Photophysics of Preassociated Pyrenes in Aqueous Polymer Solutions and in Other Organized Media

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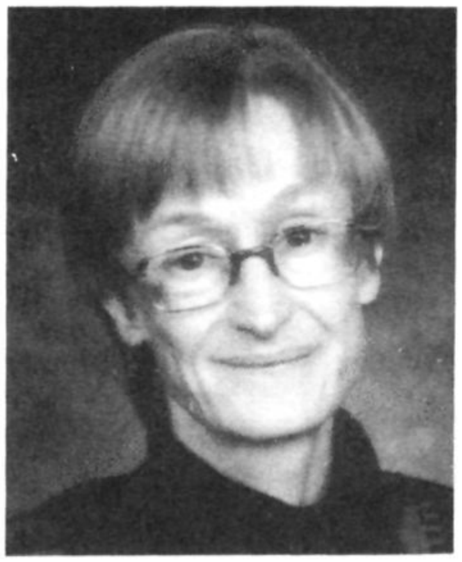
Contents

I. Introduction

In the course of a study of anthryl-labeled polyionenes in water Suzuki and Tazuke noticed rather unusual patterns in the absorption and emission spectra of anthracene.[1] The absorption spectrum of the labeled polymers in water was much broader than that of anthracene-substituted model compounds in methanol. The emission spectrum exhibited a much stronger excimer contribution, compared to structurally related labeled polyesters in organic solvents.[2] Furthermore, the excitation spectra monitored for the monomer and excimer emissions differed in shape and in the wavelengths of band maxima. To account for these effects Suzuki and Tazuke postulated that the anthracene groups attached to the polymer associate as dimers or higher aggregates.[3] Over the last 10 years instances of chromophore association have been reported for a wide range of labeled polymers in aqueous solutions. For technical reasons discussed later, pyrene (Py), rather than anthracene, has become the chromophore of choice. Attaching pyrenyl groups to water-soluble polymers may severely alter, not only the properties of the polymer, but also those of the chromophore.



Françoise M. Winnik was born in Mulhouse, France. She graduated in 1974 from the Ecole Nationale Supérieure de Chimie de Mulhouse, where Professor Jacques Streith gave her an early introduction to photochemistry. She studied under Peter Yates at the University of Toronto and received a Ph.D. in 1979. After two years of postdoctoral research in Medical Genetics in the group of Professor J. Carver at the University of Toronto, she joined the Xerox Research Center of Canada. In 1985–1986 she had a 7-month temporary assignment in the laboratory of Professor Shigeo Tazuke at the Tokyo Institute of Technology. Her research interests include the synthesis and photochemical characterization of dyes and pigments used in printing technologies and the applications of fluorescence spectroscopy to the study of water-soluble polymers.

Polymer scientists often relate the solution properties of water-soluble polymers labeled with hydrophobic dyes to those of a class of industrial polymers known as "hydrophobically-modified polymers".[4] They are synthetically-derived water-soluble polymers that contain a small number of oil-soluble (hydrophobic) substituents. When these polymers are dissolved in water the hydrophobic groups aggregate to minimize their exposure to water, a situation which parallels the aggregation of surfactants in micelles and of lipids in bilayers. The aggregation can occur within a single polymer chain, often resulting in a contraction of the polymer coil. Alternatively, networks can be created via interactions between polymer chains. Hydrophobically-modified polymers of controlled structure are sought for use as viscosity and rheology modifiers in water-based paints and in fluids for coatings applications, as flocculating agents in water treatment, as surface-active agents in personal care products, and as frictional drag reducing agents in turbulent flow. An understanding of the structure–property relationship is necessary to design new materials of improved performances. Questions relating to the molecular aspects of polymeric association in water have to be addressed. Fluorescence spectroscopy has proven to be useful in the study of these materials. Several reviews on this subject are available.[4–7]

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Scope of the Review

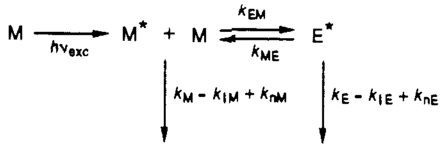
A survey of the large body of data now available provides convincing evidence that hydrophobic labels attached to water-soluble polymers exhibit common features in their absorption and emission spectra. Pyrene has been selected in this review since it is by far the most frequently used dye in fluorescence studies of labeled polymers. Pyrene has a long singlet lifetime; it readily forms excimers; it acts as an energy acceptor via nonradiative energy transfer from several dyes; and the vibronic band structure of its emission is sensitive to the environment.[8] The objectives of this review are (1) to present the salient features of the photophysics of pyrene in the following organized media: the pyrene crystal, pyrene in glassy matrices, pyrenophanes and bis-pyrenyl compounds, and pyrene in ultrathin organic films, in micelles, and in vesicles; (2) to describe spectroscopic measurements used either to detect pyrene association prior to excitation or to confirm the absence of aggregated pyrenes; and (3) to survey the structure, synthesis, and spectroscopy of the pyrene-labeled water-soluble polymers described in the literature. Excluded from this review are systems where pyrene is physically adsorbed on inorganic surfaces, such as silicas, aluminas, clays, or zeolites. The reader is directed to leading publications on this topic in the introduction to section IV.

II. Aspects of the Photophysics of Pyrene Excimers

An excimer, as defined by Birks, is a dimer which is associated in an electronic excited state and which is dissociative in its ground state.[9] The formation of a pyrene excimer requires encounter of an electronically excited pyrene with a second pyrene in its ground electronic state. According to this definition the two pyrenes must be sufficiently far apart when light is absorbed, so that the excitation is localized on one of them. This excited pyrene, often referred to as "locally-excited" pyrene gives rise to "monomer" emission. The observation of excimer emission indicates that diffusive encounter between the pyrenes has occurred. There are also instances where an excimer-like emission is observed, but there is no evidence that the pyrenes are separated when the light is absorbed. Because this emission strongly resembles pyrene excimer emission, many people refer to these excited species as "static excimers". By contrast, excimers which satisfy the Birks definition are referred to as "dynamic excimers". Under normal circumstances the distinction between static and dynamic excimers is that one can, in a time-resolved experiment, observe the growth of the excimer emission for dynamic excimers, but not for static excimers. As we see below, dynamic processes can also be observed in the case of "static excimers" if one carries out the measurement on a sufficiently fast (picosecond) time scale. An alternative distinction between dynamic excimers and those formed from (pre)associated pyrenes is that in the latter case the pyrenes are sufficiently close that they exhibit perturbed absorption and excitation spectra.

The nomenclature proposed by Birks to describe the luminescence of aromatic molecules has been modified slightly here in order to emphasize the distinction between dynamic excimers (E*) and excimers formed from associative ground-state pyrene dimers (D). Schemes 1 and 2 present the overall processes corresponding to each situation. The photophysical species and processes in the schemes are listed in Tables 1 and 2.[10]

Scheme 1



Scheme 2

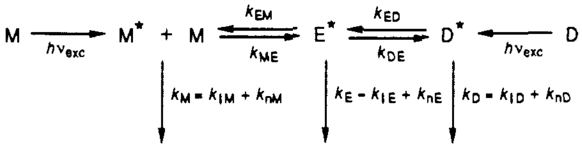


Table 1. **Description of the Photophysical Species**

symbol	description
M	chromophore in the ground state
M*	monomer in the first excited singlet state
M*-M or E*	dynamic excimer in the first excited singlet state
M-M or D	associative pyrene dimer in the ground state
D*	dimer in the first excited singlet state
E_2	sandwichtype excimer
E_1	partial overlap excimer
Q	diffusive quencher

Table 2. **Photophysical Processes**

process	description	rate, s^{-1}
$M + h\nu_{ex} \rightarrow M^*$	absorption by isolated pyrene	
$D + h\nu_{ex} \rightarrow D^*$	direct excitation of a preformed dimer	
$M^* \rightarrow M + h\nu_M$	monomer fluorescence	k_{fM}
$M^* \rightarrow M$	monomer radiationless decay	k_{nM}
$D^* \rightarrow D + h\nu_D$	excited dimer fluorescence	k_{fD}
$D^* \rightarrow D$	radiationless decay of the excited dimer	k_{nD}
$D^* \rightarrow E^*$	internal conversion of the excimers	k_{ED}
$E^* \rightarrow D^*$	internal conversion of the excimers	k_{DE}
$M^* + M \rightarrow E^*$	dynamic excimer formation	k_{EM}
$E^* \rightarrow M^* + M$	dynamic excimer dissociation	k_{ME}
$E^* \rightarrow 2\,M + h\nu_E$	excimer fluorescence	k_{fE}
$E^* \rightarrow 2\,M$	excimer radiationless decay	k_{nE}
$M^* + Q \rightarrow M + Q$	diffusive quenching of monomer	k_{QM}
$E^* + Q \rightarrow E + Q$	diffusive quenching of excimer	k_{QE}
$D^* + Q \rightarrow D + Q$	diffusive quenching of excited dimer	k_{QD}

Scheme 1 is the classic Birks two-state mechanism for excimer formation and decay. In terms of this scheme the following two equations describe the time profile of the monomer decay and the growth and decay of the excimer for a δ-pulse excitation. According to Scheme 1, since the monomer and excimer are coupled species, $\tau_1 = \tau_3$, $\tau_2 = \tau_4$, and $A_4/A_3 = 1$ (see eqs 1 and 2). These lifetimes are related to the rate coefficients

$$I_M(t) = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} \tag{1}$$

$$I_E(t) = A_3 e^{-t/\tau_3} - A_4 e^{-t/\tau_4} \tag{2}$$

in Scheme 1 by expressions which can be found in Birks' book.[11] In a kinetics experiment one determines $k_M \equiv \tau_1^{-1}$ in the limit of low concentration of M, and at higher concentration, τ_1, τ_2, and A_2/A_1. These are the four

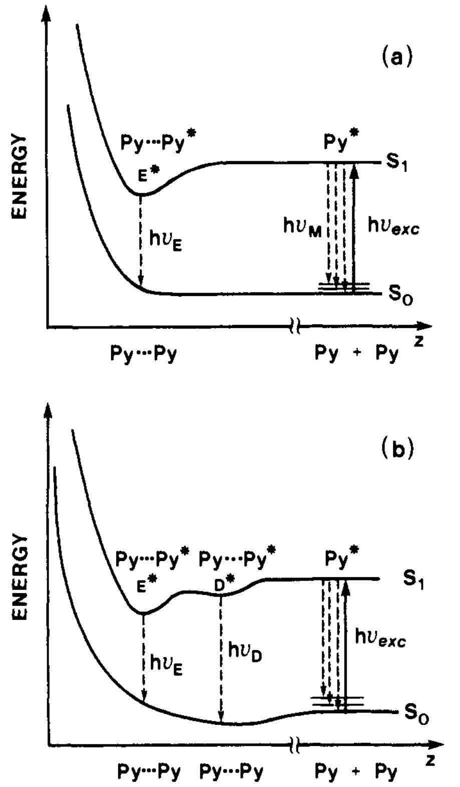


Figure 1. Schematic potential energy diagrams for pyrene excimer formation in the absence of ground-state association (a) and with pyrene ground state association (b).

