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Fluorescence properties of 1-heptanoylpyrene: a probe for hydrogen bonding in microaggregates and biological membranes

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Abstract

Fluorescence properties of 1-heptanoylpyrene in homogeneous solutions and incorporated into liposome membranes have been measured. The fluorescence intensity of the alkanoylpyrene is small in neat hydrocarbon solutions ($Q_f = 0.006$) but increases with increasing solvent polarity. On the contrary, Q_f of the 1-alkylpyrene is larger by a factor of 10 in a hydrocarbon solvent and decreases slightly in polar solvents. In alcohols the fluorescence yield of 1-heptanoylpyrene becomes considerably larger in comparison to a nonhydroxylic environment and nearly identical to that of heptylpyrene in the same solvent. The fluorescence spectra shift bathochromically in that polar environment. The fluorescence properties of 1-heptanoylpyrene incorporated in a liposome membrane indicate that the probe molecules are well embedded within the hydrophobic core of the bilayer. Semiempirical calculations were performed for 1-ethanoylpyrene and 1-ethylpyrene as model compounds in order to analyze the influence of solvent polarity and of hydrogen bonding on excited state energies and fluorescence properties of 1-acyl derivatives of pyrene. Hydrogen-bonding increases the energy gap between the lowest singlets, which are nearly degenerated, and the $^3\pi^*$-n triplet and reduced consequently the intersystem crossing efficiency. But also a change in the sequence of the excited $^1\pi^*$-π states is concluded from experimental data and should contribute to the increase of the fluorescence yield upon hydrogen bonding.

Keywords: Hydrogen bonding; Liposomes; Semiempirical calculations

1. Introduction

The photophysics of aromatic carbonyl compounds is fairly dependent on the solvent properties [1,2,3]. It is generally accepted that the π^*-π transitions are red-shifted with increasing solvent polarity, i.e. show positive solvatochromism, and this results from the increasing dipole moment in the π^*-π excited states. The π^*-n transition shows, however, negative solvatochromism, i.e. a blue-shift, under these conditions and this is attributed to a stabilization of the oxygen lone pairs of the carbonyl oxygen atom in the polar solvent [4,5]. This behaviour is also used to discern π^*-n and π^*-π transitions and is known as Kasha's test [6]. The singlet excited state decays in most cases preferentially by intersystem crossing (ISC) and its efficiency accords to selection rules known as El Sayed's rules [7]. They state that ISC is allowed if singlet and triplet are of different characters, i.e. π^*-n and π^*-π , but forbidden when they have the same character.

This is also the reason why hydrogen-bonding changes dramatically the photophysical and fluorescence properties of aromatic compounds bearing carbonyl functionalities [8,9], and this effect was demonstrated for carbaldehydes with aromatic rings of different size. For instance, pyrene-1-carbaldehyde is not fluorescent in heptane or ethyl ether, weakly in chlorobenzene or acetonitrile, but shows fairly high fluorescence in ethanol, acetic acid, or water-acetonitrile mixtures [8,9]. As the fluorescence efficiency increases, the fluorescence band shifts strongly from violet to blue, although the absorption maximum exhibits only a comparatively small red-shift. The large fluorescence shift indicates considerable geometrical rearrangement of the molecule or of the solvation shell in the fluorescent π^*-π excited state in hydroxylic, polar solvents. In binary mixtures of an inert solvent with one activating fluorescence, the fluorescence efficiency is found to increase continuously reaching its maximum value in the pure polar environment.

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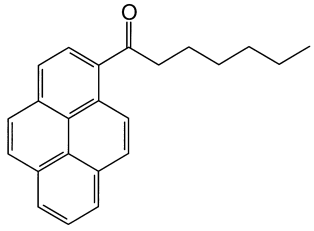
It is assumed that the splitting of π*-n and π*-π singlets is small and depends on solvent polarity. It determines the fluorescence yield of the compound as the sequence of the two states might change upon variation of the environment. This is due to two mechanisms: whereas weakly or moderatly activating solvents (e.g. ethanol) lower mainly the energy of the π*-π excited state by increasing the dielectric dipole–dipole interaction energy, strongly activating solvents (e.g. trichloroacetic acid) raise the energy of the π*-n state due to ground state hydrogen bonding [10]. In the excited state, however, the lone pair looses most of its ability to form hydrogen bonds and calculations indicate that the negative solvatochromism might be explained by changes of the molecular geometry of the hydrogen bonded system [11].

Other aromatic carbonyl compounds like acetophenone and benzophenone are commonly used as triplet sensitizers, as their triplet quantum yields are near to unity due to highly efficient spin-orbit coupling [12]. Other aromatic ketones show fluorescence, although generally with low quantum yield. Fluorenone, e.g., exhibits fluorescence with a yield $Q_f \leq 0.01$ [13,14,15,16], dependent on solvent polarity and it was concluded that the S_1 state is of π*-n character in polar but of π*-π character in nonpolar solvents [17]. A recent theoretical and experimental study [18] showed, however, that the π*-n state is the lowest singlet in nonpolar solvents and alcohols, but the π*-π state becomes lower in energy in dipolar aprotic solvents. Consequently, fluorescence quenching by alcohols is found. Similar quenching of the emission by alcohols was also found for other compounds with low lying π*-n transitions [19,20].

