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ORIGINAL PAPER

Fluorescence Characteristics of some Flavones Probes in Different Micellar Media

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Abstract The fluorescence characteristics of five hydroxiflavones (HFs) (some typical models of flavonols), (3 - HF, 6 - HF, 7-HF, 3, 6 - diHF and 3, 7-diHF) in the micellar media of non-ionic surfactant (Triton X-100), anionic surfactant (SDS) and the block copolymer Pluronic F127, have been investigated by means of UV–Vis and steady-state and time resolved fluorescence spectroscopies. Attention is paid to both excited-state intra-molecular proton transfer (ESIPT) as well as ground-state intermolecular proton transfer. The influence of the -OH groups as well as the effect of temperature on the dual fluorescence emission, the Normal and Tautomer emissions, are also investigated. The fluorescence quantum yield of the HFs in mentioned micellar media has been also determined. The results are discussed with relevance to the local environment of HFs as sensitive fluorescence probe in biological membrane systems.

Keywords Flavones · Fluorescence spectroscopy · ESIPT · Micelles

Introduction

Studies on the electronic excited—relaxation processes including excited-state proton transfer (ESPT) of flavones (typical model of flavonols), especially for the development of fluorescent probes and senzors have been extensively undertaken [1–8]. Their fluorescence properties are sensitive to the surrounding medium (polarity, hydrogen bonds, pH, temperature) [2, 9, 10] therefore, applications of the dual emission parameters of flavonols as exquisitely sensitive environmental probes have been reported [11–13]. It was found that for 3-Hydroxyflavone (3-HF) and its derivatives, the ESPT is intrinsic, proceeding across an internal H-bond of the molecule and essentially barrier free [14], while the ESPT in 7-HF, where proton donor and acceptor sites are not located adjacent to each other, strongly depends on the nature of the solvent medium. In these lines, studies of time resolved fluorescence spectroscopy and transient absorption measurements showed that the ESPT is solvent dependent, leading to the formation of different photo-tautomers in the excited state as well as in the ground state [15].

Sarkar and Sengupta, 1991 [16] reported that the intramolecular ESPT and the resulting fluorescence properties of 3-HF are used to probe microenvironments in aqueous micellar systems. In these lines the effect of reverse micelles on the intramolecular ESPT and dual luminescence behavior of 3-HF, have been investigated [17]. Amphiphilic molecules such as AOT/n-heptane at different values of water to surfactant molar ratio, were used as reverse micelles [17]. It was shown that the green tautomer emission (λ_{max}~524 nm) and blue-violet normal emission (λ_{max}~400 nm) originate from two different ground state populations of 3-HF molecule, which are located respectively in the apolar phase and at the interphase of the reverse micelles, proximal to the AOT head groups [17]. Also, it was shown that the population of 3-HF molecules which are initially located in the interfacial region proximal to the polar head groups is "pushed" out into the non-polar phase, where external hydrogen bonding perturbations are minimized [17]. By comparison, it was reported that the distribution of 7-HF molecule is predominantly localized in the bound water region of the water pool of the reverse micelles [18].

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Due to the best known molecule exhibiting intramolecular ESPT, 3-HF [1, 3, 19] has been also investigated in microemulsions and liposomes [6, 17, 20, 21]. In CTAB, SDS and Triton X-100 micelles, the origin of the long-wavelength emission of 3-HF depends on the extent of micellization [16]. While below cmc (critical micellar concentration), 3-HF resides in a predominantly aqueous environment and the long emission wavelength is assigned to an excited state anionic form (λ~ 513–515 nm) [6, 16], above cmc 3-HF resides in the regions of lower polarity in micelles, the major contribution being assigned to Tautomer form (λ~520–530 nm) [16]. Ground- and excited-state proton transfer reaction of 3-HF in aqueous micelles of CTAB, SDS, Triton X-100 and block copolimer Pluronic F68, has been reported [22]. It was pointed out that the anion responsible for the long emission band (λ~450–480 nm) is independent of intramolecular ESPT and moreover, the ESPT dynamics is similar to that in protic, hydrogen-bonding solvents like methanol and water, where dual emission is observed [22]. Demchenko et al. [23] demonstrated the possibility of obtaining, from a single fluorescence probe (and among them 3-HF) information about the broad range of intermolecular interactions in the probe environment, if the probe exists in several excited or ground states that interact differently with probe environment and when the interactions with the environment influence the energies and the relative populations of these states. The study of 3-HF in the nanoscopic polar domains within AOT/n-heptane reverse micelles has been also reported [24]. It was found that the intramolecular ESPT rate is severely retarded within these polar cavities, yielding time constants > 100 ps, the exceptionally long time constants being attributed to the formation of intermolecular H-bonded 3-HF:AOT complexes at the cost of the intramolecular H-bonding within the individual 3-HF [24].