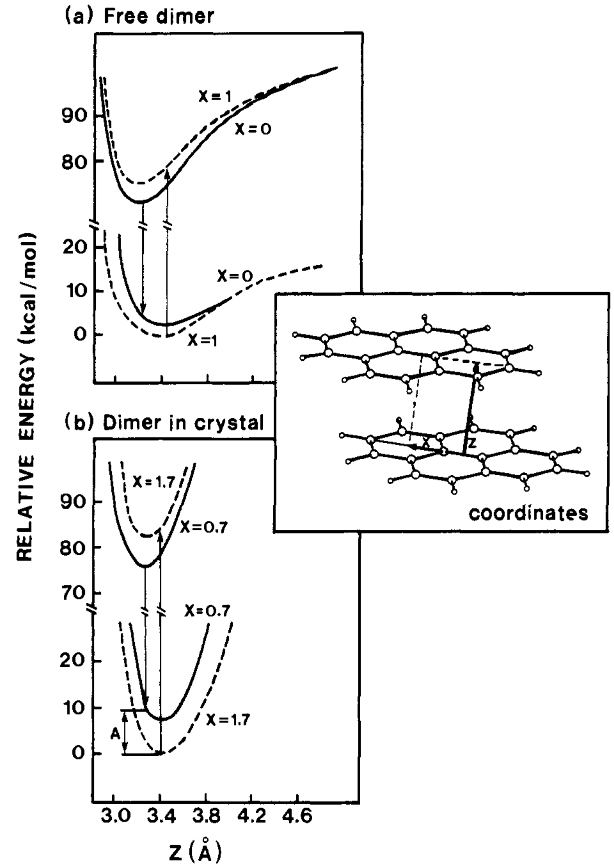


Figure 2. Calculated ground state and excimer potential surfaces for the free pyrene dimer (a) and for the dimer in the pyrene crystal (b). Reprinted from 14. Copyright 1974 Elsevier.

measurables from which the four parameters in Scheme 1 are determined.

One way of visualizing the distinction between D* and E* is in terms of the potential energy diagrams of the ground state and first excited state for a pair of Py in a sandwich dimer conformation, as a function of their intermolecular distance *z*, (Figure 1). In the case of an excimer true to Birks' definition,[11] the diagram indicates that at large distances of separation (e.g. $z > 10$ Å) in the ground state and in the excited state the energy of the pair is invariant to changes in separation distance. At a smaller separation, e.g. about 4 Å, the energy of the ground-state pair rises rapidly as a result of the repulsion between the molecules. Figure 1 shows that as Py* and Py approach, bonding between them increases, causing a minimum in the excited-state surface to occur.

The excimer state has been described theoretically by configurational mixing of exciton–resonance states and charge resonance states.[9,12] The charge resonance interaction is usually treated as nearly isotropic and only moderately dependent on the inverse distance, while the exciton resonance interaction is polarized and has a strong inverse distance dependence.[8] The latter interaction, which is predominant in all aromatic excimers except benzene and its derivatives, is responsible for the parallel arrangement of the molecular axes of the pyrenes in the excimer state. Both interactions favor the sandwich structure of the excimer since they yield the smallest distance between molecular centers.[9] The electronic stabilization of the pyrene excimer is substantial (ΔH = 10 kcal mol^{-1}).[13] Its emission, according to the Franck–Condon principle, leads to a dissociative ground state. Excimer emission is broad and structureless. Since the deactivated excimer dissociates before it can complete a vibrational cycle, too few pyrenes are at close enough distance for significant amounts of excitation of the ground-state pairs to the excimer to occur.

Figure 1b presents a potential energy diagram that allows for the formation of a stable ground-state dimer (D) and of two types of excimers, E* and D*, the latter being formed by excitation of a ground-state dimer. This diagram will be used as a working tool to serve as a focus in arguments discussed later in this review. It is supported by theoretical evaluation of the potential surfaces for excimers in pyrene single crystals[14] presented in the next section. Compared with part a of Figure 1, part b has been modified to emphasize that in this situation: (1) the ground-state surface possesses a minimum at a separation distance intermediate between that of electronically isolated molecules and the onset of π-orbital repulsion ($3 \leq z \leq 10$ Å) and (2) the excited-state surface presents a double minimum. The deeper minimum on this surface corresponds to the excimer E*, and the shallow minimum, at a slightly larger separation distance, corresponds to the excited dimer D*. The electronic stabilization of the excited dimer is depicted as larger than the corresponding stabilization of the ground-state dimer. It is worth noting that the origin of the minimum for ground-state dimer formation often derives from forces imposed by the external medium, such as defect sites in the crystal or hydrophobic interactions in water. Emission from D* is structureless, but unlike emission from E*, it leads to an associative state.

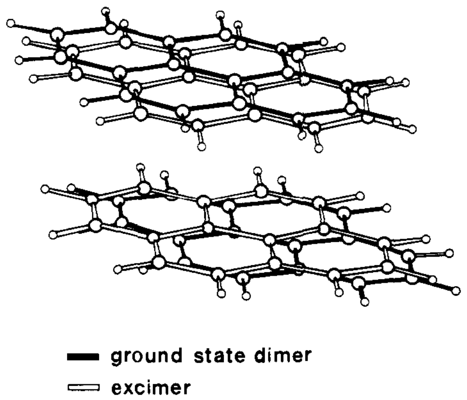


Figure 3. Calculated equilibrium geometries of the ground-state dimer and the excimer in the pyrene crystal. Reprinted from 14. Copyright 1974 Elsevier.

From calculations of the excimer potential surfaces Warshel and Huler have evaluated the equilibrium geometries of the pyrene excimers in the gas phase and in the crystal.[14] Their calculation method includes the π-electron system (quantum mechanical extension of the consistent force field),[15] the repulsive hard core of the σ electrons, and the effect of the crystal potential. The calculations led to the conclusion that the ground-state potential possesses a minimum. It is not purely "repulsive", as was assumed previously. Potential energy surfaces were evaluated for two overlapping geometries of the excimer in the case of a free dimer (Figure 2a) and of a dimer in the crystal phase (Figure 2b) (coordinate system shown in inset). For the free dimer the situation shown as $x = 0$ corresponds to the minimum, in the x direction, of the excimer energy surface, while the $x = 1$ Å surfaces are close to the ground-state minimum ($x = 1.2$ Å, $z = 3.44$ Å). The potentials in the y direction were shown to be much shallower than in the x direction. The calculated surfaces in the crystal (Figure 2b) were evaluated for $x = 1.7$ Å, the ground-state equilibrium geometry, and for $x = 0.7$ Å. The minimum of the excimer potential ($x = 0.7$ Å, $z = 3.26$ Å) does not correspond to a completely overlapping configuration, as a result of the hindering effect of the surrounding lattice. The calculated equilibrium geometries of the ground state and the excimer state in the crystal are presented in Figure 3. The main displacement upon pyrene excitation takes place in the x direction, a small displacement in the z direction is also noted. The results of the calculations are consistent with experimentally observed emission properties, e.g. the structureless envelope of the emission band and the temperature dependence of its line width, but calculated excitation energies are shifted by as much as 3000 cm^{-1}, relative to the observed values.[16] However the predictions give a reasonable description of the geometry requirements associated with pyrene excimer formation and of the relationship between excimer geometry and emission.

Calculations of the interaction potentials of the ground-state dimers and excimers of naphthalene, anthracene, and pyrene have been reported by Padma Malar and Chandra.[17] They employed a pertubative approach to calculate the interaction potential in the ground-state dimers using five contributing terms: electrostatic, π-overlap repulsion, nonbonded repulsion, charge transfer, and π–π and σ–σ dispersion energies. For the calculations of the transition energies in excimers they chose a SCF-LCAO-MO method within the framework of the supermolecule approach.[18] Their calculations confirmed that for pyrene the perfect sandwich geometry is not a stable conformation, neither in the ground-state dimer nor in the excimer. A slightly rotated sandwich geometry was favored. Binding energies of the excimers were estimated, but a satisfactory fit with the experimental value (ca. 10 kcal mol^{-1})[12] was not achieved.

III. Spectroscopic Detection of Pyrene Association in Aqueous Solutions of Labeled Polymers

Whether excimer formation takes place in a given pyrene-containing system is readily determined by measuring a steady-state fluorescence spectrum: the broad, featureless emission centered at ca. 480–500 nm is extremely easy to recognize, even when extensive pyrene monomer emission occurs, since the monomer fluorescence takes place in the 380–400-nm wavelength range (see below). Steady-state emission spectra, however, give very few clues to the actual mechanism of excimer formation. Additional measurements have to be carried out to ascertain whether pyrene preassociation takes place. They are listed in this section and illustrated with examples taken from studies of pyrene-labeled hydroxypropyl celluloses in water. The polymers, HPC-Py/438, HPC-Py/216, HPC-Py/56, and HPC-Py/26, carry varying amounts of [4-(1-pyrenyl)-*n*-butyl]oxy substituents attached at random to the carbohydrate hydroxyl groups (Figure 4). The digits associated with each acronym indicate the average number of glucose units per chromophore. Thus the polymer HPC-Py/26 has the highest level of pyrene incorporation (ca. 1.3×10^{-4} mol of Py/g of polymer). It is useful to divide the list of measurements into two types: (1) a series of straightforward tests to run if one suspects that pyrene preassociation takes place, and (2) more elaborate measurements aimed at the characterization of the excited dimer state D* in terms of its geometry, its stability, and its contribution to the total excimer emission.

A. Key Photophysical Parameters

The following photophysical parameters were found extremely useful in detecting pyrene preassociation in dilute polymer solution (Table 3).

1. The Absorption Spectrum

In the absorption spectrum, one clear indication of pyrene preassociation is the broadening of the absorption bands, compared to model systems where pyrene is molecularly dissolved. A convenient relative measure of this loss in resolution can be extracted from the ratio P_A of the absorption intensity of the most intense band to that of the adjacent minimum at shorter wavelength. For 1-substituted pyrenyl compounds, this value is usually > 3.0 for the ^1L_a band in the absence of preassociation. It decreases in relative proportion to the extent of preassociation. The broadening of the absorption bands is often accompanied by small red shifts in maxima positions and by a decrease of the extinction coefficients (hypochromism). These effects

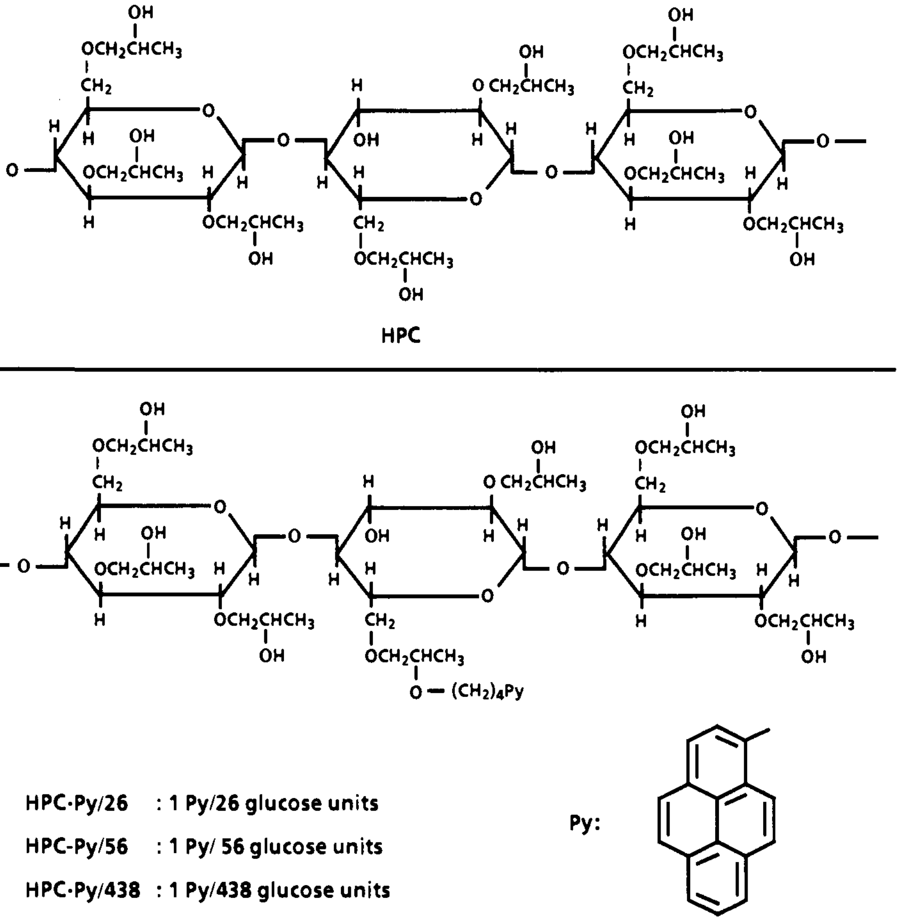


Figure 4. Chemical structures of hydroxypropyl cellulose (HPC) and pyrene-labeled hydroxypropyl cellulose (HPC-Py). Reprinted from ref 144. Copyright 1992 American Chemical Society.

are illustrated here in the case of aqueous solutions of two pyrene-labeled hydroxypropyl celluloses (Figure 5).