In this paper we report experimental and theoretical studies on one 1-acyl derivative of pyrene and show that emissivity increases in solvents forming hydrogen-bonds in comparison to nonpolar and dipolar aprotic solvents. This behaviour is explained on the basis of quantum chemical calculations showing an increase of the energy gap between the lowest π*-n and π*-π states, thus affecting the intersystem crossing yield and the radiative decay rate constant. Alkanoylpyrenes are considered as fluorescent probe molecules reporting hydrogen bonding in an organized environment like membranes and as triplet sensitizers in these media.

2. Experimental section

1-Heptanoylpyrene (Scheme 1) was obtained by Friedel–Crafts acylation of pyrene from a complex of heptanoyl chloride with aluminum chloride in a mixture of 1,2-dichloroethane and nitrobenzene. The product was purified by recrystallization from methanol after addition of charcoal. 1-Heptanoylpyrene was obtained as bright yellow crystals (18% yield, m.p. 81.5 ... 82.5°C). 1-Heptylpyrene was obtained by Clemmensen reduction of 1-heptanoylpyrene and was recrystallized from heptane and washed with cold pentane (m.p. 146.5 ... 147.5°C).



Scheme 1.

The solvents hexane (Merck, Uvasol), heptane (Fluka, HPLC grade), 1-chlorobutane (Aldrich, HPLC grade), acetonitrile (Merck, Uvasol), 1-propanol (Sigma Aldrich, HPLC grade), and methanol (Fluka, HPLC grade) were checked for absorbing and fluorescing impurities and then used without further purification. Water was deionized and three times distilled. Solutions of 1-heptanoylpyrene and 1-heptylpyrene were stored in the dark and all measurements were performed at room temperature.

Unilamellare vesicles were prepared from an egg- phosphatidylcholine (Sigma) equimolar mixture with sodium cholate by dialysis using a cellulose dialysis membrane (DIACHEMA) [21].

Absorption spectra were obtained by a Hitachi U-3300 spectrophotometer, fluorescence spectra using a Perkin–Elmer LS50B luminescence spectrometer. The lifetime measurements were performed by the single photon counting (SPC)-method (Applied Photophysics) described elsewhere [22]. Melting points were determined by capillary method (Electrothermal Engineering Limited, type IA9200, heating rate $2\,K\,min^{-1}$).

3. Computational methods

Semiempirical calculations were performed for 1-ethylpyrene and 1-ethanoylpyrene. Increasing the alkyl chain length does not change the spectroscopic properties and results solely in an improved solubility in apolar solvents [23]. Excited state calculations are started from the gas phase equilibrium geometries of the electronic ground states and are optimized using the VAMP6.1 program [24] within the AM1 Hamiltonian [25]. Excited state configuration interaction calculations were performed by the pairwise excited configuration interaction (PECI) method [26], including 14 active orbitals and all single and pairwise double excitations considered. It was shown recently that the PECI method as implemented in VAMP6.1 gives a good description of spectroscopic properties [27]. The large active space used is necessary as the oxygen lone pair orbital, contributing to the S_4 excited state in 1-ethanoylpyrene, is found as molecular orbital HOMO-5. Only vertical spectra were calculated for ground state equilibrium geometries, and effects of solvent polarity were explicitly considered using the SCRF approach. To show effects of hydrogen bonding a supramolecular ansatz was used. All semiempirical cal-

culations were performed on an Indy workstation (MIPS R4400, Silicon Graphics).

4. Results and discussion

4.1. Absorption spectra

Absorption spectra of 1-heptanoylpyrene show four distinct bands in the UV in all solvents (see Fig. 1). The $S_1 \leftarrow S_0$ band appears as distinct maximum only in heptane but as a shoulder in all other solvents. For the other transitions bandshape and absorption coefficients (ϵ) are nearly independent of the solvent and only a slight hypsochromic shift ($\leq$2 nm) is observed when the solvent polarity increases. Only the $S_4 \leftarrow S_0$ transition shows some vibrational fine structure.

Four absorption bands are also observed in the spectrum of 1-heptylpyrene; they are slightly blue-shifted in comparison to 1-heptanoylpyrene and all electronic transitions higher than $S_1 \leftarrow S_0$ show vibrational fine structure.

4.2. Fluorescence spectra

4.2.1. Fluorescence spectra in neat solvents

The 1-heptanoylpyrene samples were excited at 350 nm and fluorescence peaks are found at 413 nm in hexane, 426 nm in 1-chlorobutane, 431 nm in propanol, and 437 nm in acetonitrile as well as in methanol. Contrary to absorption a bathochromic fluorescence shift is observed with increasing solvent polarity. The fluorescence intensity decreases, however, slightly when the solvent polarity increases (compare, e.g., hexane and acetonitrile solutions shown in Fig. 2), but it is much larger in hydrogen bonding solvents (e.g. in methanol). In acetonitrile the fluorescence spectrum appears to be much broader, due to some weak long wavelength emission. As no dependence of the shape of the band on the concentration of 1-heptanoylpyrene was detected the low energy emission cannot be interpreted as dimer or excimer fluorescence.

The fluorescence spectra of 1-heptylpyrene show, in contrast to 1-heptanoylpyrene, vibrational structure and the respective peaks are located at 373, 386, and 397 nm, for all solvents considered. The solvent shift of the fluorescence spectrum is thus negligible. The fluorescence intensity is larger by a factor of 10 for 1-heptylpyrene in heptane ($Q_f = 0.06 \pm 0.01$) and decreases slightly with increasing solvent polarity.