As mentioned above, the widespread interest in the literature is done to 3-HF, so that photophysical properties of five hydroxiflavones (HFs) (some typical models of flavonols), (3 - HF, 6 - HF, 7-HF; 3, 6 - diHF and 3, 7-diHF) in homegeneous media have been recently investigated [25]. It was pointed out that the intramolecular proton transfer appears to be promoted by the solvent polarity and also by the position and number of the –OH groups of the phenyl ring and thus, an influence of the rate of the excited state proton transfer process. The attention was focussed on 3,6- and 3,7-diHF, where significant discrepacies in the data regarding proton transfer were observed [25]. Here, the influence of external hydrogen bonding perturbation on the internal hydrogen bond of the molecule, was noticed [25]. Also, the fluorescence quantum yield of the mentioned flavonols was found to be solvent dependent [25].

This work is an extension of our previous study [25] and deals with the fluorescence characteristics of the same hydroxiflavones in the micellar media of non-ionic surfactant (Triton X-100), anionic surfactant (SDS) and the block copolymer Pluronic F127, studied by means of UV–Vis and steady-state and time resolved fluorescence spectroscopies. Attention is paid to both excited-state intra-molecular proton transfer as well as to ground-state intermolecular proton transfer. The influence of -OH groups as well as the effect of temperature on the dual fluorescence emission, the Normal and Tautomer emissions, are also investigated. The quantum yield of the HFs in mentioned micellar media has been also determined. The results are discussed with relevance to the local environment of HFs as sensitive fluorescence probes in biological membrane systems.

Experimental

Materials

The structure of the studied flavones was previously reported [25]. The stock solutions of flavones were prepared in Methanol. Aliquots from stock solutions were dried at room temperature and then diluted with different aqueous solutions of surfactants: Sodium Dodecyl Sulphate (SDS) (from Fluka), Triton X-100 (TX-100) (from CALBIOCHEM) and the block copolymer Pluronic F127 (from Sigma). The used surfactant concentrations were: SDS, 3 %; Tx-100, 5 % and F127, 3 %. The flavones concentration was in the range of $4.80–6\times10^{-5}$ M.

Methods and Apparatus

The Absorption Measurements Were recorded using a Perkin Elmer, Lambda 35, UV–Vis Spectrometer at a scan rate of 480 nm/min and a spectral resolution of 1 nm.

The Fluorescence Emission and Excitation Spectra were recorded with a Jasco FP-6500 Spectrofluorometer, using 3 nm bandpasses for the excitation and the emission monochromators, the detector response of 1 s, data pitch of 1 nm, the scanning speed of 100 nm/min. The excitation wavelength was 365 nm.

The fluorescence quantum yield was determined by comparison to dilute quinine bisulfate solution in 0.1 N H_2SO_4 with 0.55 absolute quantum yield [26], using the following relationship:

$$\phi_x = (F_x A_{ref} \phi_{ref}) / F_{ref} A_x$$

where F is the area under the fluorescence emission curve, for quinine bisulfate (F_{ref}) and for the studied compound (F_x) over the wavelength region 420–650 nm, A_{ref} is the absorbance for quinine bisulfate at 346 nm.

The Excitation-Emission Matrix (EEM) Spectra were recorded by scanning the excitation wavelength in the range of 250–420 nm with a step of 5 nm and measuring the emission spectrum. Contour plots were then constructed with the dedicated software provided by the manufacturer. They contain scattering data as well ($\lambda_{ex}=\lambda_{em}$), which should be disregarded.

The Fluorescence Lifetime Decays Were recorded in a time-correlated single photon counting FLS920 system from Edinburgh Instruments, with laser excitation at 375.6 nm, a lifetime scale of 50 ns and 1,024 channels. The data were fitted with a multi-exponential decay (reconvolution) and the accuracy of the fit was checked on grounds of χ^2, which was around 1. Intensity-averaged lifetimes were calculated according to the equations [27]:

$$\langle\tau\rangle = \frac{\sum_i \alpha_i \cdot \tau_i^2}{\sum_i \alpha_i \cdot \tau_i}; \qquad f_i = \frac{\alpha_i \cdot \tau_i}{\sum_i \alpha_i \cdot \tau_i}; \qquad a_i = \frac{\alpha_i}{\sum_i \alpha_i}$$

where α_i is the pre-exponential factor and τ_i the lifetime of the *i*th component; f_i is the fractional intensity of the *i*th component and a_i is the fractional amplitude, interpreted as the relative population of the respective state.

Results and Discussion

UV-vis Absorption Measurements

Figure 1 shows the electronic absorption spectra of 3-, 6- and 7-HF in SDS 3 % (Fig. 1a) in direct comparison with their absorption in TX-100 5 % (Fig. 1b). The same shape of the absorption spectra and no shifts in the absorption wavelengths, have been observed. As it can be seen, slight changes in absorbance intensity are evidenced. The behavior is due to the nature of the surfactant. In Tx-100 5 %, the absorption band of 3-HF is well structured and slightly blue-shifted due to the presence of a hydrophobic environment, where the external H-bonding perturbation is minimized and thus an efficient intra-molecular ESPT is obtained. The absorbance intensity in TX-100 is higher by comparison to that in SDS. Considering 6 and 7-HF, the shape of the absorption spectra is changed and the absorbance intensity is lower in TX-100 than in SDS and also blue-shifted, more pronounced than in the case of 3-HF. The long wavelength absorption band, at ~350 nm, is observed only for 3-HF while for 6-HF, the absorption band is very broad and totally absent for 7-HF. That means that upon the incorporation of HFs in micelles, only for 3-HF the electron-withdrawing carbonyl group takes place. Thus, the position of the –OH group in the HFs structure on their incorporation in micelle, is taken into consideration.

