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COMMUNICATION

Excited State Chemistry of Flavone Derivatives in a Confined Medium: ESIPT emission in aqueous media

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The excited state behavior of two flavone derivatives 3-hydroxyflavone and 4'-*N*,*N*-diethylaminoflavonol in a confined medium indicates that supramolecular effects could alter the nature of the fluorescence emission. Within octaacid host the neutral unionized species of these two dyes are present showing large Stokes' shifted emission due to intramolecular proton transfer, a pattern different from that in aqueous medium.

Dyes that show photochemical and photophysical stability, as well as large Stokes' shifted emission due to tautomeric structures resulting from excited-state intramolecular proton-transfer (ESIPT) have found wide applications.[1,2] Due to ESIPT, these dyes exhibit strong solvent and/or pH dependent dual fluorescence emission.[1,2] In this context, due to its unique spectroscopic properties in solution at room temperature hydroxyflavone derivatives have been well-investigated.[3,4] The four-level excited state emission model for 3-hydroxyflavone (3HF) and 4'-*N*,*N*-diethylaminoflavonol (DEA3HF) involves a normal species (N*) and a phototautomer due to ESIPT (T*). These two structures decay emitting fluorescence at short (N*) and longer wavelengths (T*) (Fig. 1).[3,4] The driving force for ESIPT in these compounds is the redistribution of excited state electron density, which makes the OH group significantly more acidic while at the same time making the carbonyl group more basic.[5] Additionally, some aminoflavone derivatives, such as DEA3HF present interesting photophysical features that include a normal emission (N*) with significant charge-transfer character (Fig. 1, bottom).[6]

This emission being very sensitive to the dielectric properties of the environment, it could be used in optical sensing applications.[3,7] However, such applications are often limited to organic solvents. Aqueous medium unfortunately enhance aggregation of dyes, allowing undesirable interactions that can alter the photophysical features of these dyes or even quench their photoluminescence.[8] We visualize that such negative factors could be overcome through supramolecular approaches that involve confining the dyes within a water-soluble hydrophobic organic host.[9] To our knowledge thus far only cyclodextrins that form doubly-open (top and bottom) host-guest complexes have been explored in this context.[10] A recently reported organic host octa acid (OA) has been demonstrated by one of our groups to include a large number of organic guest molecules and modify the photochemistry and photophysics of included guest molecules by forming closed capsules.[11,12]

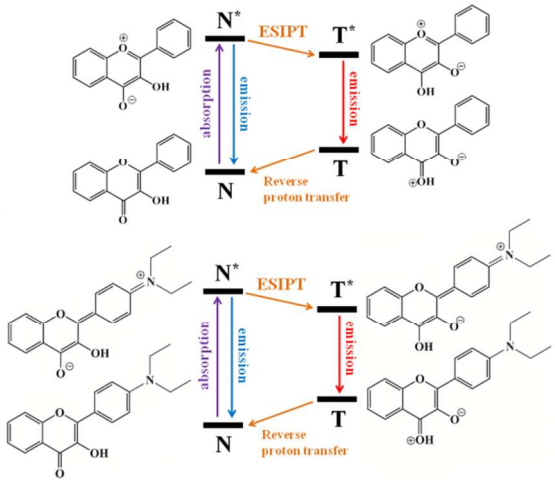
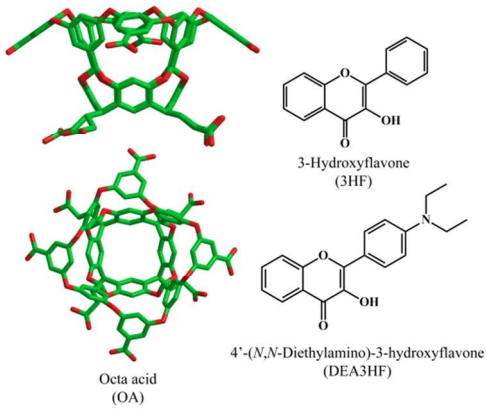


Fig. 1 Four-level model for the proton transfer in 3HF (top) and DEA3HF (bottom). N and T are the normal (N) and tautomer (T) species. The asterisk indicates the excited-state. The charge transfer emission is also presented $(N)S_0 \leftarrow (N^*)S_1$.

Prompted by these encouraging observations we have explored the excited state behavior of 3-hydroxyflavone (3HF) and 4'-*N*,*N*-diethylaminoflavonol (DEA3HF) included within the deep-cavity cavitand octa acid (OA) (Scheme 1) by steady-state and time resolved fluorescence emission techniques. In spite of the host-guest complexes being in water, remarkable single fluorescence emission in the same region as in organic solvents was observed for $3HF@(OA)_2$ with higher fluorescence quantum yield. On the other hand, the complex $DEA3HF@(OA)_2$ presented two emissions due to the N*

form with CT character and another due to the tautomeric form (T*) as in organic solvents.