4.2.2. Fluorescence spectra in aqueous binary mixtures

Fluorescence spectra of 1-heptanoylpyrene were also measured in water binary mixtures with methanol and acetonitrile in order to compare the effect of hydrogen bonding on the fluorescence properties. The spectra are plotted versus increasing molar fraction of water (x_{water}) (Figs. 3 and 4). If the water concentration is low, i.e. $x_{water} \leq 0.65$, the fluorescence intensity increases with increasing molar fraction of water in both organic cosolvents. Only monomer fluo- break rescence is observed and its maximum shifts bathochromically: In pure methanol it is found around 436 nm, but at 447 nm when the water concentration reaches $x_{water} = 0.65$. At larger concentrations of water the high energy emission decreases and a new fluorescence at lower energies rises. This new fluorescence band is found at 528 nm and might be associated with fluorescence arising most probably from ground state dimers or higher complexes of 1-heptanoylpyrene. This emission can be compared to the well-known excimer emission observed for pyrene in various solvents which is found at $\lambda = 475$ nm in isooctane solution [28]. The 528 nm emission does, however, not arise from an excited state complexation reaction, as no rise was found in time-resolved measurements and no concentration dependence is observed for substrate concentration larger than 10^{-4} M. At these low concentrations excited state reactions are most improbable to occur.

Although the main features of the influence of water on the fluorescence properties of 1-heptanoylpyrene in binary mixtures with the hydroxylic solvent methanol and the dipolar, aprotic acetonitrile are similar for both solvents, distinct quantitative differences are observed. The fluorescence intensity monitored at 440 nm is shown in dependence on the molar fraction of water for binary mixtures with methanol and acetonitrile in Fig. 5. It increases almost linearly with the molar fraction of added water but less strongly for acetonitrile than for methanol. In the latter case the fluorescence intensity increases already at minor molar fractions of added water and the fluorescence intensity reaches a value about four times larger than that in neat methanol. In the acetonitrile/water system the fluorescence intensity is almost constant at small water content but increases steeply when x_{water} exceeds 0.8. Aggregate fluorescence occurs also at smaller molar fractions of water in its binary mixture with methanol than in that with acetonitrile, i.e. at $x_{water} \geq 0.70$ in the methanol/water but $x_{water} \geq 0.85$ in the acetonitrile/water system.

4.3. Quantum yields and lifetimes of 1-heptanoylpyrene fluorescence

The fluorescence quantum yield (Q_f) of 1-heptanoylpyrene was measured in different solvents. BBOT (2,5-bis-(5-*tert*-butyl-2-benzoxazolyl)-thiophene) was used as fluorescence standard to obtain absolute quantum yields [29,30,31]. As it can be seen in Table 1, Q_f is only small in non-hydroxylic solvents but increases considerably with increasing solvent polarity of hydrogen bonding solvents.

The fluorescence lifetime of 1-heptanoylpyrene was measured and the values increase slightly with solvent polarity, in parallel with the quantum yields. The rate constant for the radiative transition to the ground state was calculated from

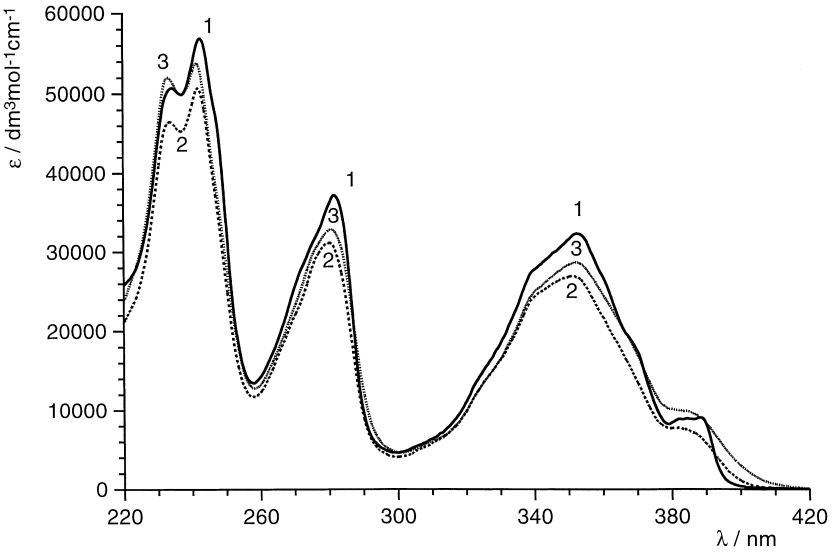


Fig. 1. Absorption spectra of 1-heptanoylpyrene in isooctane (1), acetonitrile (2), and methanol (3).