2. The Excitation Spectrum

Excitation spectra provide compelling evidence for ground-state interactions of pyrenes. Spectra monitored at the monomer emission and at the excimer emission are clearly different. While they have similar overall features, they cannot be superimposed: (1) the spectrum monitored at the excimer emission is red-shifted, compared to the spectrum monitored for the monomer, and (2) the bands in the spectrum monitored for the excimer are broadened. Relative measures of these two effects are given by (i) the peak-to-valley ratio, P_M, for the (0,0) transition in the excitation spectrum viewed at the monomer emission (usually ca. 380 nm, but other wavelengths in the 380 to 400 nm range have been selected in some studies); (ii) P_E, the peak-to-valley ratio for the (0,0) transition in the excitation spectrum viewed at the excimer emission (usually 480–500 nm). In situations where pyrene preassociation takes place P_E is always smaller P_M; and (iii) $\Delta\lambda = \lambda_{max}(\text{excimer}) - \lambda_{max}(\text{monomer})$, the shift in the wavelength maxima of the (0,0) transition in the two excitation spectra. In systems where preassociation occurs, it takes a positive value (red shift) from 1 to 4 nm.

A simple and effective means to compare a pair of emission–wavelength-dependent excitation spectra was

Table 3. Key Spectrophysical Parameters To Detect Pyrene Ground-State Aggregation

symbol	definition
	Emission Spectrum
I_E/I_M	ratio of intensities of the excimer emission at λ_E and of the monomer emission[a]
λ_E	wavelength corresponding to the maximum of excimer emission (steady-state emission or time-resolved spectrum)
	Excitation Spectrum
P_M	peak-to-valley ratio for the (0,0) transition in ^1L_a band in the excitation spectrum viewed at the monomer emission (usually ca. 380 nm)
P_E	peak-to-valley ratio for the (0,0) transition in ^1L_a band in the excitation spectrum viewed at the excimer emission (usually ca. 480 nm)
$\Delta\lambda$, nm	difference in wavelength maxima for the (0,0) transition in ^1L_a band in the excitation spectra viewed at the monomer emission and at the excimer emission [$\Delta\lambda = \lambda_{max}$(for the excimer) − λ_{max}(for the monomer)]
	Absorption Spectrum
P_A	peak-to-valley ratio for the (0,0) transition in ^1L_a band
	Time-Resolved Emission Spectrum
R	ratio $R = -A_4/A_3$ (see eq 2) of the prefactor in the excimer time-dependent profile corresponding to the decaying and rising component, respectively

[a] The I_E/I_M ratio has no absolute meaning. The value depends on how the spectra are measured and on whether the ratios are calculated from peak heights or from spectra areas.

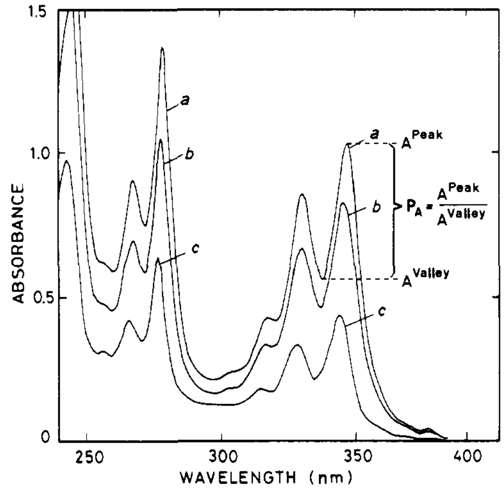


Figure 5. UV absorption spectra of aqueous solutions (26.4 ppm) of HPC-Py/26 (a), HPC-Py/56 (b), and HPC-Py/438 (c). Reprinted from ref 144. Copyright 1992 American Chemical Society.

reported recently by Zagrobelny et al. in a study of the spectroscopy of the pyrene excimer in supercritical fluids.[19] Both excitation spectra are first normalized at their respective peak maxima, then the intensity of the excimer scan is plotted versus the intensity of the monomer scan. If the spectral contours overlap completely, the correlation plots show a straight line with a slope of 1, an intercept of 0, and a correlation coefficient close to 1. When the two excitation spectra differ, the corresponding correlation plots show randomly distributed data points.

3. The Steady-State Emission Spectrum

The steady-state emission spectrum provides two parameters, the ratio, I_E/I_M, of the intensities of the excimer emission to that of the monomer emission, and λ_E, the wavelength corresponding to the maximum of excimer emission. The selection of the excitation wavelength merits consideration. If it were possible to excite preassociated pyrenes to the exclusion of isolated pyrenes (or vice versa), the profile of the excimer emission would vary with excitation wavelength. This is rarely observed; i.e., the excimer fluorescence profile is unaffected although the intensity of the excimer emission may vary with the excitation wavelength.

4. The Time-Dependent Fluorescence Spectrum

In a time-dependent fluorescence measurement the emission intensity of an excited species is monitored as a function of time after generation of an excitation pulse. The excimer fluorescence intensity $i_E(t)$ is described in general terms by eq 2. If excimer formation proceeds via Birks' mechanism (Scheme 1), when $t = 0$ the excimer concentration is equal to 0. Therefore the excimer time-dependent emission profile will present a rising component, with a negative prefactor $-A_3$, and a decaying component with a positive prefactor A_4, and the ratio $R = A_4/A_3$ will take a value of 1.0. For systems where a large fraction of the excimer originates from preassociated chromophores, time-dependent measurements carried out on a nanosecond time scale, with a typical 1–3-ns lower limit of detection, often show no rising component in the excimer profile. In cases where both excimer formation mechanisms operate (cf. Scheme 2), a rising component corresponding to excimer formation is observed in the excimer time-resolved profile, but the ratio R deviates from unity, since part of the excitation light is absorbed by the dimer D. Other technical reasons may account for a deviation of R from linearity:[20] (a) some monomer emission may take place at the observation wavelength; (b) an insufficient number of exponentials are used to fit the excimer response function; or (c) the time resolution of the experiment is inadequate. In situations of extremely fast excimer formation ($\tau < 1$ ns), time-dependent measurements carried out on the picosecond time scale are more appropriate.

Table 4. Photophysical Parameters Measured in Aqueous Solutions of Pyrene-Labeled Hydroxypropyl Celluloses[144]

	HPC-Py/26[a]		HPC-Py/216[a]	
parameter	H_2O	MeOH	H_2O	MeOH
I_E/I_M[b]	2.01	0.31	0.10	0.04
λ_E, nm[b]	489	485	489	485
P_M	2.12	2.82	2.34	2.80
P_E	1.75	2.44	1.75	
$\Delta\lambda$, nm	4		2	
P_A	1.89	3.20	2.16	3.50
R				

[a] Polymer concentration: 0.1 g L^{-1}. [b] λ_{exc} = 330 nm.

B. The Case of Pyrene-Labeled Hydroxypropyl Cellulose

1. The Absorption Spectrum

Pyrene association is revealed by perturbations of the three vibronic bands of the $S_2 \leftarrow S_0$ and $S_1 \leftarrow S_0$ transitions. They are shifted to the red and broadened. The extent of spectral broadening due to pyrene association in water is reflected by the large differences in P_A values (see Table 4) for spectra of HPC-Py/26 in water ($P_A \approx 1.9$) and in methanol ($P_A \approx 3.1$). The magnitude of the red shift (0–2 nm) gives a qualitative measure of the extent of pyrene aggregation. Two polymers labeled with high and low level of pyrene, HPC-Py/26 and HPC-Py/438, exhibit marginally different absorption maxima at 345 and 329 nm and 344 and 328 nm, respectively.[21] This red-shift is accompanied by a strong hypochromic effect.[22] In the case of HPC-Py/26 the extinction coefficient per pyrene chromophore decreases from its value of 45 000 in methanol to 25 000 in water.[23] Hypochromism of the pyrene chromophore was observed previously.[24] A case especially relevant is that of poly(vinylpyrene) (PVP) for which a strong hypochromism and larger red-shifts of the $^1L_b \leftarrow {}^1A$ (4 nm), $^1L_a \leftarrow {}^1A$ (5 nm), and $^1B_b \leftarrow {}^1A$ (3 nm) bands were reported in tetrahydrofuran solution, compared to the monochromophoric 1-pentylpyrene used as the reference compound.[24] Both the red-shifts and the hypochromism contain information on the geometry of the ground-state pyrene dimers and aggregates.

2. The Excitation Spectrum

Excitation spectra monitored for the monomer (379 nm) and the excimer (489 nm) emissions are shown in Figure 6 for an aqueous solution of HPC-Py/26 and

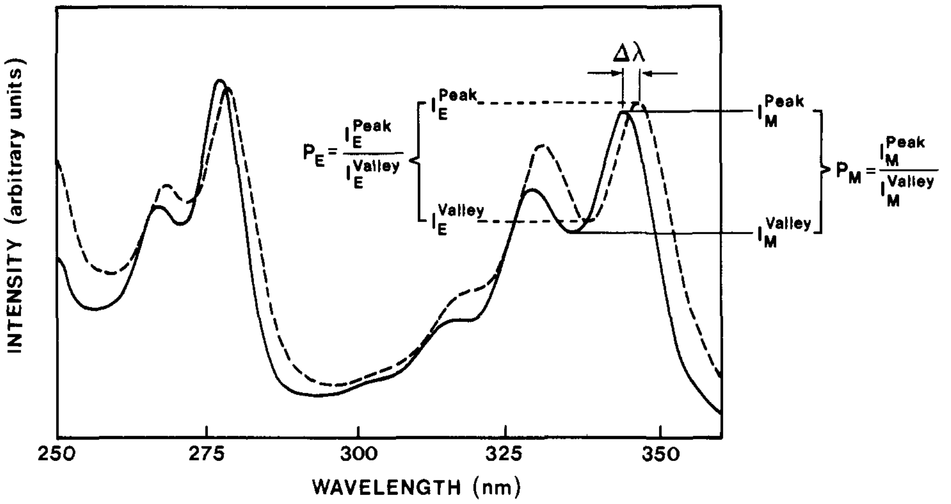


Figure 6. Normalized excitation spectra of HPC-Py/26 in water, monitored at 380 nm (monomer, full line) and at 489 nm (excimer, dashed line). Reprinted from 23. Copyright 1987 American Chemical Society.