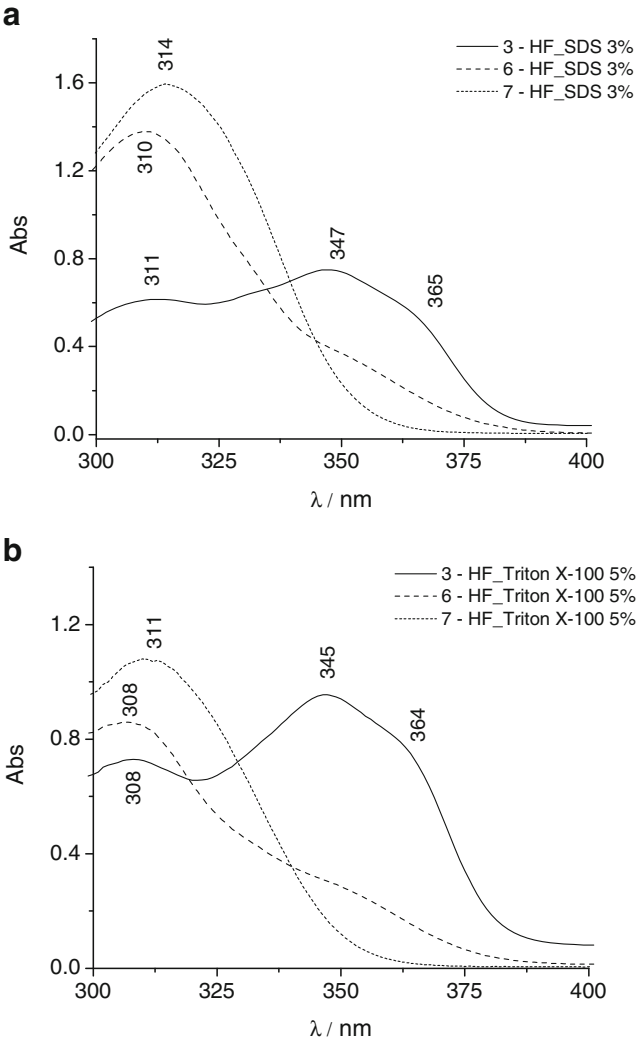

Fig. 1 UV–Vis absorption spectra of the 3-HF, 6-HF and 7-HF in SDS 3 % (**a**) and in Triton X-100 5 % (**b**)

Also, the number of the – OH groups have influence on the electronic absorption spectra; Absorption characteristics of all HFs studied are presented in Table 1.

Steady-State Fluorescence Measurements

Figure 2 displays the fluorescence emission (Fig. 2a) and excitation (Fig. 2b) of HFs in the block copolymer Pluronic F127 3 % at room temperature for an excitation wavelength of 365 nm. The Normal fluorescence emission, N^*, is not significant, and the fluorescence emission is predominantly from Tautomer form, T^*, at ~530 nm. The photo-tautomer emissions of 3, 6-diHF in F127 3 % shows a 9-fold enhancement in fluorescence intensity compared with the N^* form. The gradual red-shift comparing to the fluorescence band of 3-HF, λ_{em}=533 nm for 3, 6-diHF and λ_{em}=535 nm for 3, 7-diHF,is due to the number and the position of the – OH groups. An efficient intra-molecular

Table 1 Absorption (λ_{Abs} and ε), Emission (λ_{em} and Fluorescence Quantum Yield (ϕ_f)) and Stokes Shift ($\Delta\nu$) of the flavonols in different surfactant media. λ_{ex}=365 nm

Probe	Surfactant	λ_{Abs} (nm)	ε (L M^{-1} cm^{-1})	λ_{em} (nm)	$\Delta\nu$ (cm^{-1})	ϕ_f
3-HF	F127	312; 345; 416sh	883	456; 480; 528	10039	0.178
	SDS	312; 347; 365sh	12500	447; 523	9731	0.190
	TX-100	308; 345; 364	15833	534	10014	0.098
6-HF	F127	270; 310; 359sh	5500	445*; 516sh	-	-
	SDS	272; 310; 357sh	23000	454*; 530sh	-	-
	TX-100	308; 353	14167	474*; 510sh		
7-HF	F127	314; 450bb	1876	440*; 528**	-	-
	SDS	315	29831	533*	-	-
	TX-100	312	20075	530*	-	-
3, 6-diHF	F127	331; 365sh; 427	4167	533	11518	0.127
	SDS	333; 371	18333	450; 523	10992	0.037
	TX-100	332; 372	17500	534	11317	0.058
3, 7-diHF	F127	324; 345	1854	477; 535	12267	0.110
	SDS	322; 346; 416	14583	450; 525	9816	0.197
	TX-100	320; 345; 362sh	15855	537	10283	0.113