Scheme 1 Structures of host (left) and guest molecules (right).

Complexation of guests 3HF or DEA3HF with host OA was accomplished by vigorous stirring a dimethylsulfoxide solution of the guest with a sodium tetraborate buffer solution (10 mM, pH: 9.0) of the host for a few minutes. The complexation was confirmed by monitoring the ^{1}H NMR signals of the host and guest (Fig. 2).[13] The signals due the guest 3HF could not be located by this method. However that due to OA were disturbed suggesting inclusion of 3HF within OA. On the other hand, methyl and the methylene hydrogens of DEA3HF (asterisk in Fig. 2) were recorded at -2.0 ppm and 1.1 ppm, >2 ppm upfield shifted with respect to DMSO-d^6, as presented in Fig. S8 (ESI). The guest-host ratio was estimated to be 1:2 (flavone@$(OA)_2$) by recording the ^{1}H NMR spectra of the solution upon gradual addition of the flavones to a 10 mM buffer solution of OA (Fig. S11 and S12 in ESI), (Scheme 2).

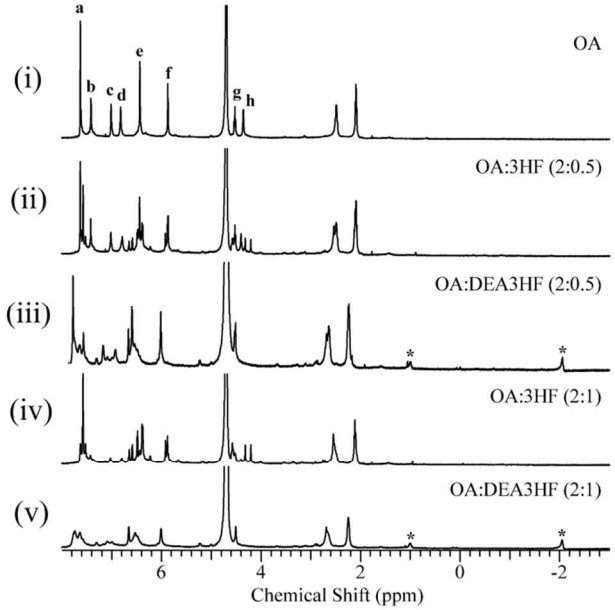
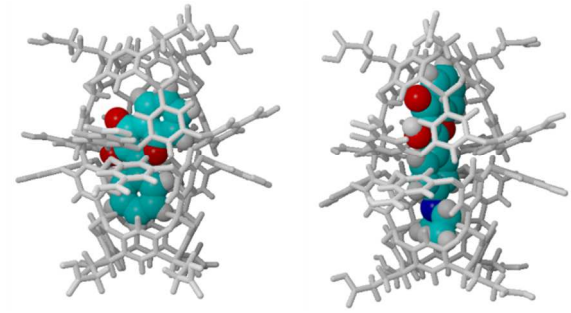


Fig. 2 ^{1}H NMR titration plots, where (i) OA (1 mM in 10 mM borate buffer - pH 9.0 in D_2O), 2.5 µL of (ii) 3HF and (iii) DEA3HF and 10 µL of (iv) 3HF and (v) DEA3HF, both in of 60 mM solution of DMSO-d^6 to 0.6 mL in 10 mM borate buffer - pH 9.0 in D_2O. Guest ^{1}H NMR signals are labeled as a-h.



Scheme 2 Representation of the inclusion complexes 3HF@$(OA)_2$ (left) and DEA3HF@$(OA)_2$ (right). (Yasara Software)

The UV-Vis absorption spectra of the free guests as well the host-guest complexes are provided in Fig. 3. Relevant data from the UV-Vis absorption spectra are tabulated in Table S1 (ESI). The guests 3HF and DEA3HF have an absorption maxima at 344 nm and 409 nm, respectively in organic solvents.[14] The molar absorptivity coefficient ε values, as well as the radiative rate constant (k_e^o), calculated from the absorption spectra using the Strickler-Berg relation,[15] indicate the absorption to be due to spin and symmetry allowed π-π* transition. No significant solvatochromism in the ground state was observed for these two dyes. The absorption spectra for 3HF@$(OA)_2$ and DEA3HF@$(OA)_2$ consisted of two distinct bands, one at shorter wavelength (λ_{abs} 280 nm) due to OA and the other with λ_{abs} at 346 nm for 3HF@$(OA)_2$ and 400 nm for DEA3HF@$(OA)_2$ due to the guest dyes.