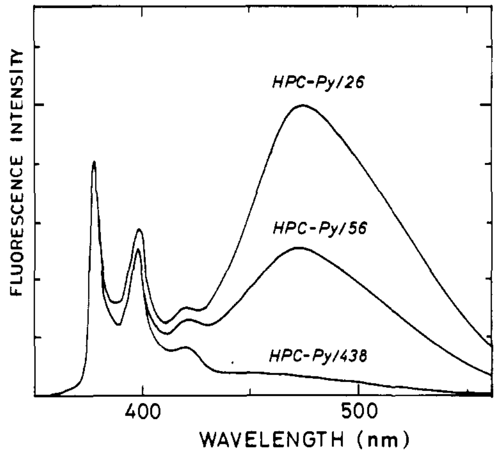


Figure 7. Fluorescence spectra of HPC-Py (0.026 g L^{-1}) in water; λ_{exc} = 330 nm; 20 °C; spectra were normalized at 377 nm. Reprinted from ref 144. Copyright 1992 American Chemical Society.

corresponding P_M, P_E, and Δλ values are listed in Table 4 for solutions of this polymer in water and in methanol. Notice how the two excitation spectra differ both in the positions of the band maxima and in the widths of bands. A mild dependence of the red-shifts (Δλ values) on the level of pyrene incorporation gives a qualitative indication on the extent of pyrene aggregation in aqueous solutions.

3. The Steady-State Emission Spectrum

Steady-state emission spectra, normalized at 377 nm, of HPC-Py in water (0.026 g L^{-1}) present the characteristic pyrene monomer emission, with the (0,0) band at 376 nm, and a broad excimer emission centered at 480 nm. Excimer emission, relative to the monomer emission, decreases as the amount of label decreases (Figure 7). Not shown on this figure are the changes in I_E/I_M observed as a function of excitation wavelength. Exciting the sample at 360, 370, and 375 nm led to incremental enhancement of I_E/I_M, but the shape and the maximum position of the excimer band were not affected. Elaborate concentration-dependent measurements gave no clear answer to the question of the relative extent of interpolymeric and intrapolymeric association among pyrenes.[23]

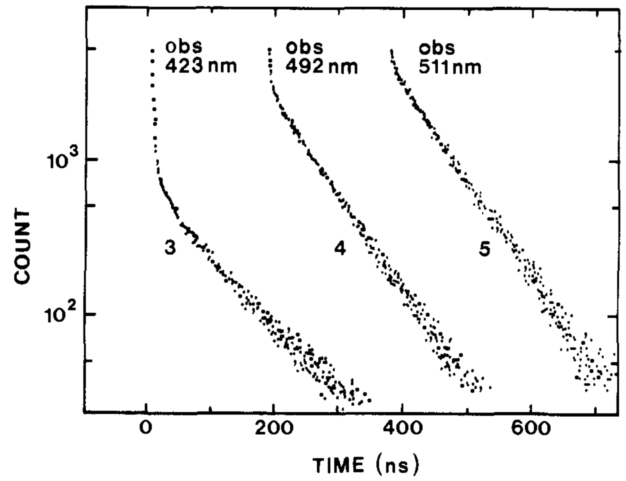


Figure 8. Fluorescence decay curves of the excimer monitored at different wavelengths in a solution of HPC-Py/56 in water, 20 °C. Reprinted from 21. Copyright 1987 American Chemical Society.

4. The Time-Dependent Spectrum

Time-dependent profiles of the excimer emission for aqueous solutions of these polymers have no rising component when measured in the nanosecond time domain. Nonetheless decay profiles in the nanosecond time scale contain useful information, especially when one compares decay profiles viewed at several wavelength within the excimer emission range. The excimer decay profiles monitored at three wavelengths are presented in Figure 8 for an aqueous solution of HPC-Py/56.[21] The decay curve monitored at 511 nm can be fitted to a biexponential decay with a major component (τ = 72.0 ± 2.2 ns) and a minor component (τ = 3.77 ± 0.18 ns). At 492 nm, the fast decaying component becomes dominant. At 423 nm the decay is dominated by a very short-lived, probably subnanosecond, component. This heterogeneity in decay profiles within the excimer emission signals the existence of several excimer-like emissive species. A detailed analysis of the excimer emission profile performed by picosecond time-resolved fluorescence spectroscopy confirmed this conclusion.

Emission spectra of HPC-Py/56 measured with increasing time after excitation are shown in Figure

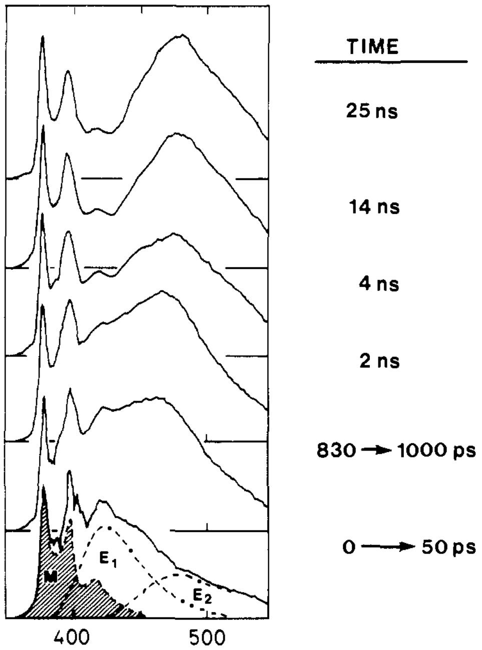


Figure 9. Time-resolved fluorescence spectra of an aqueous solution of HPC-Py/26 at 25 °C. The time 0 corresponds to the time at which the excitation pulse reaches maximum intensity. Spectral analysis was performed assuming three components: a pyrene monomer (M) and two pyrene excimers (E_1 and E_2). Reprinted from ref 144. Copyright 1992 American Chemical Society.

9.[21] The spectra were normalized at the wavelength of maximum intensity. Time 0 corresponds to the time at which the excitation pulse reached its maximum intensity. Focusing our attention first on the spectra measured in the nanosecond time domain (top three frames of Figure 9), we observe that the spectrum recorded about 25 ns after excitation closely resembles the steady-state spectrum shown in Figure 7. Differences can be detected among the three spectra recorded in the nanosecond domain, and these in turn differ significantly from the spectra recorded in the picosecond time domain. All spectra were simulated successfully by a superposition of three components: (1) a monomer band (M) with vibrational peaks at 376, 397, and 421 nm, (2) a broad structureless band (E_1) with a maximum at 420 nm, and (3) a second excimer band (E_2) with a maximum at 470 nm. The contours of the three components are shown in dashed lines in the bottom frame. The blue-shifted excimer E_1 is the major contributor to the excimer emission in the short time domain (<100 ps), it all but vanishes in the longest time range. This emission was assigned to a higher energy excimer species with only partial overlap of the pyrene moieties, by analogy with previous observations of stearic acid LB films doped with a pyrene-labeled fatty acid (see section IV.D). The red-shifted excimer, E_2, grows in within about 100 ps. It was assigned to a sandwichtype excimer.

IV. Pyrene Excimers in Organized Media

Ground-state pyrene association has been described in several systems prior to its detection in aqueous solutions of labeled polymers. Absorption of light by ground-state pyrene complexes was detected first in frozen solutions.[25] Clear evidence for ground-state association exists also for pyrene adsorbed on silicas, aluminas, clays, and zeolites.[26] The photophysical behavior of pyrene adsorbed on such surfaces is rich in information pertaining to orientation, distribution, and association in the sorbed state. Most practical situations however are plagued with artifacts, as discussed in great detail by Lochmüller and Wenzel.[27] To obtain reliable information on the spectral properties of excimers originating from preassociated pyrenes, it is best to turn to well defined physical entities. Five such systems will be discussed here: (1) pyrene crystal; (2) glassy solutions or mixtures containing pyrene as a guest compound; (3) pyrenophanes, where the two chromophores are held face to face by two hydrocarbon bridges and bis-pyrenyl compounds having a single chain linkage between the two chromophores; (4) ultrathin organic films; and (5) micelles and vesicles.

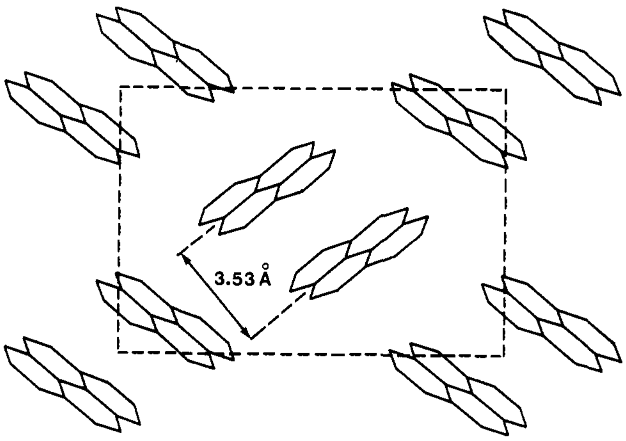


Figure 10. Arrangement of molecules in the (0,0,1) plane of a pyrene crystal. Reprinted from 28. Copyright 1947 Chemical Society, London.

A. Pyrene Crystal

The structure of the pyrene crystal is one composed of partially-overlapping card-packed dimeric units in which the pyrene molecules of each pair are separated by a distance of 3.53 Å (Figure 10).[28,29] When a pyrene molecule in the crystal absorbs light, two parallel molecules will tend to move toward one another and toward more complete overlap. These movements are resisted by the environment. As a result an excimer in the crystal may not attain the geometry it would in solution. Fluorescence properties of pure single crystals have been reviewed by Stevens[30] and an overview of the excimer emission in crystals has been given by Cohen in terms of the "reaction cavity" concept.[31] It was revealed by early experiments that the pyrene crystal shows excimer emission, without any monomer emission,[32] at all temperatures between 4 and 423 K.[33,34] Its absorption spectrum is structured, with band maxima only slightly red-shifted compared to molecular pyrene.[35] This suggests that the absorbing species is effectively monomeric. Recent reports from several laboratories have pointed out that the spectroscopy of pyrene crystal is more complex than previously believed. There are in fact two excimer emissions detectable by time-resolved spectroscopy. These are reviewed here, since they provide a basis for the interpretation of the spectroscopy of pyrene in other constrained environments.

Steady-state and time-resolved spectra of pyrene crystals have been monitored as a function of temperature, in the high temperature domain (298–423 K)[33] and in the low temperature domain (4–290 K).[34] From 298 to 403 K the spectra do not change significantly. Above the melting point (424 K) the excimer emission band is conserved, but it undergoes slight shifts of the peak maximum and a broadening in the long wavelength region. A small shoulder at 420 nm appears at 458 K and it becomes more distinct with increasing temperature. It was assigned tentatively to a monomer vibronic band. Excimer decay times decrease with increasing temperatures from 108 ns (296 K) to 51 ns (423 K) and 10 ns in the liquid state. Below room temperature, in steady-state experiments, one observes that the excimer emission broadens with increasing temperature and monotonically decreases in intensity. In time-resolved experiments, the excimer rise time (picosecond time scale) increases abruptly at ~120 K,[36] then decreases with increasing temperature. The temperature of the sharp increase in rise time corresponds to a structural phase transition at $T_p \approx 120$ K of the pyrene crystal.