*, ** are very weak signals; *sh* is shoulder and *bb* is broad band

ESPT is considered with a high quantum yield of the T^* fluorescence band, around ϕ=0.1, Table 1, which is higher than that in different solvents (ϕ=0.039), in Acetonitrile; ϕ=0.080, in Ethanol; ϕ=0.053, in Dioxan [25]. That means that HFs molecules are mainly encapsulated in the hydrophobic environments of the micelle which favors proton abstraction leading to an efficient intra-molecular ESPT process. For 3, 6 - and 3, 7-HF the fluorescence emission were observed to be very low (the results are not shown). In Fig. 2b the corresponding fluorescence excitation spectra are presented. The excitation profile is the same, with significant red-emission region. This fact is attributed to the numbers and the position of the –OH groups. For 3, 6-diHF, it is 12 nm red-shifted (332 nm) by comparison with 3-HF (310 nm). In direct comparison with Fig. 1b (in TX-100 5 %), the excitation profile for 3-HF (in F127 3 %) is significantly red-shifted. As can be observed there are two bands: at 347 nm and 373 nm, in F127 3 %, compared with 345 nm and 364 nm, in TX-100 3 %. The behavior is attributed to the microenvironment of the 3-HF. That means that 3-HF is encapsulated in the F127 3 %, at an external interface in the proximity of a hydrophilic group.

The fluorescence emission of 6-and 7-HF in F127 3 % was found to be very low (the results are not shown) while in SDS 3 % (Fig. 3), the fluorescence emission from both N^* and T^* bands were observed. As can be seen, the fluorescence emission of both probes is very weak, the ratio of fluorescence intensity of the N^* and T^* bands being strongly dependent on the position of the –OH group. As is evident from Fig. 3, the value of I_{N*}/I_{T*} ratio is ~3 for 6-HF in SDS 3 % as compared with its value of ~1, for 7-HF in SDS 3 %. Also, the encapsulation of HFs in the proximity of a hydrophilic region, which do not favors the proton abstraction, is considered.

The Effect of Temperature on the Intra-Molecular ESPT

The effect of temperature (in the range on 25–60 °C) on the fluorescence emission of the mentioned HFs in the micellar media of non-ionic surfactant (Triton X-100, 5 %), anionic surfactant (SDS, 3 %) and the block copolymer Pluronic F127, 3 % was investigated.

In F127 3 % micelle, with the increasing of temperature, it was observed that the fluorescence emission from both N^* (λ_{em}~445 nm) as well as from T^* forms (λ_{em}~535 nm) for all HFs is very weak and decreases, no significant changes occurring in the N^* and T^* emission, Table 1. It was found that in F127 3 %, the fluorescence intensity of 3-HF is 4- times greater than the one of the 6-HF and 10-times greater than that of 7-HF.

In TX-100 5 % micelle, the fluorescence emission of the 3-HF and 3, 6-diHF also decreases with the increasing of the temperature, the major fluorescence emission being from T^* form (λ_{em}~534 nm). Compared with pluronic F127 3 %, the fluorescence emission of 3-HF and 3, 6-diHF in TX-100 5 % is higher and no changes in the fluorescence emission from T^* form were observed (λ_{em}~534 nm). Slight changes were observed for 3, 7-diHF for which λ_{em}~537 nm and this fact is due to the 7- position of the –OH group. Here, the motion of the 3, 7-diHF on the excited state in the micelle is taken into consideration.

The fluorescence emission of 3-HF; 3, 6-diHF and 3, 7-diHF, in SDS 3 % vs. temperature, has been also investigated. In this case, the fluorescence emission from both N^* and T^*