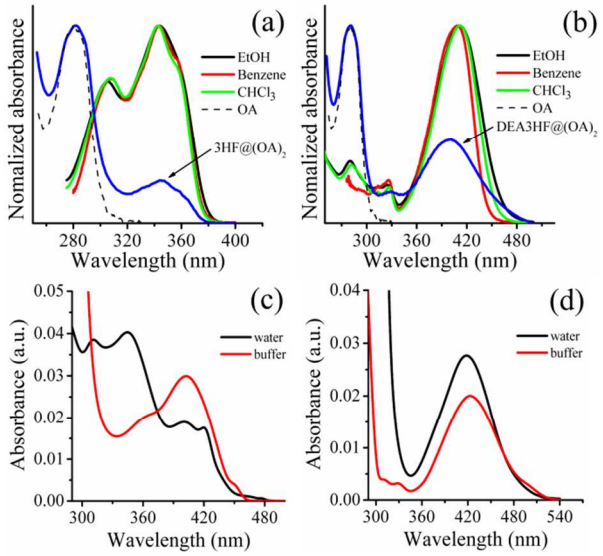


Fig. 3 Normalized UV-Vis absorption spectra in solution of (a) 3HF and (b) DEA3HF and respective inclusion complexes (~10^{-5} M) in buffer (10 mM, pH: 9.0). The OA in buffer is also presented (dash line). Absorption spectra of the guests in water and borate buffer (10 mM, pH: 9.0) are presented in (c) HF and (d) DEA3HF (~10^{-7} M).

Similarity in the absorption spectra of 3HF and DEA3HF in organic solvents and in OA suggested that in these media these flavonols remain in the neutral unionized form (N in Fig. 1).[16] On the other hand, the absorption spectra (Fig. 3c and 3d) in water and tetraborate buffered water without OA, suggested the flavonols to be ionized in the ground state (see Fig. 1 ESI).[16,17] Based on the absorption spectra we conclude that the confinement by inclusion within OA has

provided a hydrophobic environment to the dye in an aqueous medium.

To probe the influence of OA on the excited state properties of 3HF and DEA3HF fluorescence emission spectra and lifetimes were recorded. The emission spectra were recorded by exciting the dyes at their absorption maxima (see Table S1 (ESI)). The fluorescence emission spectra of free and OA complexed 3HF and DEA3HF are presented in Fig. 4. The relevant data from fluorescence emission are summarized in Table S2 (ESI).

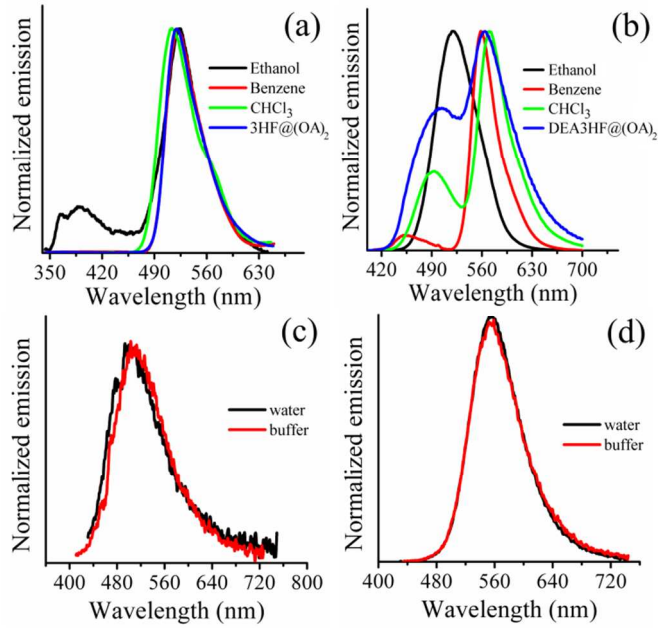


Fig. 4 Normalized steady-state fluorescence emission spectra in solution of (a) 3HF and (b) DEA3HF and respective inclusion complexes in buffer. Emission spectra of the guests in water and borate buffer (10 mM, pH: 9.0) are presented in (c) 3HF and (d) DEA3HF.

