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Fluorescence from the second excited singlet state of 3-hydroxyflavone in supercritical CO_2

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Abstract

Fluorescence from the second excited singlet state (S_2) of 3-hydroxyflavone (3HF) has been observed for the first time in supercritical carbon dioxide (sc-CO_2) environment. Steady-state experiments reveal that the intramolecular proton transfer is less effective from the S_2 state of 3HF compared to that from the S_1 state.

1. Introduction

The density of a supercritical fluid (SCF) can be easily tuned by changing its pressure under isothermal conditions, between the density of a gas and that of a normal liquid. Consequently, medium properties can be continuously varied without a change in the molecular nature of the solvent. Local density effect on the solute molecules is one of the most important properties of SCF [1–4]. Because of its high sensitivity, fluorometric technique is gradually establishing its importance in characterising the SCF properties along with other techniques [5–9]. In a recent communication, we have shown that the room temperature fluorescence and phosphorescence from the relaxed state of benzil can be used to study the kinematic behaviour of supercritical CO_2 [5]. In the same article, we have also shown that supercritical CO_2 serves as a microheterogeneous environment towards the fluorophore.

3-Hydroxyflavone (3HF) is a well known probe that undergoes an excited-state intramolecular proton transfer (ESIPT) reaction from its first excited singlet state (S_1) [10–17]. Since the discovery of ESIPT with 3HF by Sengupta and Kasha [10], this molecular system has been extensively exploited for different purposes. In non-hydroxy solvents, the fluorescence quantum yield of 3HF from S_1 state is extremely low because of the competition of the fluorescence with an efficient (barrierless) and extremely fast ESIPT process. This leads to the observation of principally the tautomer fluorescence in such solvents. In hydroxy solvents, however, intermolecular hydrogen bonding between the fluorophore and the solvent restricts the ESIPT process resulting in an increase in the S_1 fluorescence. Dual emission is therefore observed in these environments. In spite of being extensively studied, to the best of our knowledge, there is no report of fluorescence from the S_2 state of 3HF although S_2 emission from the thio-analogue of the compound as well as other thiones have been reported [18–22]. A recent work of Uchiyama et al. [23], where they have determined the solubility of the flavone and 3-hydroxyflavone from the mass of solute trapped by decompressing and the volume of CO_2, has shown that 3HF is soluble in supercritical CO_2. In the present Letter, we report, for the first time, a fluorescence from the second excited singlet state of 3HF in supercritical CO_2 environment. In the following Letter, we have exploited the relative intensity of the S_2 to the tautomer

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fluorescence (S_2/T) to probe the microenvironments available in the supercritical fluids [24].

2. Experimental

3HF (Aldrich, 99%) was purified by repeated recrystallisation from ethanol. Trace of water was removed from the purified sample by putting it into an evacuator with occasional heating. Purity of the sample was checked through thin layer chromatography (with a 3:1 mixture of dichloromethane *n*-hexane as elluent) and spectroscopic measures. For preparation of the mother solution, spectroscopic grade diethylether (Aldrich) was used after drying it further by distilling over metallic sodium. The dry ether was then stored over molecular sieves.

A Shimadzu UV-2001 spectrophotometer and a Spex Fluorolog 3.22 spectrofluorometer were used for the absorption and fluorescence measurements, respectively. For the time-resolved study, we used time-correlated single photon counting (TCSPC) technique with an apparatus already described elsewhere [25]. The time-resolution of our apparatus is ca. 200 ps. HyperChem 6.01 software package [26] was used to calculate the optimised structure and the energy states of 3HF by semi-empirical (AM1-ZINDO/S) method.

The supercritical cell with its accessories has been described in one of our recent publications [5]. For spectroscopic measurements, the sample was prepared in the following way: a solution of 3HF (1×10^{-5} M) was made in diethylether and was loaded in the clean SCF cell. The solvent ether was then completely evaporated. The cell was evacuated and filled with CO_2 after thermostating at the experimental temperature.

3. Results and discussion

Fig. 1 presents the normalised absorption and fluorescence spectra of 3HF in sc-CO_2 at a temperature of 311 K and pressure of 85 bar. The absorption spectrum consists of two bands, with maxima at 333 and 300 nm (30 030 and 33 333 cm^{-1}), respectively. These bands are also observed in the gas phase and in liquid solvents. The maximum of the lowest energy absorption band in the gas phase occurs at 30 800 cm^{-1} [14] and in organic solvents it changes from 29 498 in methylcyclohexane to 28 985 cm^{-1} in octanol [12]. The two absorption bands correspond to $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions.