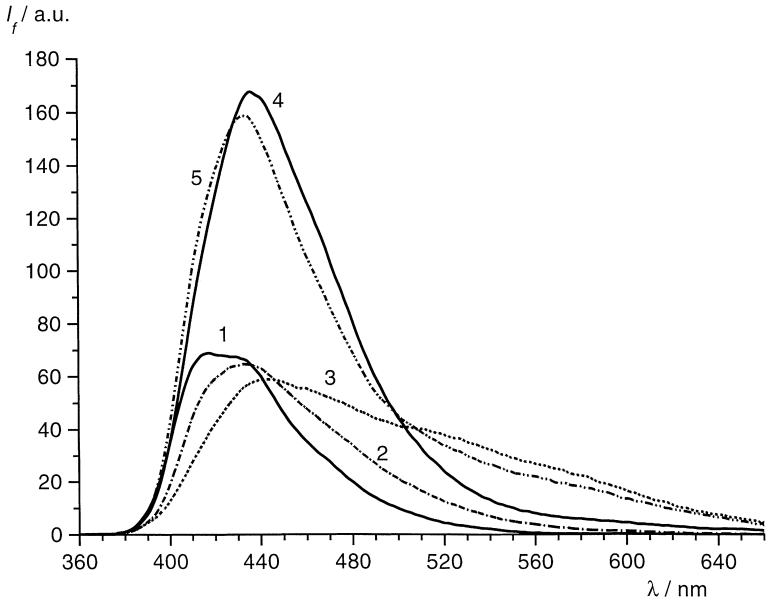


Fig. 2. Corrected fluorescence spectra ($\lambda_{exc} = 350$ nm) of 1-heptanoylpyrene in isooctane (1), 1-chlorobutane (2), acetonitrile (3), methanol (4), and 1-propanol (5).

$k_f = Q_f/\tau_f$ (see Table 1) and the obtained values are rather small, in accordance with the small fluorescence quantum yields, and do not depend essentially on the solvent.

In alcohols, however, two decay times were recovered; one of them is nearly identical to that found in other solvents and the second is considerably shorter. No wavelength dependence of the decay times was found and also for the relative percentage of the emissions associated with these lifetimes. The contribution of the short component increases as the aliphatic chain of the alcohol decreases. From this it was concluded that the two species can be ascribed to an uncomplexed excited molecule similar to non-hydroxylic solvents and a second, i.e. one with the shorter decay time, to a hydrogen bonded complex. In this case the fluorescence rate constants for the complexed species can be calculated from the following equation:

$$Q_f = k_f^{(1)}\tau_f^{(1)} + k_f^{(2)}\tau_f^{(2)} \quad (1)$$

the index (1) defining the non-complexed and (2) the complexed species. Assuming $k_f{}^{(1)} = 3.9 \times 10^6\ s^{-1}$ for both alcohols, $k_f{}^{(2)} = 13 \times 10^6\ s^{-1}$ and $k_f{}^{(2)} = 11 \times 10^6\ s^{-1}$ were obtained for 1-propanol and methanol, respectively. The radiative decay rate constant is thus larger by a factor 2. . . 3 for the hydrogen bonded complexes than for the bare molecule.

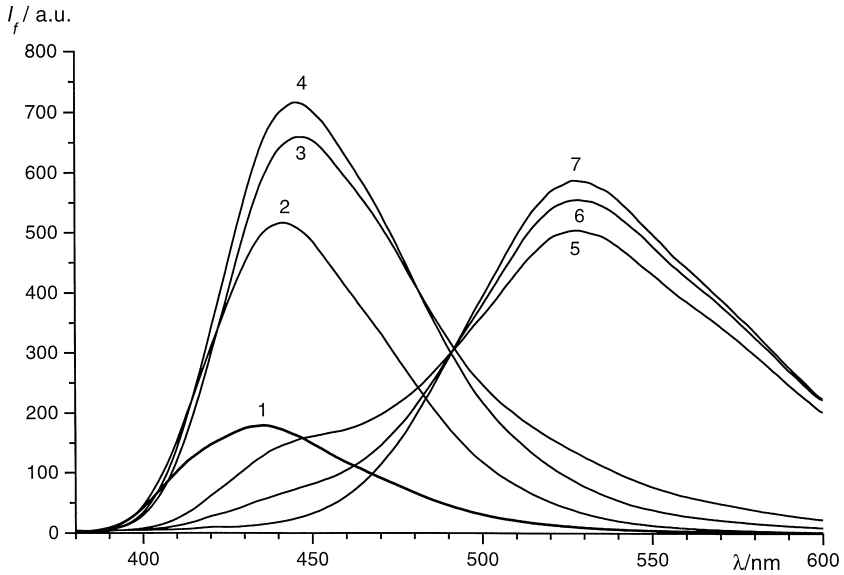


Fig. 3. Fluorescence spectra of 1-heptanoylpyrene in water binary mixtures with methanol (the molar fraction of methanol is given in parentheses): 1 (1.00), 2 (0.51), 3 (0.34), 4 (0.31), 5 (0.25), 6 (0.16), 7 (0.08).

