such important manifestations of solvent relaxation are the red shift of the emission maximum with increase in mobility of surrounding solvent molecules, as well as with increase in the polarity of the medium. We have previously shown that, for 7HF molecules in both the neutral and anionic form, solvent dipolar reorientation around the excited fluorophore is an important relaxation pathway [7–9]. The phenomenon of REES is mostly observed in very viscous solutions or condensed phases where τ_r is comparable with or greater than τ_f. The manifestation of REES is a function of the mobility of the surrounding solvent [10–13]. It has therefore been utilized by us to monitor the motional restriction of the surrounding water molecule of 7HF in AOT–*n*-heptane reverse micelles as the water content is increased.

Fig. 3 shows the variations in the blue–violet emission maximum and $\Delta\lambda$ with increasing W_0 where $\Delta\lambda$ represents the REES shift in the emission maximum when the excitation wavelength is changed from 310 to 350 nm, the red edge of the absorption spectrum. (The possible selective excitation

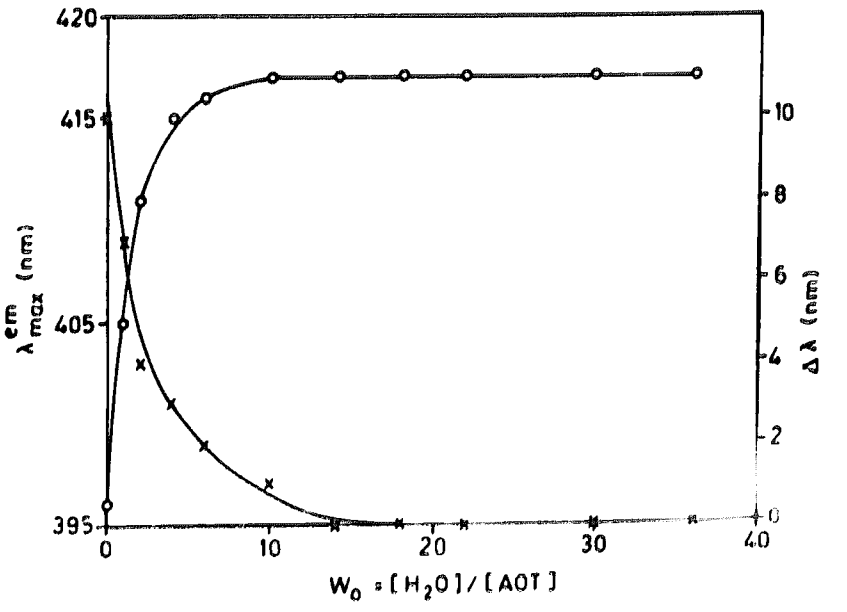

Fig. 3. λ^{em}_{max} of 7HF in AOT–*n*-heptane reverse micelles at different W_0 (–○–) ($\lambda_{exc} = 310$ nm; [AOT] = 55.6 mM) and $\Delta\lambda$ vs. W_0 (– × –), where $\Delta\lambda$ represents the shifts in λ_{max} when λ_{exc} is changed from 310 to 350 nm.

of the ground-state anion of 7HF when λ_{exc} is 350 nm, which falls in the region of absorption of the anionic species [6], will not be reflected in the blue–violet emission band having $\lambda_{max} \approx 445$–450 nm, since the emission maximum for the anionic form of 7HF is about 515–520 nm.) At $W_0 = 0$, the observed REES shift $\Delta\lambda$ is 10 nm. As the water content is increased, $\Delta\lambda$ rapidly decreases. From $W_0 \approx 8$–10 onwards, $\Delta\lambda$ vanishes with the emission maximum occurring at 417 nm for both midband and red-edge excitation. The change in $\Delta\lambda$ with W_0 is accompanied by a concomitant change in the emission maximum (with $\lambda_{exc} = 310$ nm) to longer wavelengths from 396 nm (at $W_0 = 0$) to 417 nm (at $W_0 = 10$). However, beyond $W_0 \geq 10$ there is no further shift in emission maximum to a longer wavelength. It should be noted that in bulk water the emission maximum is about 425 nm [4]. The red shift in the emission maximum with increasing W_0 reflects not only the increase in mobility of the surrounding solvent but also changes in polarity. On the contrary the decrease in $\Delta\lambda$ with W_0 can solely be attributed to the increase in mobility of the surrounding water molecules. The increase in polarity of the surrounding medium with increasing W_0 is also reflected in the absorption spectrum (not shown) where we observe a red shift in the lowest energy absorption maximum from 306 nm at $W_0 = 0$ to 311 nm at $W_0 = 36$. No change has been noted, however, in the absorbance values with W_0, which rules out the possibility of trivial effect of additional solubilization.

The variations of I_T/I_N, $\Delta\lambda$, $\lambda^{em}{}_{max}$ and $\lambda^{abs}{}_{max}$ with increasing W_0 show that the immediate environment of 7HF molecules in the water pool changes rapidly in both polarity and mobility up to $W_0 \approx 10$. An increase in W_0 beyond 8–10 does not significantly modify the immediate environment of 7HF. The fact that even at high water content, e.g. $W_0 = 36$, the values of I_T/I_N, $\lambda^{em}{}_{max}$ and $\lambda^{abs}{}_{max}$ are not similar to those observed in bulk water [3,4] indicates that the nature of the surrounding water molecules of 7HF is different from that of "free" bulk water. Previous workers have shown that, in reverse micelles of AOT–*n*-heptane [15–18], peculiar properties of water which are different from those of free "bulk water" are manifested in the range $0 < W_0 \leq 10$. These properties include restricted mobility (translational and rotational), extent of H bonding and effective dielectric constant which are all significantly different from that observed in bulk water [17]. These properties of "bound" water continuously change until complete solvation of the sodium and sulphonate ions occurs at $W_0 \geq 10$, beyond which "free" water with properties resembling those of normal bulk water is found at the centre of the water pool. An increase in W_0 beyond 8–10 can no longer significantly modify the properties of the "bound"-water region but increases the volume of the "free"-water core. The changes observed here in I_T/I_N, $\Delta\lambda$, $\lambda^{em}{}_{max}$ and $\lambda^{abs}{}_{max}$ with increasing W_0 indicate that 7HF molecules predominantly reside in the bound-water region of the reverse micelles, in proximity to the polar head groups.

4. Concluding remarks

We have used the dual-emission and REES effect to explore the local environment of 7HF molecules in the AOT–*n*-heptane–H_2O reverse micelles. The variations in relevant spectroscopic parameters with increasing W_0 suggest that the 7HF molecules are predominantly localized in the bound-water region of the water pool near to the AOT head groups.

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