Excitation at 333 nm in sc-CO_2 leads to two emission bands, one structured band in the 370–450 nm region ('violet' band) with three distinct peaks (27 100, 26 300 and 25 700 cm^{-1}) and several shoulders, and one broad band peaking at 520 nm. It is well known that excitation of 3HF in the gas phase and in methylcyclohexane leads

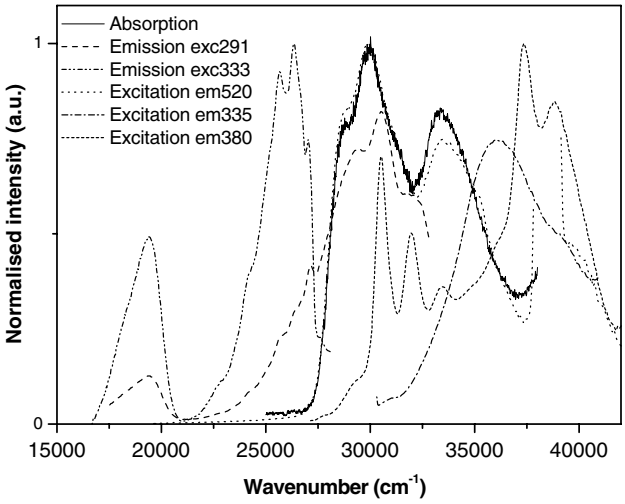

Fig. 1. Normalised absorption, fluorescence and excitation spectra of 3HF in sc-CO_2 ($T = 311$ K, $P = 85$ bar). The fluorescence and excitation spectra are normalised to the corresponding absorption band maxima.

to one single band ca. 520 nm, but a dual emission is observed in the presence of hydrogen bonding or solvent polarisation [12,13]. The maximum of the low-energy emission changes from ca. 24 500 in alcohols to 25 700 cm^{-1} in acetonitrile. Shifting of the violet band in sc-CO_2 towards the higher energies relative to organic solvents is consistent with the shift of the absorption band, as discussed above. The lifetime of the violet band is beyond the time-resolution of our instrument (ca. 200 ps), which also agrees with the tens of picoseconds of the lifetimes values measured in organic solvents. It is tempting to assign the electronic origin of the violet band in sc-CO_2 and in polar solvents to the same excited state, but it must be stressed that the intensity of the violet band in sc-CO_2 is much larger than that in polar, non-hydrogen bonding solvents, and that the fluorescence excitation of this band in sc-CO_2 differs from the corresponding absorption spectrum. On the other hand, the large Stokes-shifted and unstructured emission, also observed in the gas phase and in liquid solutions, at 520 nm can be confidently assigned to the tautomer of 3HF. This assignment is further confirmed by the excellent match between the fluorescence excitation monitored at 520 nm and the absorption spectrum, and by its fluorescence lifetime, which is 1.0 ± 0.05 ns independently of the wavelength of excitation of 3HF.

The tautomer emission is also observed with excitation at 291 nm, as expected from its fluorescence excitation, but a new structured emission, with vibrational progressions of 1350 cm^{-1}, is observed in the 300–350 nm region ('UV' band). The fluorescence excitation of this new emission is a single band centred at 36 000 cm^{-1}, which is not apparent in the absorption spectrum. The lifetime of the UV band is beyond the time-resolution of our instrument. Similar bands are not observed

in organic solvents. However, this new and strong emission cannot be assigned to an impurity for the following reasons: (i) excitation of the same 3HF sample at 291 nm in liquid solvents does not lead to any emission at 330 nm; (ii) excitation of sc-CO_2 at 291 nm in the absence of 3HF does not lead to any emission at 330 nm; (iii) this new emission was consistently observed in different samples of 3HF and using CO_2 with different degrees of purification; (iv) the position of the peaks does not change when the excitation is changed as shown in Fig. 2. The same arguments apply to the violet band observed upon 333 nm excitation. Thus, both the UV and violet bands are originated from the excited 3HF in sc-CO_2.