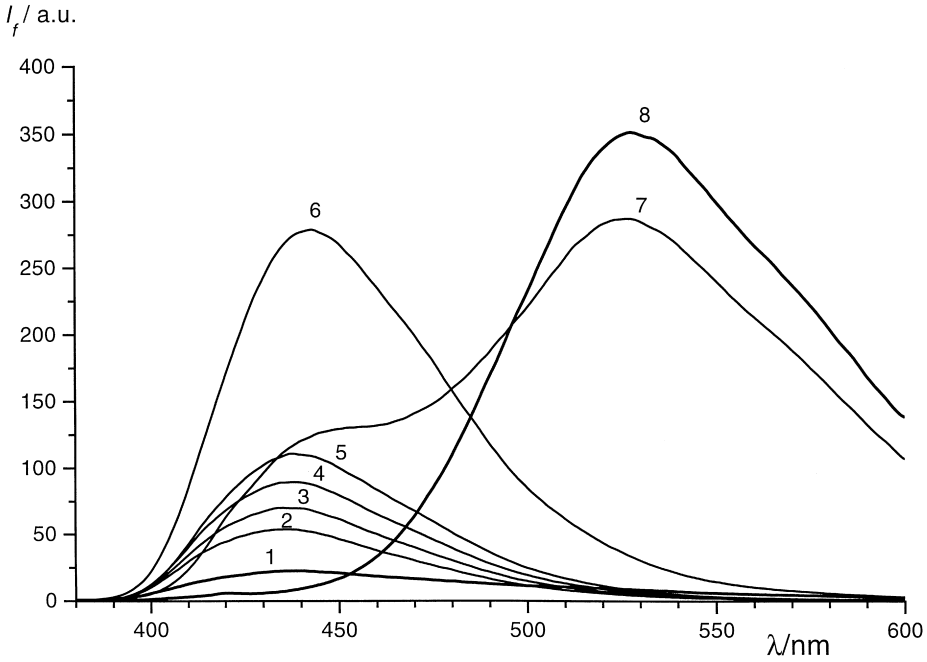


Fig. 4. Fluorescence spectra of 1-heptanoylpyrene in acetonitrile binary mixture with water (the molar fraction of acetonitrile is given in parentheses): 1 (1.00), 2 (0.41), 3 (0.35), 4 (0.29), 5 (0.22), 6 (0.16), 7 (0.11), 8 (0.00).

Table 1
Fluorescence lifetimes, quantum yields, and radiative rate constants of 1-heptanoylpyrene in several solvents

Solvent	$\tau_f^{(1)}$ (ns)	$\tau_f^{(2)}$ (ns)	Q_f	$k_f^{(1)}$ ($10^6\,s^{-1}$)
Hexane	1.82		0.0057	3.1
1-Chlorobutane	1.82		0.0072	3.0
tert-butyl methyl ether	2.49		0.0066	2.7
Tetrahydrofuran	2.18		0.0086	3.9
Acetonitrile	2.80		0.011	3.9
Propanol-1	1.72 (63%)	0.50 (37%)	0.020	
Methanol	2.66 (52%)	0.68 (48%)	0.018	

4.4. Theoretical investigations on 1-ethanoylpyrene and 1-ethylpyrene

Excited state calculations on 1-ethylpyrene and 1-ethanoylpyrene were performed to analyze the fluorescence properties of 1-acyl derivatives of pyrene and the influence of hydrogen bonding. The MOPAC method applied describes geometries for hydrogen bonded systems rather well but the binding energies result generally too much small. All molecules were fully optimized in the ground state. To obtain the geometries for the complexes a methanol molecule

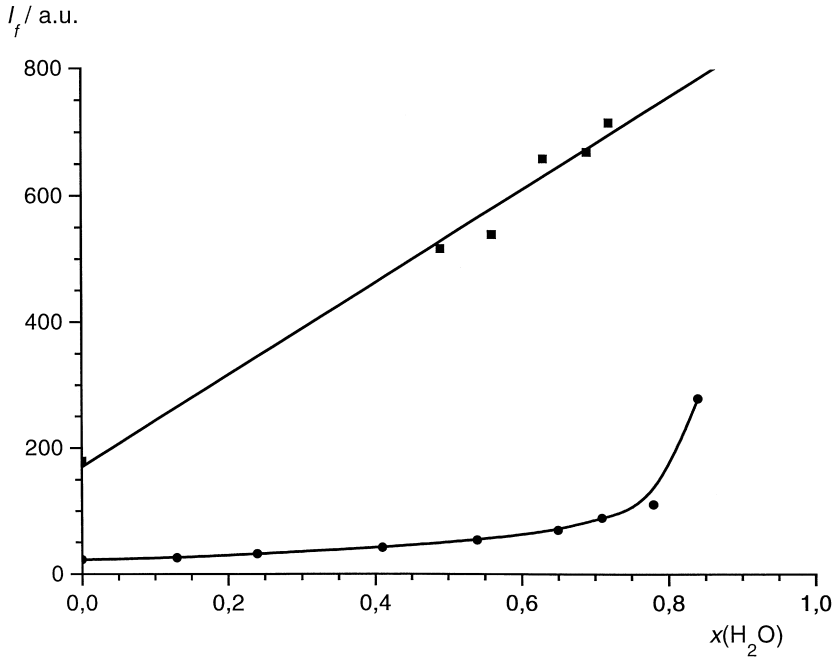


Fig. 5. Dependence of the fluorescence intensity of 1-heptanoylpyrene, monitored at 440 nm, on the molar fraction of water in binary mixtures with acetonitrile (1) and methanol (2).