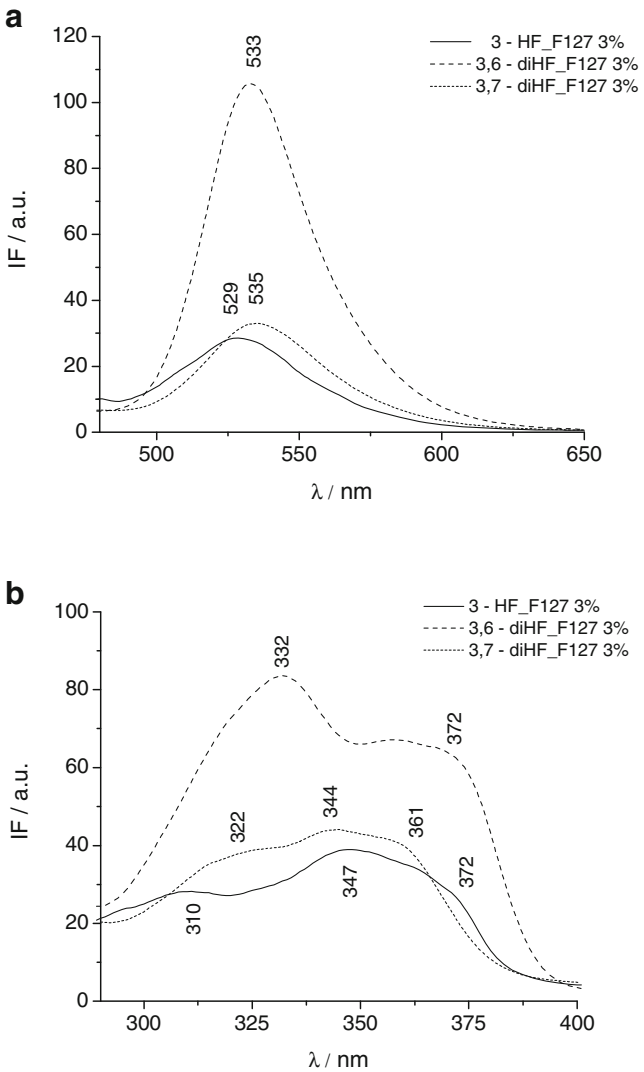

Fig. 2 Fluorescence emission (**a**) and excitation spectra (**b**) of flavonols in pluronic F127 3 % at room temperature. λ_{ex}=365 nm; λ_{em}=530 nm

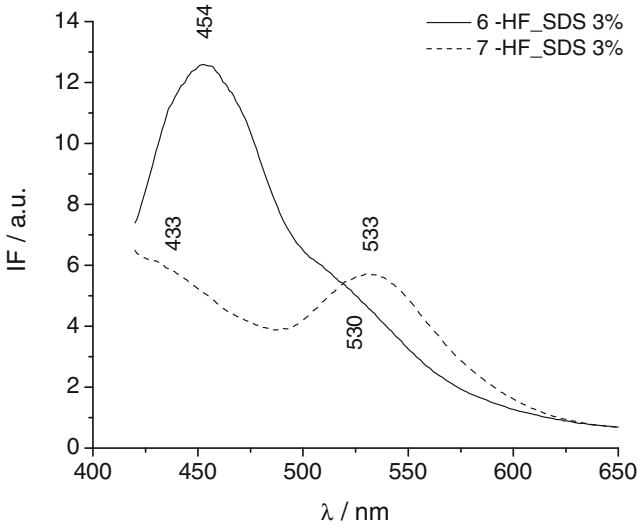

Fig. 3 Fluorescence emission spectra of 6 - HF and 7 - HF in SDS 3 % at 25°. λ_{ex}=365 nm

forms, have been observed meaning that the intra-molecular ESPT takes place in the excited state. For 3-HF, the fluorescence emission from N^* (λ_{em}=447 nm) and T^* (λ_{em}=523 nm) form is unchanged with the increasing of temperature in the range of 25–60 °C. In direct comparison with 3-HF, for 3, 6-diHF, a 3 nm red-shift of the fluorescence emission from the N^* form (λ_{em}=450 nm) is evidenced, the emission from T^* form being unchanged, λ_{em}=523 nm. Also, the fluorescence intensity of both N^* and T^* forms decreases as temperature increases. For 3, 7-diHF, a slight red-shift of the emission from T^* form is observed, λ_{em}=525 nm, and the fluorescence intensities of both emission forms are higher as compared with 3-HF and 3, 6-diHF. In addition, for all HFs, the ratio of the intensity of the blue-violet band of the normal form (I_{N*}) to the green ESPT tautomer emission band (I_{T*}) increases with temperature, in the range of 25÷45 °C, as follows: I_{N*}/I_{T*}=0.179÷0.281, for 3-HF; I_{N*}/I_{T*}=0.418÷0.446, for 3, 6-diHF and I_{N*}/I_{T*}=0.342÷0.524, for 3, 7-diHF. From 56÷60 °C, the intensity ratio I_{N*}/I_{T*} decreases.

In direct comparison with the above micellar media, the fluorescence emission of 3-HF; 3,6-diHF and 3,7-diHF in TX-100 5 % is predominant from T^* form (λ_{em}=534 nm) and the fluorescence intensity increases as follows: 3-HF > 3,7-diHF > 3,6-diHF. The fluorescence intensity for all HFs decreases as temperature increases. Also no shifts in the λ_{em} were observed.

For exemplification and to summarize all the data, Fig. 4 shows the T^* fluorescence intensities of 3-HF; 3, 6-diHF and 3, 7-diHF vs. temperature in the mentioned micellar media, at a temperature of 50 °C for an excitation wavelengths of 365 nm with their corresponding fluorescence excitation spectra.