The fluorescence emission (λ_{em} ~524 nm) due to tautomeric T* form (resulting from ESIPT) of guest 3HF was observed in non protic organic solvents. In ethanol, as expected, this emission was replaced by the one at 400 nm due to N* state (normal emission, CT state).[18] Interestingly, in spite of being in water, the host-guest complex 3HF@$(OA)_2$ showed a single fluorescence emission in the same region as in benzene and chloroform, suggesting that within the OA cavity 3HF remains in its neutral form shielded from the bulk water. This is consistent with earlier conclusion that OA capsule is 'dry'.[19] Literature reports on the emission of 3HF included in cyclodextrins indicate the presence of normal (N*) and tautomeric (T*) emissions.[20] It is important to note that within OA fluorescence emission from the neutral form of 3HF based solely from the ESIPT mechanism (T*) alone was observed; the normal emission (N*) was absent. Most importantly, the fluorescence quantum yield of T* emission (Table S3 (ESI)) within OA increased by ~50 and ~1.4 times with respect to that in water and benzene, respectively.

Due to strong intramolecular charge transfer (ICT) character, the photophysical properties of DEA3HF are different from that of 3HF.[21] DEA3HF dye shows two emissions in polar aprotic solvents, one with a λ_{max} ~ 483 nm, due to the N* form with CT character and another with a λ_{max}~565 nm due to the tautomeric form (T*). In ethanol, the pre-existing intramolecular hydrogen bond is replaced by intermolecular hydrogen bonding with the solvent and this leads to a single, redshifted and enhanced normal emission (λ_{max}~518 nm).[6,22] Unlike 3HF@$(OA)_2$, DEA3HF@$(OA)_2$, shows two emissions in borate buffer, one due to N* and the other due to T*. Regarding the solvatochromic effect, the charge transfer band (N*) shifted its maximum from 453 to 503 nm from benzene to octaacid medium, which could be attributed to a higher dipole moment of N* in comparison to the N state and higher polarizability of the cavitand interior. As already observed in the case of 3HF, the total fluorescence quantum yield of DEA3HF (Table S3 (ESI)) within OA increased by ~78 times with respect to that in water. The fluorescence quantum yield in OA (~ 0.078) is close to that in benzene.

In the absence of OA, in water, with or without borate buffer, upon excitation of 3HF at 402 nm one main emission band located ~506 nm was observed (Fig. 4c). This emission we believe is due to the anionic species of 3HF.[23] Under similar conditions, the same emission profile was observed for DEA3HF (Fig. 4d), where the fluorescence maxima located at ~556 nm could be ascribed to the anionic species.[10b] However, no such emission was observed within OA. These suggest that within OA the two flavonols remain in an unionized neutral form.

Having gained knowledge concerning the excited state structures preferred by 3HF and DEA3HF within OA, we carried out fluorescence lifetime measurements to probe how confinement by OA influences the excited state dynamics of the guests. A nonlinear least square method was employed to fit the decay to a sum of exponentials. The value of χ^2 and a visual inspection of the residuals and the autocorrelation function were used to determine the quality of the fit, as presented in Fig. S13-44 (ESI). The relevant results are summarized in Table 1. Additional data from time-resolved fluorescence results are provided in Table S4 (ESI). The fluorescence decay profiles of 3HF in aprotic organic solvents are mono-exponential in the range of 1.75-3.01 ns, which are in the same order of magnitude as the ones reported in the literature.[4b,8b,14,24] In ethanol, since a dual emission could be observed, the time-resolved fluorescence was monitored at 402 and 532 nm, related to the N* and T* emission maxima, respectively. Values of 3.53 ns ± 0.02 ns (N*) and 2.56 ns ± 0.02 ns (T*) were obtained. Upon inclusion within OA, the lifetime of 3HF was significantly longer (8.30 ns ± 0.01 ns @520nm) than that in pure homogeneous solvents.[18,25] This dynamics can be associated with the inhibition of non-radiative processes due to the confined medium. Since DEA3HF presented a dual fluorescence emission, the fluorescence decay profiles were monitored at the charge transfer band (blue shifted band) and at the tautomeric band (redshifted band). In solution of aprotic solvents it could be observed a mono-exponential time decay for each emission band. In ethanol, the hydrogen-bonded species presented one lifetime (2.03 ns ± 0.01 ns @520 nm) as expected. On the other hand, the inclusion complex DEA3HF@$(OA)_2$ presented for each observed wavelength (502 nm and 564 nm) a two-exponential decay fit (1.92 ns ± 0.02 ns/3.77 ns ± 0.04 ns @502 nm and 1.45 ns ± 0.03 ns/3.69 ns ± 0.02 ns @ 564 nm) probably due to the large spectral band width observed in this environment. The short lifetime was ascribed to the charge transfer state and the longer one to the ESIPT mechanism.