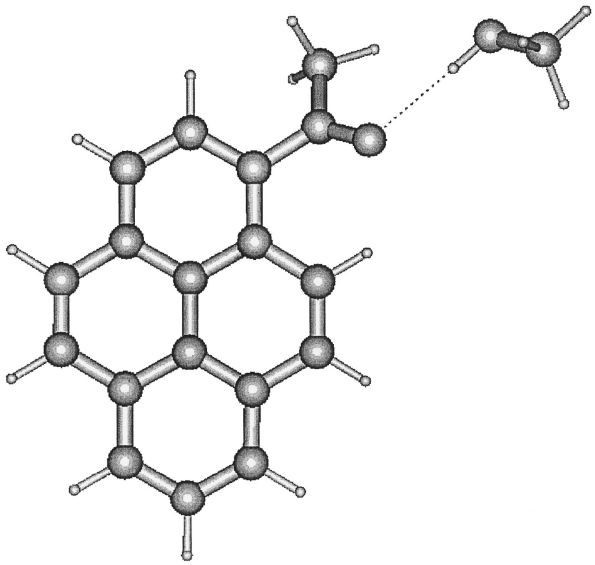


Fig. 6. Configuration of the energetic minimum of 1-heptanoylpyrene with one attached methanol.

was placed at various starting positions around the carbonyl group of 1-ethanoylpyrene. After full relaxation of all geometric parameters, only two relevant ground state configurations were found: in the first configuration, the methanol molecule is positioned *quasi trans* to the pyrene ring, relative to the carbon–oxygen double bond (see Fig. 6). The second stable configuration has only a negligibly higher potential energy and the alcohol molecule is found in a *quasi cis* position to the pyrene ring. Most of the calculations, however, except those with starting geometries within a small region around the *cis* configuration, lead to the *trans* arrangement, most likely due to steric hindrance with the neighboring ring system. This arrangement where the methanolic oxygen is placed ≈ 0.3 nm from the oxygen atom of the carbonyl group (see Fig. 6) is thus accepted as the most stable geometry and all calculations on complexes were performed for this configuration.

A comparison of the state energies of 1-ethylpyrene, 1-ethanoylpyrene, and the 1-ethanoylpyrene complex with one methanol molecule is shown in Fig. 7.

In all three cases the S_2 state results from the HOMO $\rightarrow$ LUMO π^*-π transition, whereas the S_1 state results from a combination of the HOMO–1 $\rightarrow$ LUMO and HOMO $\rightarrow$ LUMO+1 π^*–π transitions. For 1-ethanoylpyrene, the carbonyl group in conjugation to the aromatic pyrene ring leads to another low-energy excited state which can be described as the π^*-n state. It appears as third excited state for the isolated molecule. For the photophysical properties the respective π^*-n triplet state which appears as seventh state (T_7) in the triplet manifold, is important, as it is found in the vicinity of the two lowest S_1, S_2 singlet excited states. Attaching a methanol molecule to the carbonyl group of the substrate, the $^1(\pi^*$-n) state shifts considerably to higher energies and becomes the S_4 state, however, only 0.02 eV above the S_3. More important, the π^*-n triplet shifts likewise to higher energies and is now found well above the lowest S_1 and S_2 singlet excited states. All other states do not change their energetic position significantly.

In Table 2, the energy gap between the S_1 and S_2 excited states are presented. Due to the small energy differences these two states are quasi-isoenergetic in all cases. This is in good agreement with the absorption spectra,

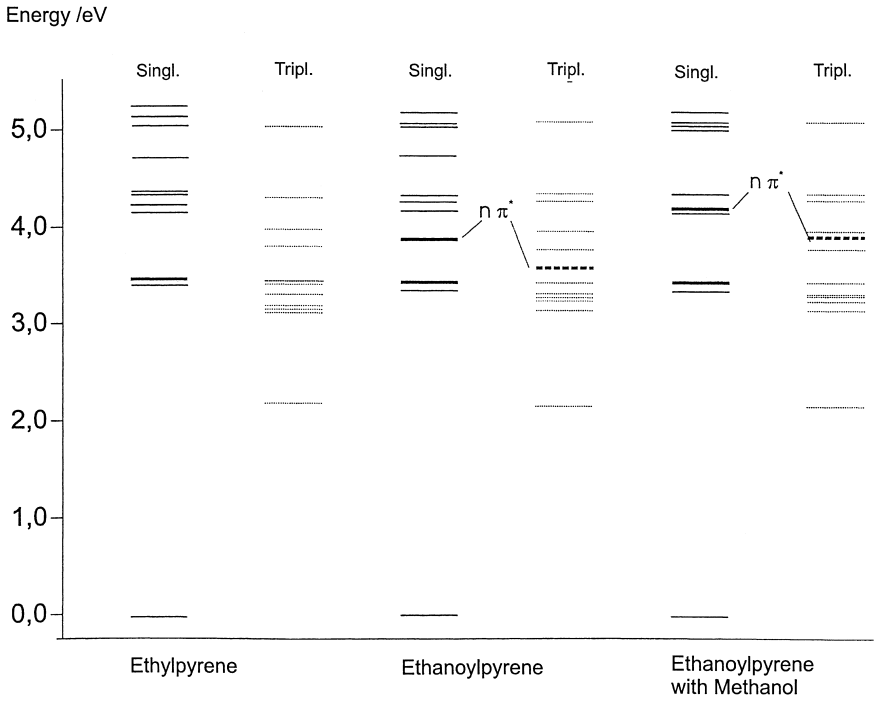


Fig. 7. Comparison of the excited state energies of singlet and triplet transition for ethylpyrene, ethanoylpyrene, and ethanoylpyrene plus one methanol molecule.