Time-resolved measurements performed above T_p reveal the contribution to the total emission of a blue-shifted component formed within less than 15 ps after excitation and depopulated across a barrier of 350 ± 30 cm^{-1} toward the excimer state, with an intrinsic lifetime longer than 100 ns.[37] The occurrence of two types of excimer emissions in the high temperature phase of pyrene resembles that of α-perylene. It is supported by similar temperature-dependent measurements reported by Matsui and co-workers.[38] Cooling the pyrene crystal below T_p triggers the immediate disappearance of the blue-shifted emission, except when special conditions are used to grow pyrene crystals. Mizuno and Matsui succeeded in growing a metastable crystal with the high temperature-phase structure maintained below T_p.[39] In this crystal the blue-shifted emission persisted upon cooling below T_p, down to ~60 K at which temperature a new emission was detected. Seyfang et al. reported recently the preparation of microcrystalline pyrene films deposited on silicon substrates.[40] These microcrystals reveal at high-temperature features typical of the single crystal, but they do not show a phase transition. Their spectroscopic properties are preserved upon cooling below T_p, and below 50 K a new blue-shifted emission appears. It becomes predominant in spectra recorded at lower temperature. Its features seem to be very similar to those observed during cooling of a metastable single crystal. The assignment of this emission is still controversial. Sumi proposed a model to describe this situation, as well as the low-temperature emission in α-perylene crystals.[41]

The spectroscopic data in the $T_p < T < 150$ K temperature domain were interpreted by Seyfang et al.[37] in terms of a two-step excimer formation process: the blue-shifted emission was assigned to a metastable B state which is populated after excitation into the singlet state manifold S; it decays either directly into the ground state, by radiative or radiationless processes, or via thermal activation into the excimer state E; the E state is populated only via the latter process; it decays by radiative or radiationless processes to the ground state. On the basis of this scheme an energy diagram was drawn for the three electronic states involved (Figure 11).

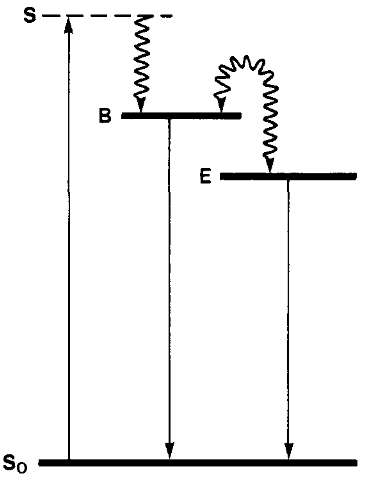


Figure 11. Energy diagram for the excited state relaxation in pyrene crystal, high-temperature domain. Reprinted from 37. Copyright 1988 Elsevier.

Recent room temperature femtosecond time-resolved transient grating experiments reveal the occurrence of an extremely fast relaxation process following electronic excitation via two-photon absorption.[42] Formation of a long-lived state was observed within several picoseconds following absorption of a 65-fs excitation pulse. Excimer formation was judged complete in less than 5 ps and likely in less than 500 fs. This emission was interpreted as corresponding to the relaxation of the initially excited exciton into the self-trapped distorted excimer state.

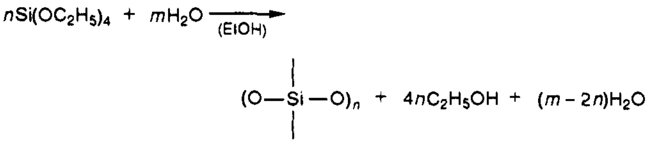
B. Pyrene in Glassy Matrices

The motion of molecules trapped in glassy mixtures is limited by the rigidity of the matrix. Thus, given the distance requirements for pyrene excimer formation in the crystal, it will be necessary to prepare glassy mixtures of high guest concentration in order to observe excimer formation, assuming a randomly mixed solution. Nonetheless excimer fluorescence from frozen cyclohexane solutions has been observed in glasses containing as low as 10^{-4} mol L^{-1} of pyrene.[43] More concentrated low-temperature solvent glasses are difficult to prepare, due to solubility limits, but it is possible to achieve a higher loading of randomly distributed guest molecules in glassy polymeric materials.

1. Pyrene in Silica Glasses

Over the last decade important advances in the preparation of inorganic glasses have taken place through the application of the sol/gel method. The basic idea is to prepare inorganic glasses by polymerization of suitable monomers rather than by high temperature melting of oxides and salts.[44] For example silica glasses are prepared at room temperature by the hydrolysis/condensation of tetraethyl orthosilicate (see Scheme 3). Organic molecules can be trapped within the ceramic matrix if they are dissolved in the medium during gelation.[45] Dyes have been used as probes to monitor the gelation process and as permanent guests imparting to the glass specific photoreactivity, such as photoconductivity,[46] photochemical hole-burning capabilities,[46] and photochromism.[47] Kaufman and Avnir

Scheme 3



$$n\mathrm{Si(OC_2H_5)_4} + m\mathrm{H_2O} \xrightarrow[\mathrm{(EtOH)}]{} (\mathrm{O{-}\overset{|}{\underset{|}{Si}}{-}O})_n + 4n\mathrm{C_2H_5OH} + (m-2n)\mathrm{H_2O}$$

used pyrene (initial concentration: 1×10^{-3} mol L^{-1}) to monitor structural changes along the sol/gel transition of a mixture of tetramethyl orthosilicate, water, and methanol.[47] During the gelation process extreme changes occurred in the fine structure of the monomer emission and in the relative ratio of excimer to monomer intensities. At the end of the process, in the xerogel state, the excimer was barely detectable. Studies at higher pyrene concentrations have been reported recently by a Japanese group.[48] Pyrene was present in the gelling solution at a concentration of 1×10^{-2} mol L^{-1}. A measurable excimer contribution was observed in the steady-state fluorescence spectrum of the pyrene-doped glass obtained after drying at 40 °C for 1 week. The concentration of pyrene in this glass, about 4×10^{-2} mol L^{-1}, was higher than in the starting mixture, as a result of solvent evaporation during curing. The excimer emission was shown to originate from aggregated pyrenes on the basis of evidence obtained from (1) comparison of the excitation spectra monitored for the excimer and monomer emissions,[47] (2) fluorescence decay measurements,[49] and (3) time-resolved spectroscopy.[50] Two excimer emissions were detected in the time-resolved spectra: a blue-shifted excimer, formed within the time of excitation (laser light pulse of 60 ps half-width), with a decay time of ca. 5 ns attributed to the emission of an excited ground-state dimer D and a slower decaying excimer with a decay time of 35 ns, which corresponds to the excimer observed in steady state spectra.

2. Pyrene in Organic Polymeric Glasses

The photophysics of pyrene in organic glassy polymers were examined nearly 20 years ago by Avis and Porter for PMMA glasses,[51] and in 1980 by Johnson for PS glasses.[52] Films were prepared by solution casting from benzene or toluene ([Py] = 0.1–1.3 mol L^{-1}). Excimer emission was observed in all cases. In PMMA the absorption spectra of pyrene were broadened and slightly red-shifted, compared to the absorption spectrum of pyrene in the casting solution, and differences were observed between the excitation spectra monitored for the excimer and monomer emissions. Excimer decay times at high pyrene concentrations were ca. 65 ns in PMMA[51] and 45–60 ns in PS.[52] The excimer fluorescence response curves reached a maximum after ca. 20 ns,[51] a rise time much longer than that measured in pyrene crystal. Avis and Porter suggested that the slow rise in the excimer fluorescence response curves is due to the step of converting an excited pyrene dimer to the excimer.[51] Johnson however argued that the slow excimer formation rate may rather be due to the step of transferring electronic excitation from an isolated pyrene to a pyrene dimer.[52] The excited dimer would then form the excimer within 1 ns, a delay similar to the pyrene crystal.

The emission of pyrene in glassy polymeric matrices was investigated recently by total internal reflection (TIR) fluorescence spectroscopy. In this technique an evanescent wave is used to excite selectively in a matrix a chromophore located in the vicinity of the substrate/matrix interface.[53,54] The thickness of the interfacial layer probed depends on the excitation wavelength, the absorbance of the chromophore and the penetration depth of the evanescent wave. In the case of pyrene in PMMA excited at 310 nm, this thickness ranges from ca. 50 μm ([Py] $\approx 8 \times 10^{-4}$ mol/MMA unit) to ca. 0.5 μm ([Py] $\approx 8 \times 10^{-2}$ mol/MMA unit).[55] Films of varying pyrene content in PMMA and PS were cast or spin-coated on a sapphire substrate.[54,55] Steady-state spectra and time-resolved measurements were carried out for each film under "normal" conditions and under TIR illumination conditions. In PMMA the following trends were observed at pyrene concentrations high enough to allow for detection of excimer emission: (1) the excimer to monomer intensity ratio was smaller at the interface than in the bulk; and (2) the excimer and monomer decay times were shorter at the interface, compared to the bulk (Figure 12).[55] The authors attributed the decrease in lifetime near the interface to pyrene self-quenching via formation of nonemitting dimers.[56] They suggested that the concentration of such dimers is higher near the interface than in the bulk. A comparison of the detailed structure of the excimer emission profile in the bulk for pyrene in PMMA and in PS revealed the presence of two excimer species in PMMA, but not in PS.[55] In PMMA a small contribution of a blue-shifted emission was attributed to a partial overlap excimer. In PS the emission of this excimer was not detected, confirming data reported earlier by Johnson.[52] Further fluorescence studies including time-resolved spectroscopy on the picosecond time scale may help to clarify this important issue of the effect of polymer molecular structure on the aggregation of host molecules.

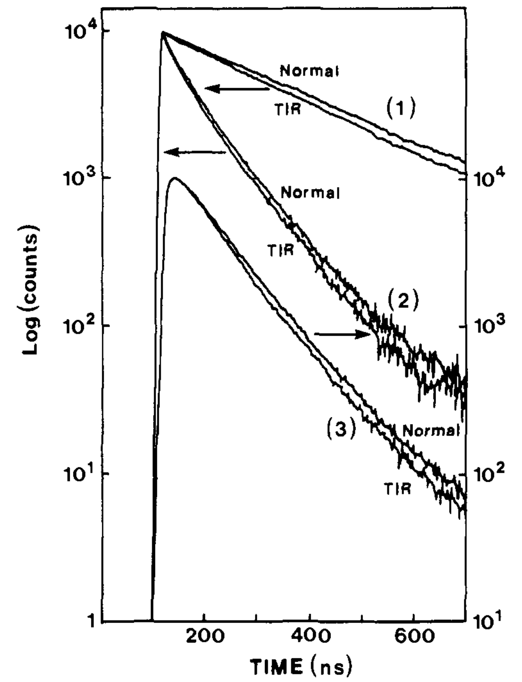


Figure 12. Fluorescence decay curves of the monomer (374 nm) and excimer (520 nm) of pyrene in PMMA matrices measured under normal conditions (normal) and under total internal reflectance (TIR) conditions: (1) [Py] = 2.48×10^{-3} mol/MMA unit mol. (2) [Py] = 5.58×10^{-2} mol/MMA unit. (3) [Py] = 4.90×10^{-2} mol/MMA unit mol. Reprinted from ref 55. Copyright 1990 Society of Polymer Science, Japan.