The excitation spectra were recorded at the T^* fluorescence emission and as can be seen (Fig. 4b, d, f), the predominant absorbing form in the ground state is the neutral form of the HFs. The shape of the excitation spectra is different with significant shifts of the peak positions in the region of 300–400 nm. For 3-HF in SDS 3 %, the excitation band at 356 nm (Fig. 4b) is attributed to a location of HF molecule in a non-polar medium. The band at 356 nm is split in two excitation bands: at 344 and 362 nm, for 3-HF in TX-100 (Fig. 4d) and 347 nm and 362 nm, for 3-HF at interface of the micelle.

Overall, the fluorescence emission of the studied HFs in micellar media of anionic surfactant (SDS) and non-ionic surfactant (TX-100) is major from T^* form and the fluorescence intensity increases in the order: 3-HF > 3, 7-diHF > 3, 6-diHF. The fluorescence emission depends on the polar group from SDS as well as on the number and position of the –OH groups.

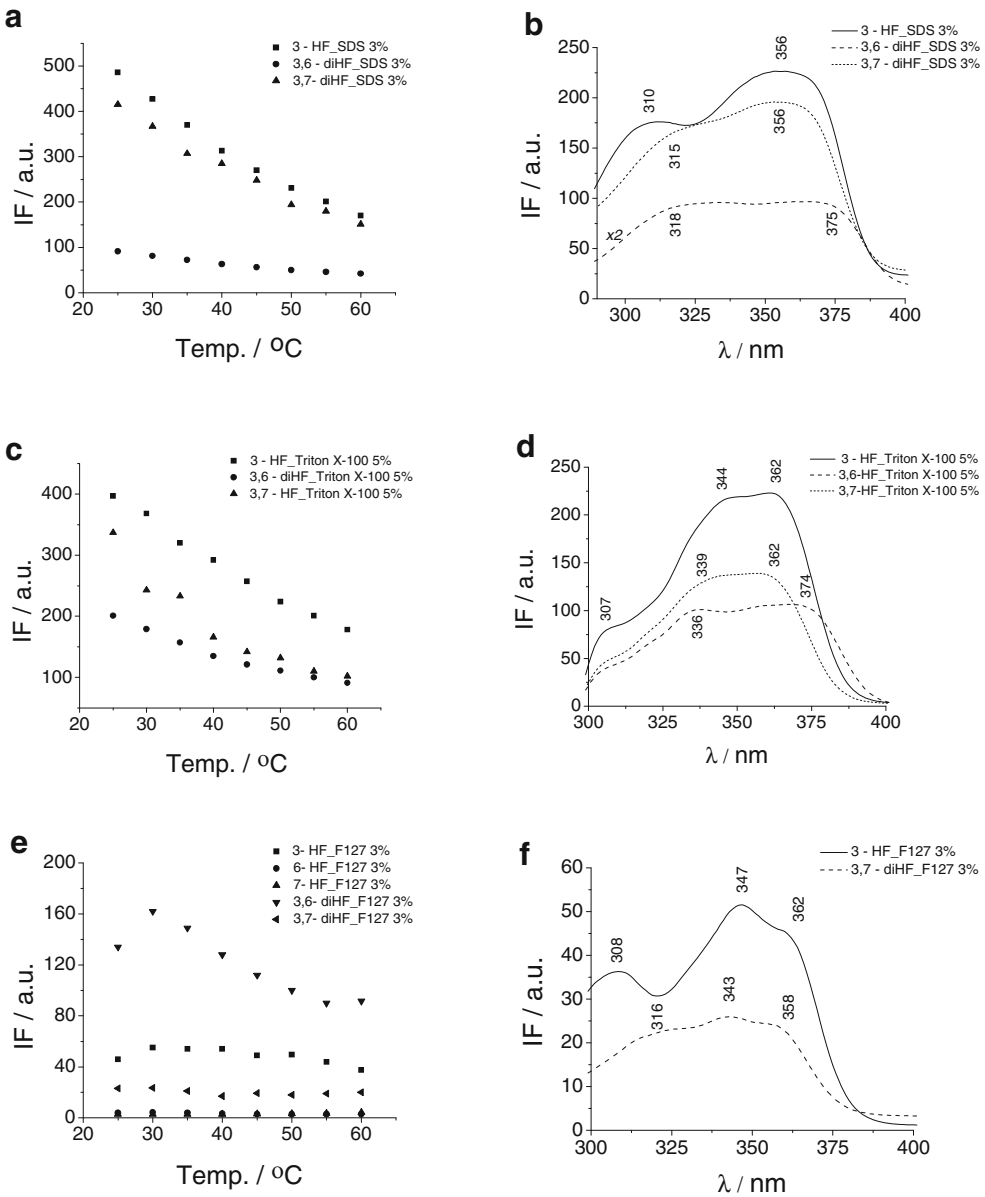

Fig. 4 Tautomer fluorescence intensities of HFs vs. temperature in SDS 3 %, TX-100 5 % and F127 3 % (at λ_{ex}=365 nm) and their corresponding fluorescence excitation spectra (at 50 °C, λ_{em}=530 nm)

Also, the fluorescence intensity in SDS is 10-fold higher than that in the block copolymer Pluronic F127 and λ_{em} is ~9 nm blue-shifted. Comparing with SDS 3 %, in TX-100 5 %, a 11 nm red-shift of the fluorescence emission from T^* form is observed. As temperature increases, an influence of the micellar aggregates formation on the encapsulation of HFs, is considered.