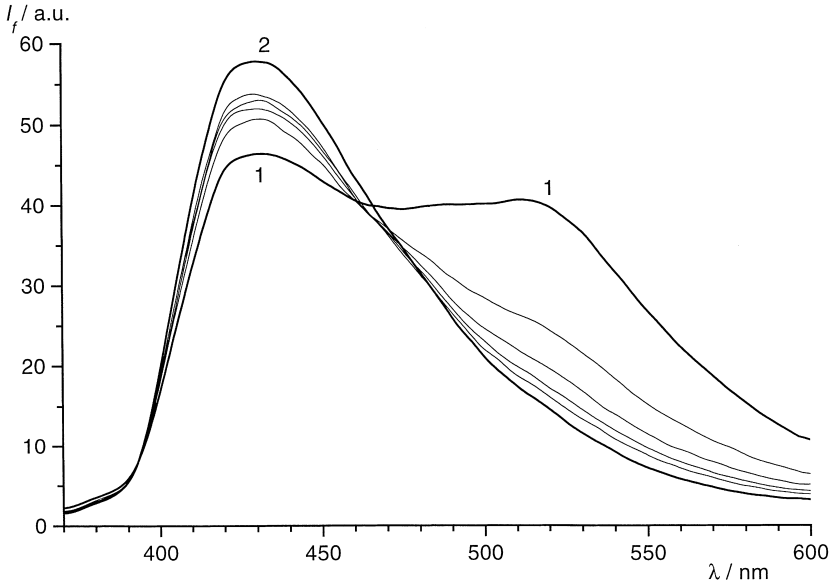


Fig. 8. Kinetics of incorporation of 1-heptanoylpyrene into PC-liposomes. Fluorescence spectrum a few seconds after addition of the marker (1), after 1 h (2).

Table 2
Energy gap between S_1 and S_2 excited states and oscillator strengths for absorption of the two lowest excited states of 1-ethylpyrene, 1-ethanoylpyrene, and the hydrogen bonded complex between 1-ethanoylpyrene and one methanol molecule

	ΔE (eV)	f ($S_1 \leftarrow S_0$)	f ($S_2 \leftarrow S_0$)
1-ethylpyrene	0.06	0.003	0.196
1-ethanoylpyrene	0.05	0.042	0.164
1-ethanoylpyrene plus methanol	0.09	0.027	0.179

where no significant shifts of the two lowest transitions are observed.

4.5. Kinetics of incorporation of 1-heptanoylpyrene into phosphatidylcholine liposome membranes

The incorporation of 1-heptanoylpyrene into PC liposome membranes was monitored by stationary fluorescence spectroscopy. Fluorescence spectra were measured over several

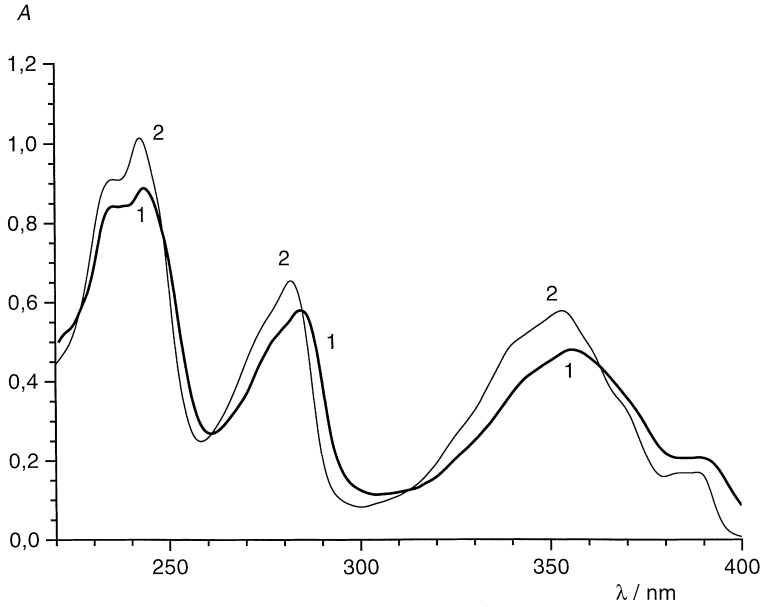


Fig. 9. Absorption spectra of 1-heptanoylpyrene in a liposome dispersion (1) and in heptane solution (2) of the same molarity.