Table 1. Lifetimes (ns) from fluorescence decays of 3HF, DEA3HF and inclusion complexes 3HF@$(OA)_2$ and DEA3HF@$(OA)_2$.

System	Solvent	τ_1 (Rel. %)	τ_2 (Rel. %)
OA	Buffer @344 nm	2.80(100)	-
	Buffer @418 nm	4.07(100)	-
3HF	Ethanol @402 nm	3.53(100)	-
	Ethanol @532 nm	2.56(100)	-
	Chloroform @513 nm	1.75(100)	-
	Benzene @522 nm	3.01(100)	-
DEAF3HF	Ethanol @520nm	2.03(100)	-
	Chloroform @479 nm	1.44(100)	-
	Chloroform @553nm	0.943(100)	-
	Benzene @453 nm	1.85(100)	-
	Benzene @559 nm	1.97(100)	-
3HF@$(OA)_2$	Buffer @520 nm	8.30(100)	-
DEA3HF@$(OA)_2$	Buffer @502 nm	1.92(48)	3.77(52)
	Buffer @435 nm	2.85(100)	-
	Buffer @564 nm	1.45(22)	3.69(78)
	Buffer @700 nm	3.19(100)	-

Conclusions

This work has established that the photophysics of 3HF is altered within OA in water: (a) single emission due to T* is observed, (b) the quantum yield of emission is enhanced with respect to water, borate buffer and benzene and (c) the lifetime of the emissive state is lengthened. The photophysics of DEA3HF is also altered within OA in water, where (a) dual fluorescence emissions due to N* and T* as in organic solvents are obtained, (b) the quantum yield of emission is enhanced with respect to water and borate buffer and (c) the lifetime of the emissive state is moderately lengthened. In spite of the dyes being in water it behave as though it is in an organic solvent. Within OA container the dyes seem to be shielded from the bulk water. OA capsule provides an opportunity to explore the excited state behavior of sparingly water-soluble organic compounds in water. OA mimics the environment of an organic medium. We plan to continue to exploit this feature in modifying the excited state behavior of organic molecules.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Anionic structures of the flavonols, detailed spectroscopic characterization, ^{1}H NMR titration plots, relevant data from the photophysical study, time resolved fluorescence decay curves and details from the materials and methods can be found in the ESI. See DOI: 10.1039/c000000x/

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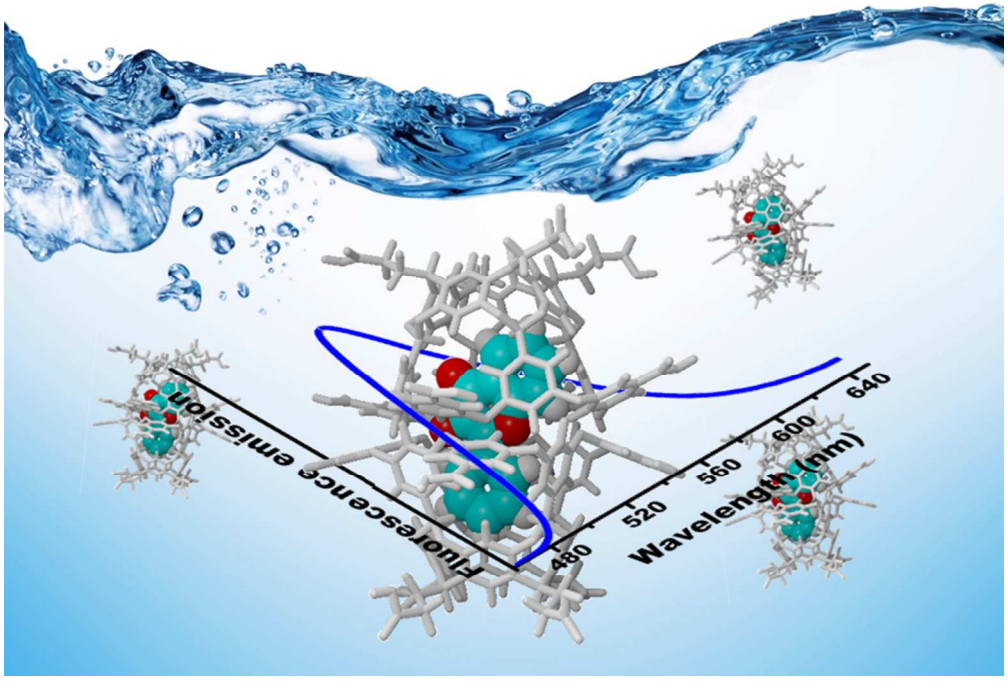

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