Farid and co-workers have reported the effect of glassy polymer hosts on the spectral position of the excimer band produced by high concentrations of methyl 4-(1-pyrenyl)butyrate.[57] Band positions in the glassy polymer were compared to the band positions in solution for the following hosts (and solvents): PS (toluene), PMMA (methyl isobutyrate), and poly(vinyl benzoate) (methyl benzoate). In the three solvents the excimer emission occurred at 480 nm. In all polymers the emission was blue-shifted by 35 (PS) to 45 nm (PVB). Farid and co-workers attributed the blue-shift to improper orientation of the excimer components in the polymer matrices. No lifetimes were reported in this study. Kosbar and Frank reported recently a study of pyrene excimer formation in novolac (cresol formaldehyde) films doped with pyrene, to unravel the influence of the casting parameters on the aggregation of guest molecules in a resist material used in semiconductor manufacturing.[58]

C. Pyrenophanes and Bichromophoric Compounds

1. Pyrenophanes

In phanes two aromatic rings are held face-to-face by at least two methylene bridges. These substances represent a unique set of materials for studying the effects of fixed orientations and distances of two chromophores on the absorption and emission properties of the pair.[59] The spectroscopic properties of several pyrenophanes will be reviewed briefly here.

Molecular Structures. The synthesis and properties of eight different pyrenophanes have been reported. Their chemical structures are shown in Figure 13. The nomenclature rules used here are those established for all phane compounds. For example, [2.2](2,7) pyrenophane (1) consists of two pyrene groups linked by a $(CH_2)_2$ bridge attached at the 2-position of each ring and by a $(CH_2)_2$ bridge attached at the 7 position of each ring. Crystal structures are known for this pyrenophane and two of its homologs 3 and 4, in which the pyrene groups are linked by propylene and butylene bridges, respectively. In [4,4](2,7) pyrenophane (4)[60] the two pyrene units are of almost ideal planarity. The transannular distance between the pyrene planes is 3.48 Å. It is a strain-free molecule with regard to transannular $\pi \cdots \pi$ interactions. The pyrene units are not in an ecliptic arrangement to each other. There is a considerable lateral displacement perpendicular to the long pyrene axis, but the parallel shift along this axis is small. The butylene bridges adopt a zigzag conformation with staggered orientations of vicinal hydrogen atoms. In the [3.3](2,7) compound 3 the pyrene units are slightly bent out of planarity, with transannular interplanar distances ranging from 3.18 Å between the bridgehead carbon atoms to 3.48 Å in the center of the molecule. The pyrene groups are also eclipsed. As in the case of 4, the displacement perpendicular to the long pyrene axis is much more pronounced than lateral displacement. In the [2.2](2,7) compound 1,[61] the pyrene units are bent considerably. The transannular interplanar distances range from 2.79 Å, between the bridgehead carbons to 3.80 Å in the center of the molecule. In contrast to the [3.3] and [4.4] homologs the carbon atoms are nearly perfectly eclipsed.

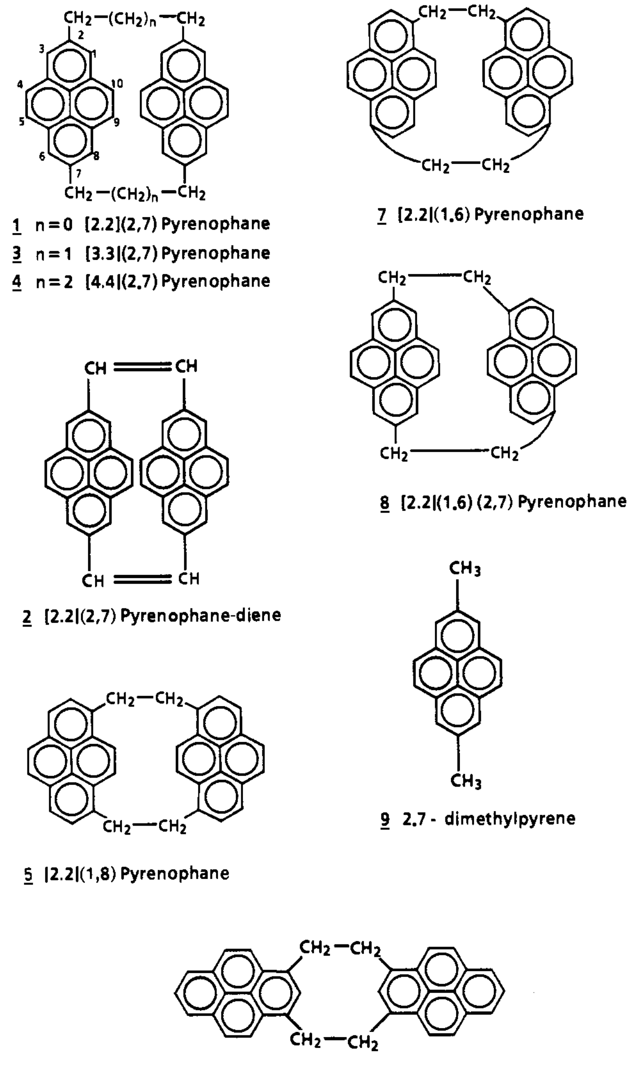


Figure 13. Chemical structures of pyrenophanes.

Solution ¹H NMR spectra ($CDCl_3$, toluene-d_8) of [n,n](2,7)pyrenophanes (n = 2–4) reflect the differences in distances and orientations of the pyrene groups in this series of molecules.[20] Relevant chemical shift values of the pyrenophanes and 2,7-dimethylpyrene (**9**) are listed in Table 5. First, notice the differences in chemical shifts for the aromatic protons of the pyrenophanes compared to those of 2,7-dimethylpyrene ($\Delta\delta$). Reynders et al. attributed these to ring current effects.[62] Second, several significant features of the chemical shifts of specific protons in each molecule can be interpreted in terms of the molecular structure of these pyrenophanes in solution. In [4,4](2,7)-pyrenophane the differences $\Delta\delta$ have equal values for two sets of equivalent protons (e.g. H_3/H_6) and (H_4/H_5). This is not the case for [3,3](2,7)pyrenophane. This observation implies that in solution, as in the crystalline phase, the pyrenes are nearly eclipsed in the dibutylene compound, but not in the dipropylene derivative. The large differences between $\Delta\delta(H_3)$ and $\Delta\delta(H_4)$ for [2.2](2,7)pyrenophane are consistent with the extensive distortion from planarity of the pyrene groups in this isomer.

In Figure 14 are depicted predicted structures of several pyrenophanes in a top view along the pyrene planes (a) and in two side views (b and c) along

Table 5. Chemical Shifts δ^a and Differences $\Delta\delta$ as Compared to Those of 2,7-Dimethylpyrene of [*n*,*n*](2,7)Pyrenophanes[20]

compound	$\delta(H_3)$	$\delta(H_4)$	$\Delta\delta(H_3)^b$	$\Delta\delta(H_4)^b$	$\delta(\alpha\text{-}CH_2)$	$\delta(\beta\text{-}CH_2)$
2,7-dimethylpyrene (**9**)	7.970	7.976			(2.789)^c	
[2.2](2,7)pyrenophane (1)	7.196	7.449	−0.774	−0.527	3.492	
[3.3](2,7)pyrenophane (3)	7.386	7.413	−0.684	−0.563	3.22	2.55
[4.4](2,7)pyrenophane (4)	7.320	7.327	−0.650	−0.649	2.782	2.179

^{a} In ppm. ^{b} $\Delta\delta = \delta_{2,7\text{-dimethylpyrene}} - \delta_{[n.n](2,7)\text{pyrenophane}}$. ^{c} Chemical shift of the methyl group.

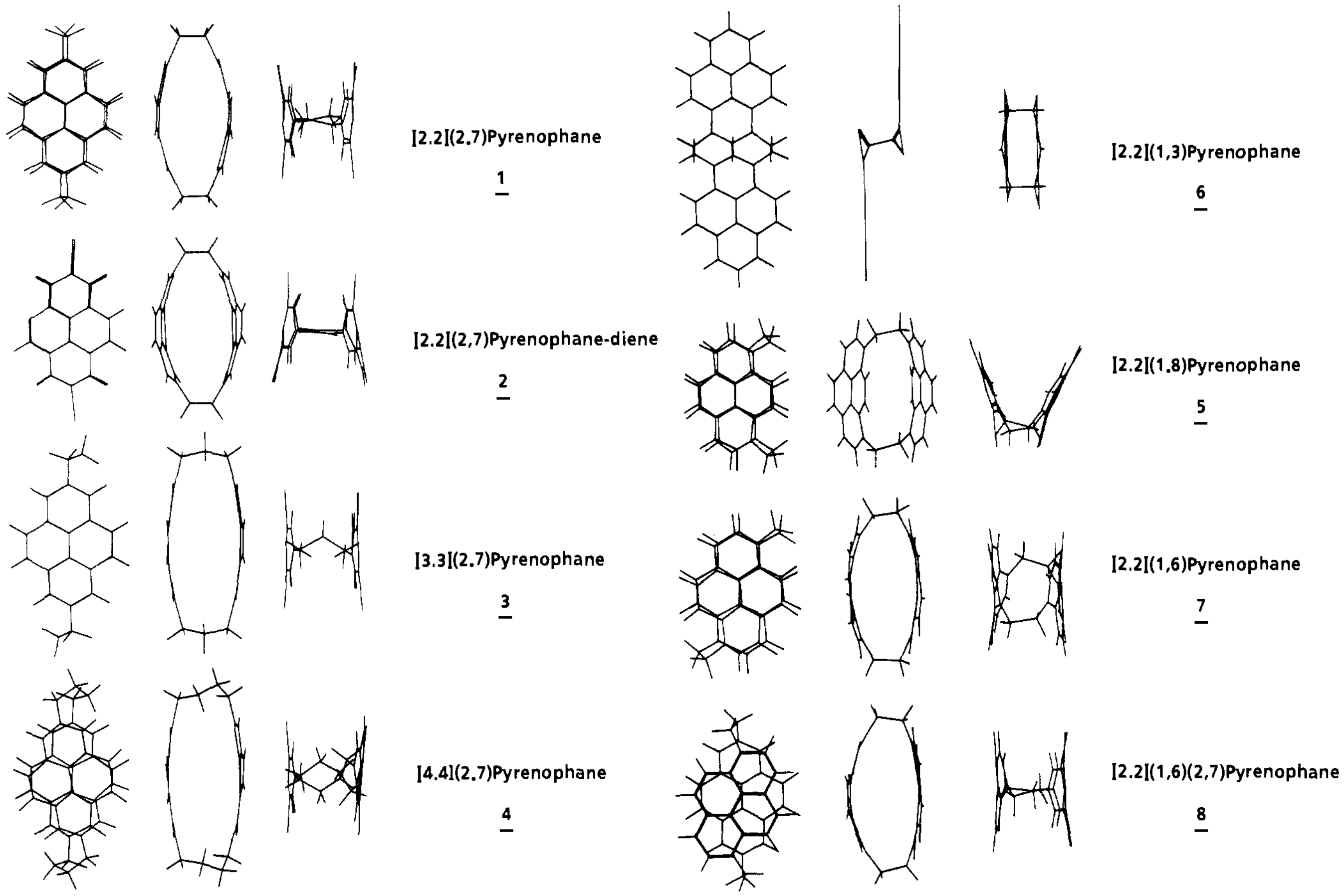


Figure 14. Predicted preferred conformations of pyrenophanes.

projections of the bridges. These structures were generated by performing single molecule geometry optimization calculations using the AM1 semiempirical quantum mechanical method contained in MOPAC, version 6.0.[63] The calculations were performed without any symmetry constraints and using the MOPAC keywords AM1 and PRECISE. In all cases, the initial structures were generated by performing molecular mechanics geometry optimizations using the MM2 force field,[64] as encoded in the POLYGRAF molecular mechanics code.[65] The AM1-minimized geometry showed the effect of the typically strong π–π interaction that in this case leads to more distortion of the pyrene rings from planarity than in the structures computed with MM2 level molecular mechanics. In the crystal, lattice-packing interactions could also alter the planarity of the molecules, in the opposite direction however.