Semi-empirical calculations on the energies of different electronic states of 3HF can help to assign the emission bands. We optimised the ground state geometry using the AM1 method. ZINDO/S was then applied to get the energies of different excited electronic states. 145 configurations were considered for the singly excited CI calculations. The calculated energies for the S_1 and S_2 states relative to the ground state are 26 100 and 31 690 cm^{-1}, respectively. From the intersection of the normalised lowest energy absorption band and structured fluorescence centred at 26 300 cm^{-1}, we obtain the energy of 27 500 cm^{-1}, in good agreement with the energy calculated for the S_1 state. Similarly, from the intersection of the normalised higher energy absorption band and structured fluorescence centred at 30 500 cm^{-1}, we obtain an energy of 32 000 cm^{-1}, consistent with the energy calculated for the S_2 state. These energy values are in very good agreement with the $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ energies reported by Premvardhan and Peteanu [27]. Thus it appears that the violet band is the normal fluorescence from the S_1 state of 3HF, and that the UV band is the normal fluorescence from the S_2 state of 3HF. The 600 cm^{-1} higher energy of the normal S_1 fluorescence band observed in sc-CO_2 relative to acetonitrile solution is consistent with the increased energy of the corresponding absorption bands in sc-CO_2 and methylcyclohexane.

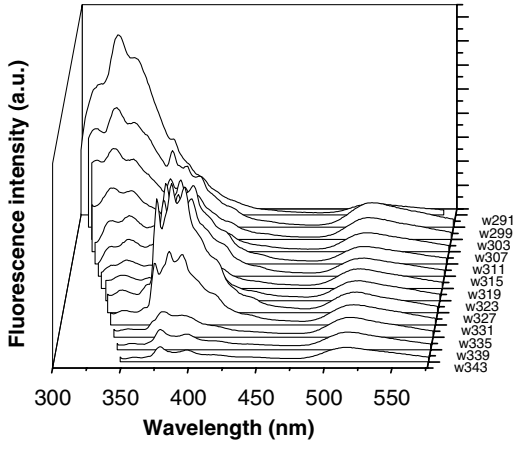

Fig. 2. Fluorescence spectra of 3HF in sc-CO_2 ($T = 311$ K, $P = 85$ bar) as a function of excitation wavelength. For the clarity of the figure only some spectra, selected arbitrarily, are presented.

Fig. 2 presents the variation of the fluorescence behaviour with excitation energy, from 291 to 343 nm. It is revealed that the fluorescence band positions and band patterns do not change with a change in the excitation wavelength for all the three emissions (S_2, S_1 and tautomer). As the excitation wavelength increases, the S_2 emission decreases gradually and the S_1 fluorescence intensity increases. The 520 nm emission, ascribed to the tautomer, is enhanced relative to the normal emission when 3HF is excited to the S_1 state compared to when it is excited to the S_2 state. This qualitative feature is not changed when the emission is corrected for the change in absorption intensity with the excitation wavelength. This reveals that the intramolecular proton transfer process is less efficient in the S_2 state compared to the S_1 state. Further decrease in the excitation energy within the S_1 absorption envelope shows a relative increase in the 520 nm emission relative to the violet (S_1) emission. Excitation wavelength dependence of the relative yield of the S_1 and the tautomer emissions has already been observed by Itoh and Kurokawa [14] in the vapour phase study of 3HF and has been ascribed to ESIPT from an upper vibrational level of the S_1 state.

Although the energies and lifetimes of these structured emissions are consistent with their assignments, our observations raise three additional questions: why do the fluorescence excitation spectra of the UV and violet bands differ from the absorption spectrum of 3HF? Why is the ratio of the normal to tautomer emission so high in sc-CO_2? Why is the radiative rate of the S_2 emission competitive with the internal conversion to S_1?