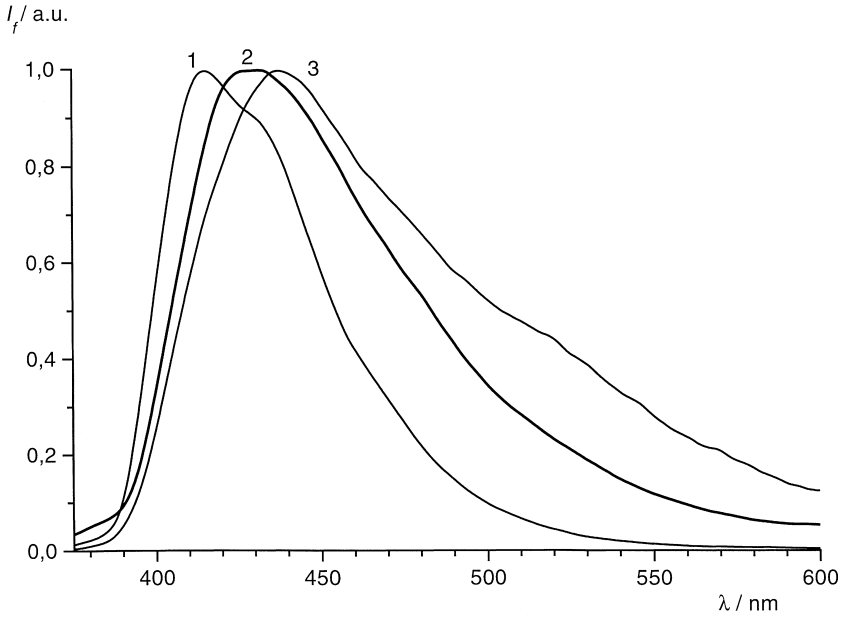


Fig. 10. Normalized fluorescence spectra of 1-heptanoylpyrene in isooctane (1), phosphatidylcholine liposomes (2), and acetonitrile (3).

hours in time intervals of 3 min after the addition of the probe to a buffered (pH = 7.0, T = 295 K) aqueous dispersion of PC liposomes and the results are shown in Fig. 8. The incorporation of 1-heptanoylpyrene into the lipid bilayer is clearly indicated by the disappearance of the long wavelength emission found in aqueous solutions and the increase of the monomer emission around 430 nm. After 1 h no further change in the spectrum occurred and the probe was quantitatively complexed within the membrane. This is also indicated by the absorption spectrum where after this time a spectrum constant in time was found (see Fig. 9).

The absorption spectrum of an equilibrated solution of 1-heptanoylpyrene in the vesicles differs, however, considerably from that obtained in neat solutions of different polarity (compare Figs. 1 and 8). The position of the absorption maxima shifts to longer wavelengths and the optical density decreases as the spectra broaden considerably. The observed shift is considerably larger than that for any other solvent in comparison to hexane solution. This observation might be due to stronger dispersion interactions of the excited probe with the compact membrane in comparison to that in the ground state. In organized media van der Waals contacts with the environment are more frequent than in solution. The polarizability of pyrene increases in the excited state which gives also rise to excimer formation, and dispersion interactions in-

creases, thus, also and give rise to a distinct bathochromic shift.

The fluorescence spectrum is compared to neat solvents in Fig. 10 and the spectrum found in the vesicles is similar in position and shape to that in 1-chlorobutane. As, however, an additional shift of the fluorescence should arise from the strengthened intramolecular dispersion interactions, the probe is most likely located at a position within the membrane of very low polarity. The small fluorescence yield indicates that hydrogen bonding to water molecules is most unlikely and that 1-heptanoylpyrene is well embedded within the hydrophobic core of the membrane. Its triplet state properties will be reported in a subsequent publication.

5. Conclusions

1-Heptanoylpyrene was synthesized and its absorption and fluorescence spectroscopic properties were measured in various solvents and solvent binary mixtures and compared to the respective 1-heptylpyrene. Special attention was paid to the issue of this molecule as probe for hydrogen bonding in self-assembled media and in biological membranes. Whereas the absorption spectra are nearly independent of the environment with the exception of loss of vibrational structure in polar solvents, the fluorescence exhibit both spectral and intensity changes. In non-hydroxylic solvents the fluorescence shifts bathochromically with increasing solvent polarity but its yield remains constantly small. In alcohols, however, the fluorescence quantum yield becomes considerably larger, although its value does never exceeds 0.02. A biexponential fluorescence decay observed in alcohols was attributed to fluorescence originating from hydrogen bonded and non-complexed species.

Hydrogen bonded complexes were modeled by semiempirical calculations and the spectral properties were calculated and compared to the non-complexed molecule and to an alkylpyrene. The weak π^*-n state is not observed experimentally because of its small transition dipole and is located very near to the two lowest π^*-π states. They are very near in energy and have considerably different transition dipole moments. The π^*-n state shifts to higher energies upon formation of hydrogen bonded complexes and this could reduce intersystem crossing. The result, that the lifetime of the excited state decreases and the radiative decay rate increases in the complex indicates that the two lowest π^*-π singlet states interchange in the excited complex, most probably due to geometrical rearrangement.

Water binary mixtures with methanol and acetonitrile were also studied: The fluorescence intensity increases with water concentration but considerably more in the alcohol than in acetonitrile binary mixture. In both cases a new fluorescence band which can be associated with a ground state dimer and correlates to the well known excimer fluorescence, is found at high water content. In the methanol–water system these complexes are stable over a rather wide range of water concentration. In the acetonitrile case, however, such solutions are unstable.

1-heptanoylpyrene is quantitatively incorporated into phosphatidylcholine liposomes, at least above their gel to liquid crystal transition temperature. This reaction is slow and mainly determined by the low solubility of this hydrophobic compound in aqueous solution. Nevertheless, the probe occupies a well-defined site within the membrane and is not hydrogen bonded as the fluorescence intensity remains low and similar to non-hydroxylic solvents. From the spectral shift it might be concluded that the site has medium polarity but it is more probable that van der Waals interactions with the lipid chains increase in the excited state because of the increased polarizability of pyrene in its excited versus its ground state, an effect which also causes a significant red shift of the fluorescence spectrum.

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