The pyrenophanes, especially the [3.3] and [4.4] varieties, can exist in several conformations. The structures reported in Figure 14 are believed to represent the lowest energy conformers. Repeated annealing steps using molecular dynamics simulations were used to sample the conformation space and determine the lowest energy conformations. Only for the lowest energy conformers, based on the MM2 calculations, were the geometries optimized further with the semiempirical quantum mechanical methods. We cannot rule out the possibility that a higher energy MM2 conformation may have lower final quantum mechanical energy.

Absorption Spectra. Transannular π–π interactions are reflected in the absorption spectra of pyrenophanes by two characteristic features: a new broad featureless band appears in the 380–450-nm range and, compared to the absorption of pyrene or 2,7-dimethylpyrene (**9**), the bands corresponding to the ^1L_b and ^1L_a transitions are broadened and experience a bathochromic shift. These effects are most pronounced in the spectrum of [2.2](2,7)pyrenophane (Table 6).[60,66,67] They are reduced in the absorption spectrum of [3.3](2,7)pyrenophane.[60] For [4.4](2,7)-pyrenophane the onset of the absorption spectrum is shifted to shorter wavelengths (410 nm) and the vibrational structure is almost as sharp and pronounced as in the spectrum of the model compound.[60] Thus the absorption spectra reflect the decreasing transannular interactions in the series [2.2], [3.3], and [4.4]. Among

Table 6. Pyrenophane Structures and Spectral Properties

compound	UV absorption, nm (solvent)	Fluorescence, nm, (solvent, T)	ref
2,7-dimethylpyrene (9)	277, 308, 322, 338, 358, 376 (dioxane)	387 (MTHF, 1.3 K) 387, 490 (MTHF, room temperature)	60
[2.2](2,7)pyrenophane (1)	277, 314, 328, 380–430 (dioxane)	556 (MTHF, 1.3 K) 540 (sol, room temperature), 568 (crystal)	67
[2.2](2,7)pyrenophanediene (2)	327, 370–420 (THF)	510 (sol, room temperature)	66
[3.3](2,7)pyrenophane (3)	271, 314, 328, 368, 382 sh (dioxane)	515 (MTHF, 1.3 K) 510 (crystal)	60
[4.4](2,7)pyrenophane (4)	273, 314, 327, 342, 365 sh, 380 (dioxane)	470 (MTHF, 1.3 K) 481 (crystal)	60
[2.2](1,8)pyrenophane (5)	336, 386 sh (THF)	410 (room temperature)	68
[2.2](1,3)pyrenophane (6)	350, 365, ca. 390, sh (THF)	475 (CH_3CN)	69
[2.2](1,6)pyrenophane (7)	336, 370–430 (THF)	440–450 (THF, room temperature)	68
[2.2](1,6)(2,7)pyrenophane (8)	336, 380–430 (THF)	480–505 (THF, room temperature)	68

the other known isomeric pyrenophanes linked by two ethylene bridges, the effects are severe in the spectra of the [2.2](1,6) and [2.2](1,6)(2,7).[68] In contrast the [2.2](1,8)[68] and [2.2](1,3)[69] isomers exhibit only small bathochromic shifts and band broadening, compared to pyrene. Hence the interaction between the π-electronic systems is relieved in these isomers.

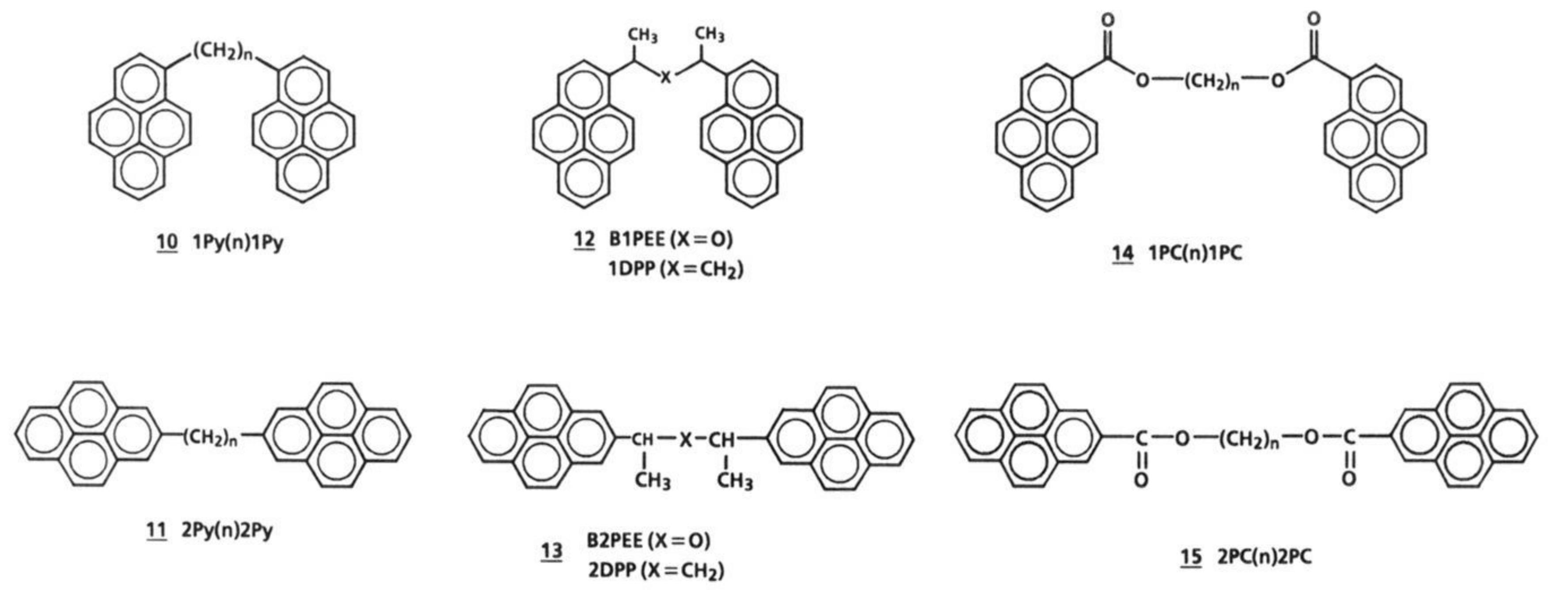
Emission Spectra. Structural and orientational effects are differentiated very clearly in the emission spectra of the pyrenophanes. Fluorescence spectra of [2.2](2,7)-, [3.3](2,7)-, and [4.4](2,7)pyrenophane in methyltetrahydrofuran (MTHF) solutions and glasses (1.3 K) show the broad structureless emission characteristic of the pyrene excimer, with no contribution from locally excited pyrene. Within this series the excimer emission undergoes a bathochromic shift from 470 nm [[4.4](2,7)][60] to 560 nm [[2.2](2,7)],[65] reflecting the increase in transannular π–π interactions. The spectrum of the [2.2](1,6)(2,7) isomer also exhibits only excimer emission in MTHF glass and room temperature solution. On the basis of limited data it appears, however, that under identical conditions the spectra of the [2.2](1,8) and [2.2](1,6) isomers exhibit only emission from locally excited pyrene. Note that the emission of 2,7-dimethylpyrene in a MTHF glass of identical chromophore concentration also exhibits only pyrene monomer emission. At room temperature more concentrated solutions of 2,7-dimethylpyrene display an excimer emission centered at 490 nm in addition to pyrene monomer emission. The red shift of the excimer emission in [2.2]phanes, with respect to the corresponding monomers, has been observed also in naphthalenophanes,[70] diphenylophanes,[71] fluorenophanes,[67] and phenanthrenophanes.[72] Among them, it is the pyrene excimer that experiences the most pronounced bathochromic shift.[73]

A marked solvent-dependence is displayed by the emission of [2.2](1,3)pyrenophane.[69] In nonpolar solvents, such as methylcyclohexane the fluorescence originates entirely from locally excited pyrene at all temperatures. In polar solvents, such as acetonitrile and acetone, an intense excimer emission centered at 475 nm is observed in addition to pyrene monomer emission. The dynamic nature of this excimer was postulated from the fact that identical excitation spectra were recorded for the excimer and monomer emissions.[69] Also, the ratio of monomer to excimer emission quantum yields exhibits a marked temperature dependence. For example in acetone at −58 °C the excimer emission is precluded. Note however that in time-resolved experiments (nanosecond time scale) the excimer profile was fitted to a single exponential decay law, with no detectable rising component. The formation of an intramolecular excimer in [2.2](1,3)pyrenophane requires an anti–syn isomerization to occur during the lifetime of the excited pyrene. This isomerization is very difficult on the ground-state surface. The barrier to ring inversion in [2.2]metacyclophane was estimated to be greater than 26–28 kcal/mol^{-1}, from temperature-dependent ^{1}H NMR studies.[74] Hayashi and co-workers[69] suggest that in polar solvents this barrier for isomerization is overcome by transannular interactions in a charge transfer state formed upon pyrene excitation.

2. *Bis-pyrenyl Compounds*

The bis-pyrenyl compounds discussed in this section are compounds in which the two chromophores are linked by a short flexible chain. In solutions of these materials, the local concentration of the chromophores is greatly enhanced, compared to monochromophoric derivatives, since they are not distributed randomly, as would be the case in solutions of isolated chromophores. Therefore intramolecular excimer emission from bichromophoric compounds is detected at very low overall chromophore concentration (ca. 10^{-5} mol L^{-1}). The extent of excimer emission is limited by the probability for a molecule to reach, within the lifetime of the excited state, a conformation suitable for excimer formation and by the stabilization of the excimer.[75] Conformational aspects of the entire molecule have to be considered, as discussed for example in reviews by De Schryver[75,76] and in a comprehensive study of the excimer emission from the diastereomers of *meso*- and *rac*-2,4-di(1-pyrenyl)pentane (**12**, X = CH_2).[78] When two identical chromophores are linked by three sp^3 carbons in a propylene chain or by a C–O–C bond in the ether analogs (**12**, X = O), the conformational change of the backbone chain is always faster than the excimer-forming step, except in molecules having conformers where the two chromophores already overlap in the ground state.[76] If the chromophore is nonsymmetrically substituted, as for example in the case of 1-pyrenyl derivatives, more than one excimer can be formed. This is a consequence of the existence of several rotational isomers (rotamers) around the C–C bond that links the chromophore to the chain ends.

This unfavorable circumstance has hampered the detailed and quantitative analysis of the wealth of data accumulated in studies of molecules end-substituted with 1-pyrenyl chromophores. These constraints are



10 1Py(n)1Py

12 B1PEE (X=O) 1DPP (X=CH2)

14 1PC(n)1PC

11 2Py(n)2Py

13 B2PEE (X=O) 2DPP (X=CH2)

15 2PC(n)2PC

Figure 15. Chemical structures of bis-pyrenyl compounds.