The different excitation spectra of the violet and the tautomer emission bands in sc-CO_2 suggest the presence of more than one different ground-state precursors. The density of sc-CO_2 at 311 K and 85 bar is about half of that of ethanol. At such low densities sc-CO_2 may form clusters of different sizes and the same cluster may contain more than one 3HF molecules. We propose, as a working hypothesis, that the ground-state precursors differ in their solvation and/or aggregation. One type of precursor, in a more flexible cluster, can undergo very rapid ESIPT and yield the tautomer that emits in the nanosecond time scale. This explains the emission and the fluorescence excitation of the tautomer. The other excited-state species finds itself in a more rigid solvation cluster, that it may share with another probe molecule, and an unfavourable geometry for intramolecular proton transfer may facilitate the observation of the normal fluorescence. The absorption indicates that the excited-state energy of 3HF in this cluster is 535 cm^{-1} (1.5 kcal/mol) higher than in methylcyclohexane. This may reflect

a solvation cluster that restrains 3HF to a conformation that is unfavourable for intramolecular proton transfer and leads to normal fluorescence. It is appropriate to mention here that the absorption spectrum of the 3HF derivative with a methyl group in the 2′ (ortho) position have the same blue shift and lead to much more intense normal fluorescence spectra [16]. The good match between the absorption and fluorescence excitation spectra of the tautomer indicates that 3HF molecules are preferentially located in solvation clusters that allow for ESIPT. It is interesting to remark that both the absorption and the fluorescence excitation of the tautomer and violet bands show clear vibration progressions of 1350 cm^{-1}, typical of π, π^* states [28], indicating that this is the character of the S_1 state. This vibrational progression can also be recognised in the violet fluorescence, but it is mixed with at least another vibrational progression. An in-plane OH bending mode is a good candidate for that mixing [29].

The working hypothesis formulated above to explain the fluorescence excitation of the violet band and its intensity, also accounts the observations regarding the UV band. The two ground-state precursors differ in their solvation clusters. One of them leads to very fast ESIPT, but the restricted access to this deactivation channel in the other one gives the opportunity for other deactivation channels to operate. The internal conversion to the S_1 state is much faster than the S_2 radiative rate, except when there is a large energy spacing between these two states. In the case of 3HF in sc-CO_2 this energy spacing is ca. 4500 cm^{-1}. Observation of S_2 fluorescence from compounds with an $S_1 - S_2$ spacing of 2500–3000 cm^{-1} has already been reported [30]. Observation of the S_2 emission due to the thermal population of this state from the S_1 state seems to be impossible as the population in the S_2 state will be insignificant compared to the population in the S_1 state (S_2 population less than 10^{-9} times S_1 population). Furthermore, the S_2 emission spectrum does not depend on the excitation wavelength indicating that the S_2 fluorescence spectrum results from the vibrationally equilibrated S_2 state. Some of the S_2 species also leak down to the S_1 state through the non-radiative deactivation processes [31,32]. This is reflected by the feeble structure visible on the tail of the S_2 fluorescence band.

It is important to notice the relative intensities of the different fluorescence bands. Fig. 1 shows that the relative yield of the tautomer fluorescence to the molecular fluorescence is much less for the S_2 emission than for the S_1 fluorescence under the same environment. In both the cases, however, the tautomer emission is remarkably less than that expected for a non-hydrogen bonding solvent. It thus appears that sc-CO_2 can form hydrogen bonds with 3HF, and that they can be rather important in certain cluster sizes. The hydrogen bonding ability of CO_2 has already been reported in the literature [6,33]. The relatively small tautomer fluorescence from 3HF excited to S_2 suggests that the ESIPT process is less efficient from the S_2 state. The difference in the efficiency of formation of the tautomer from S_1 and S_2 can be justified considering the nature of the two electronic states. S_1 and S_2 states of 3HF correspond to $\pi\pi^*$ and $n\pi^*$, respectively [11,27]. So reorganisation of the π-electrons associated with the ESIPT process is much more efficient in the S_1 state because of its $\pi\pi^*$ character while it is much less efficient in the S_2 state.

It is interesting to note that the S_2 fluorescence yield of 3HF in sc-CO_2 is ca. 20% higher than the S_1 yield. This suggests that CO_2 molecules are less effective than normal liquid solvents in quenching the $n\pi^*$ state. The absence of high-frequency CH or OH bonds, frequently associated with the promoting modes of non-radiative transitions [34] or involved in H-abstraction reactions [35], may explain that inefficiency. The S_2 fluorescence does come from a relaxed S_2 state (Fig. 2), and we propose that it emanates from the 3HF in a solvent cluster where the intramolecular H-bond required for proton transfer is hindered. The inertness of supercritical CO_2 contributes to the enhancement of S_2 fluorescence yield.

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