Time-Resolved Fluorescence Measurements

The intensity-time profiles for 3, 6-diHF in the three media are depicted in Fig. 5. The lifetimes are presented in Table 2, together with the emissive fraction, f, and the pre-exponential factor, B, at an excitation wavelength of 375.4 nm and an

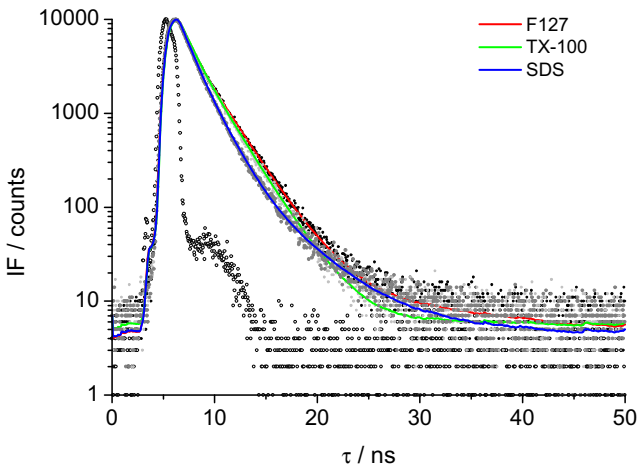

Fig. 5 Fluorescence decays for 3, 6-diHF in different surfactant media

Table 2 Fluorescence lifetimes (ns), preexponential factors and fractional intensity of the flavonols in different surfactant media; λ_{em}=530 nm

Surfactant	B_1	f_1	τ_1	B_2	f_2	τ_2	B_3	f_3	τ_3
3,6-diHF									
F127	-	-	-	0.038	39.45	1.232	0.026	60.55	2.757
SDS	-	-	-	0.062	85.83	1.490	0.004	14.17	3.910
TX-100	-	-	-	0.034	37.8	1.283	0.029	62.2	2.509
3-HF									
F127	0.094	35.05	0.359	0.028	41.36	1.416	0.01	23.59	4.906
SDS	0.032	14.75	0.371	0.068	81.87	0.960	5.60E-004	3.38	4.846
TX-100	0.464	17.66	0.037	0.061	57.78	0.917	1.20E-002	24.56	1.946
3,7-diHF									
F127	0.077	30.88	0.431	0.019	27.14	1.500	0.013	41.98	3.571
SDS	0.054	33.13	0.494	0.048	57.39	0.965	0.002	9.48	4.830
TX-100	0.058	25.9	0.377	0.048	61.2	1.090	0.003	12.9	3.456

emission one of 530 nm. 3-HF and 3,7-diHF have three lifetime components, one of less than 0.1 ns (short lifetime component), the second of around 1 ns (median lifetime component) and finally the third around 3 ns (long lifetime component), while 3,6-diHF presents two components in all the media, i.e. the median and the long lifetime ones. SDS always favors the median lifetime species: large emissive fractions are observed. For 3-HF and 3, 7-diHF the short lifetime component is predominant in pluronic F127 and TX-100.

It can be seen from the EEM spectra presented in Fig. 6 that the most intense fluorescence is measured at λ_{em}=530 nm and λ_{ex} in the range 300–375 nm. It corresponds to the T^* fluorescence. Other significant peaks are at λ_{em} around 480 nm and λ_{ex} 375 nm and correspond to the anion. They appear for all the studied HFs in SDS. Other less visible peaks are those from N^* emission ones, appearing at λ_{em}=410/λ_{ex}=295 nm for 3-HF, λ_{em}=450/λ_{ex}=315 nm for 3, 6-diHF and λ_{em}=372/λ_{ex}=290 nm for 3, 7-diHF. These peaks are overlapped with a surfactant peak in TX-100.

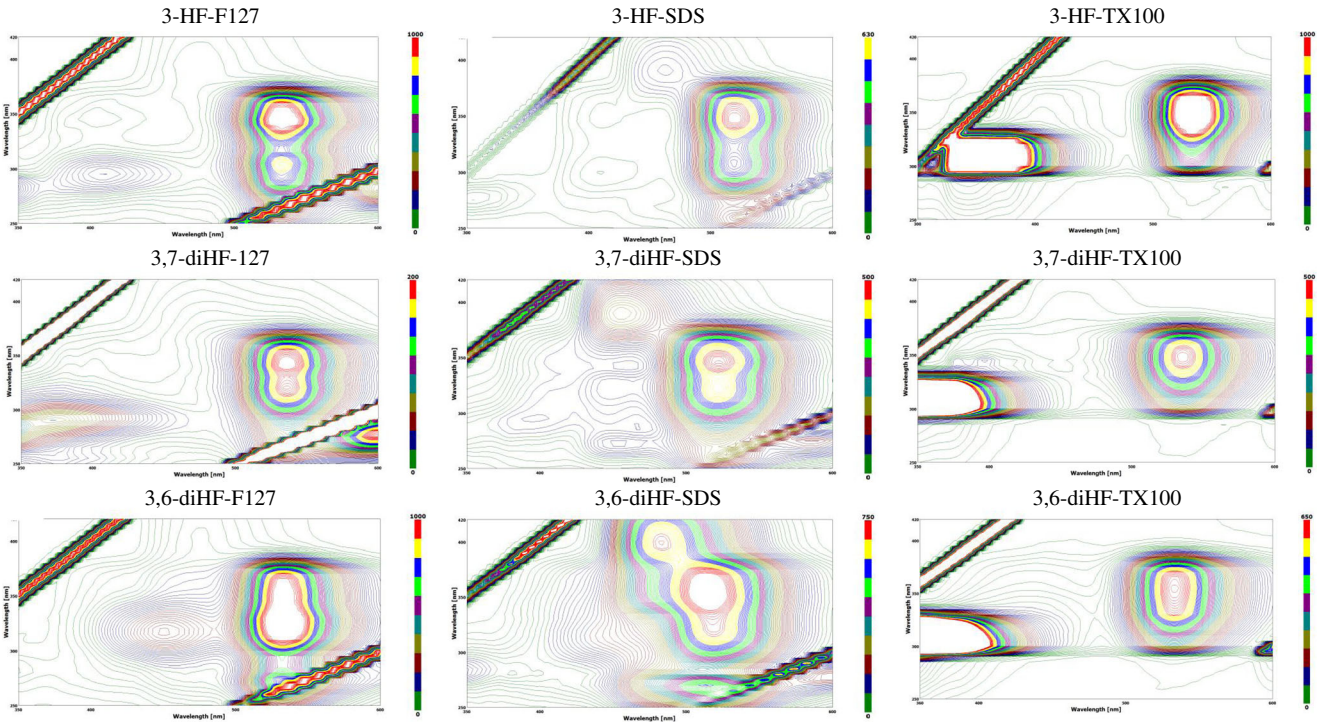

Fig. 6 Contour plots constructed from the EEM spectra measured for the studied flavones in F127 (*left*), SDS (*middle*) and TX-100 (*right*). The emission and excitation wavelengths (nm) are depicted on the x and y axes, respectively. The scattered light should be disregarded (λex=λem)

The three species found from the lifetime measurements can be identified in the EEM spectrum. Let us remember that the excitation wavelength for the lifetime measurements is 375.4 nm, so we are placed on the descending region of the T^* emission in the EEM spectra. The median component is the easiest to identify, as it is the most abundant in SDS according to the lifetime measurements. The EEM spectra reveal that a peak is present at 390/465 nm for 3-HF, 400/484 nm for 3, 7-diHF and 390/457 for 3, 6-diHF only in SDS. This species, having the largest excitation wavelength correspond to the anion and is the median lifetime species. The long-lived species has a 60 % emissive ratio for 3, 6-diHF in F127 and TX-100 and must correspond to the intense band at 530 nm, which is the T^* emission. This leaves us with the short-lived species as being the N^* excited one.

Conclusions

Of the studied HFs, 3-HF; 3, 6-diHF and 3, 7-diHF undergo an intra-molecular proton transfer across the internal H-bond in the micelle that leads to the formation of the tautomer (T^*) fluorescence emission in the micellar media of non-ionic surfactant (Triton X-100 5 %), anionic surfactant (SDS 3 %) and the block copolymer Pluronic F127 3 %;

The fluorescence intensity of the T^* form increases in the following order: 3-HF > 3, 7-diHF > 3, 6-diHF. The fluorescence emission depends on the polar group from SDS as well as on the number and position of the –OH groups;

In pluronic F127, the fluorescence quantum yield of HFs decreases in the following order: 3-HF (ϕ=0.178) > 3,6-diHF (ϕ=0.127) > 3,7-diHF (ϕ=0.110);

Time-resolved fluorescence analysis showed that SDS always favors the median lifetime species: large emissive fractions are observed. For 3-HF and 3, 7-diHF the short lifetime component is predominant in pluronic F127 and TX-100;

From the EEM spectra, the most intense fluorescence is measured at λ_{em}=530 nm and λ_{ex} in the range 300–375 nm, corresponding to the fluorescence emission of T^* form;

The fluorescence intensity of HFs, in all used micellar media, decreases as temperature increases. The decreasing is more significant in F127 3 % than TX-100 5 % and SDS 3 % and the feature is due to the aggregates formation and thus different encapsulation of HFs in the micelles;

The characteristic fluorescence emission of the studied HFs (especially 3-HF, 3, 6-diHF and 3, 7-diHF, respectively) in different micellar media apparently is predominant in the visible region from Tautomer form (λ_{em}~530 nm) suggesting the possibility of using them as an intra-molecular and sensitive fluorescence probe in biological membrane systems.

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