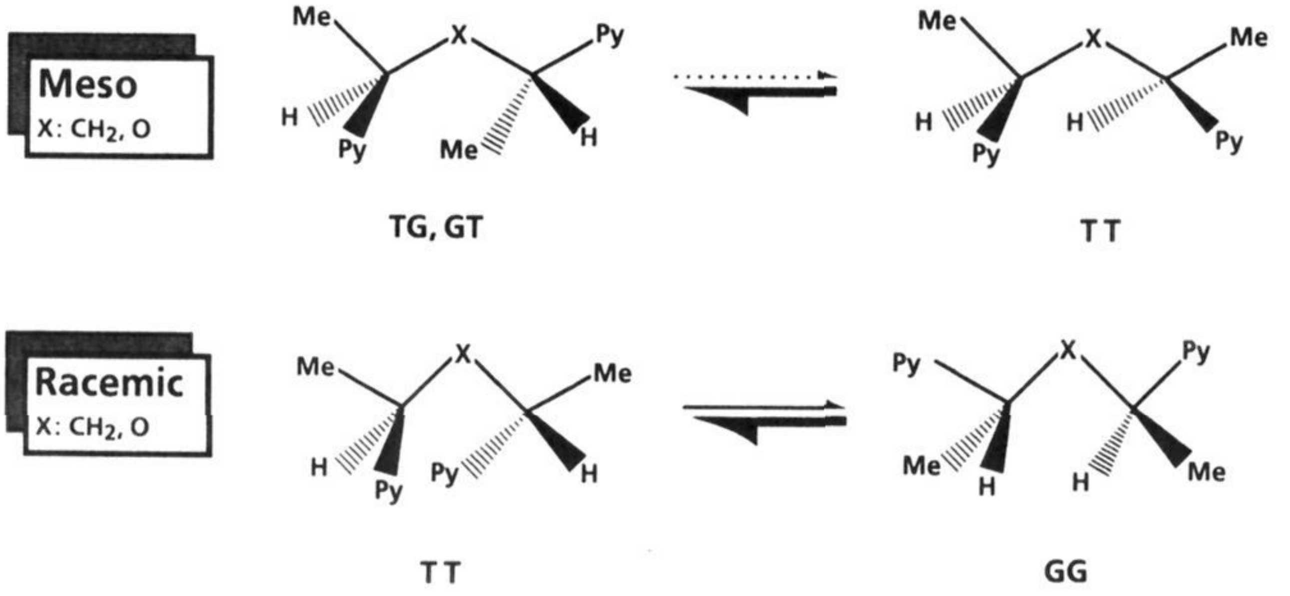


Figure 16. Most stable conformers of meso and racemic B2PEE.

relieved when the chain ends carry a symmetrical chromophore, such as a 2-pyrenyl group. All the rotamers around the C–C bond linking the C_2-pyrenyl carbon and the chain ends are equivalent. For each chain conformer, excimers formed by the various rotamers are indistinguishable spectroscopically. Therefore spectroscopic measurements performed with these derivatives generate data that can be analyzed with confidence in great detail. The synthesis and purification of bis(2-pyrenyl) molecules have long presented a challenge to organic chemists. Only recently have they become available in a few laboratories. In a number of these compounds stable pyrene dimers have been detected in solution. As described next, this conclusion was reached primarily by analysis of solution ^{1}H NMR spectra. It was corroborated by spectroscopic results as well.

i. Analytical Tools. *^{1}H NMR Data.* The analysis of ^{1}H NMR data is based on the assumption that ring current effects generated by the dimer will perturb the chemical shifts of the aromatic protons. Comparison of the chemical shifts in bichromophoric compounds to those of the corresponding aromatic protons in monomeric model compounds and in pyrenophanes will yield evidence for chromophore proximity. In extremely favorable cases Zachariasse and co-workers were able to extract from the data the mole fraction of ground-state dimers formed in solution.[79]

Electronic Spectra. Ground-state dimer formation in solutions of bis(2-pyrenyl) compounds was detected by steady-state measurements of excitation spectra, time-resolved fluorescence measurements carried out on the nanosecond time scale, and picosecond time-resolved absorption spectroscopy.

ii. Specific Examples (see Figure 15). *Bis[1-(2-pyrenyl)ethyl] Ether (B2PEE, **13**, X = O) and 2,4-Di-2-pyrenylpentane (2DPP, **13**, X = CH_2).* The most stable conformers of the meso- and racemic 2DPP and B2PEE (Figure 16) were estimated from measurements of the vicinal coupling constants of the methylene and methine protons in the ^{1}H NMR spectra of $CDCl_3$ or toluene-d_8 solutions.[80] The racemic derivatives exist mostly in two conformations: the predominant conformer is in the TT conformation (60–70 mol %), the GG conformer is the second most stable isomer. The symbol T indicates a methyl group between two hydrogens of the methylene group, the symbol G indicates a pyrenyl group between the two methylene groups (Figure 16). For meso compounds the TG/GT conformation is the most stable at room temperature. Only small amounts of the TT conformer are present.

Emission spectra of solutions of racemic 2DPP and B2PEE exhibit a significant excimer conformation (λ_E = 480 nm). Excimer rise times range from ca. 15 ns (2DPP) to ca. 20 ns (B2PEE). The excimer contribution to the total emission is much stronger in the case of the meso derivatives, indicating a much more efficient excimer formation process than in the case of the racemic derivatives. Time-resolved measurements indicate that in the meso compounds the excimer formation is completed within 1 ns, 180–300 ps for the excimer rise time,[81] which corresponds to the time required for a 120° rotation of the backbone chain (TG/GT $\rightleftharpoons$ TT isomerization). A contribution to the total emission of an excimer formed via excitation of a ground-state pyrene dimer (TT conformer) was detected in time-resolved emission measurements and by picosecond transient absorption spectroscopy. The lower limit of its mole fraction in 2DPP solutions (ca. 10% at room temperature) was calculated from ^{1}H NMR data and fluorescence decay profiles.[79]

Bis(2-pyrenylcarboxy)alkanes (2PC(n)2PC, ***15****).*[20] Zachariasse and his co-workers studied a large series of α,ω-bis(2-pyrenylcarboxy)alkanes. Evidence for stable intramolecular pyrene dimers was obtained for 2PC(n)2PC with n = 3–16, 22, and 32. This conclusion was based on the observation in the ^{1}H NMR spectra of shieldings of all the aromatic protons with respect to model 2-substituted pyrenes. These shieldings were attributed to ring current effects in conformers in which the pyrenylcarboxy groups adopt a dimer configuration. For 2PC(n)2PC with $n > 8$, the dimer was assigned a symmetrical sandwich structure, whereas with n = 3 to 8, the dimers were attributed a geometry in which the pyrenyl moieties are shifted along their long axis. A comparison of specific chemical shift differences (e.g. $\Delta\delta(H_4)$) for 2PC(n)2PC with those for [4.4]-(2,7)pyrenophane allowed values to be estimated for the molar fractions of preformed dimers. For n = 10, at 24 °C, in $CDCl_3$ about 6% of the molecules exist in conformations with preformed pyrene dimers. For n = 3 to 8, this fraction was judged to be higher. Existence of preformed pyrene dimers in these solutions was confirmed by monitoring the photophysics of the excimer.[80] Particularly revealing were (1) the dependence of the excimer emission on the excitation wavelength (2PC(3)2PC) and (2) the time-dependent excimer rise and decay profiles.

D. Pyrene in Ultrathin Organic Films

The Langmuir–Blodgett (LB) technique provides a unique approach to achieve supramolecular architectures of layered assemblies of suitably designed amphiphilic molecules, including long-chain carboxylic acids (fatty acids), phospholipids, and macromolecules.[82] In the course of the LB film preparation process, the amphiphilic molecules are spread as a solution on the air–water interface of a Langmuir trough. With the evaporation of the solvent, they rapidly form islands of two-dimensional structures which are then compressed by the action of a barrier into a two dimensional continuous solid. For many studies this layer is then transferred onto a solid substrate.[83] The technique has grown into a powerful and versatile method to produce supramolecular architectures useful to test molecularly-controlled processes, especially the photophysics of chromophores experiencing restricted motional freedom. Pyrene excimer formation in LB assemblies has been investigated over the past 10 years in several laboratories. In most systems the pyrene group was linked covalently to an amphiphilic molecules: a fatty acid, a phospholipid, or a macromolecule. The labeled amphiphile was then incorporated within an organized layer during the LB process.

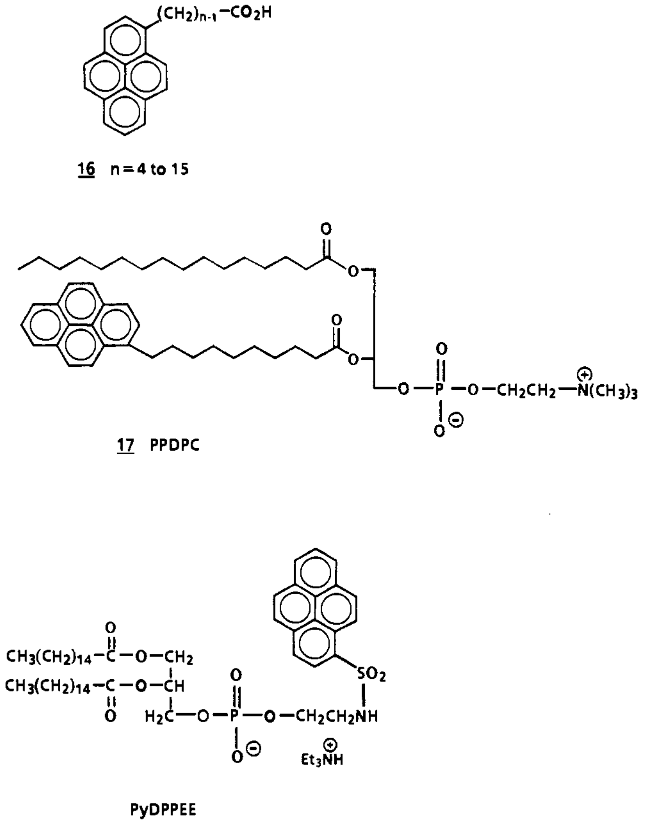


Figure 17. Chemical structures of pyrene-labeled fatty acids and phospholipids.

1. Pyrene-Labeled Fatty Acids

End-labeled n-alkylcarboxylic acids $Py(CH_2)_{n-1}CO_2H$, n = 10, 12, and 16 (see Figure 17 and Table 7) have been incorporated into mixed monolayers of single- or double-chained amphiphiles. Among the early experiments are those reported by Subramanian and Patterson,[84] on the fluorescence of 10-(1-pyrenyl)decanoic acid (PyDecA, **16**, n = 10) incorporated into a monolayer of a phospholipid, such as distearoylphosphatidylcholine (DSPC). Probe to phospholipid molar ratios ranged from 1:3 to 1:1. In these mixed compressed monolayers a strong pyrene excimer emission was observed at the air/water interface. The excimer has a very short rise time, faster than the detection limit of the experimental setup (ca. 1 ns) and a decay time ranging from 18 to 33 ns, depending on the PyDecA mol fraction in the monolayer. The same authors also reported related experiments performed on mixed monolayers of 12-(1-pyrenyl)dodecanoic acid (PDA, **16**, n = 12) and 4-(1-dodecyl)aniline.[85] Pyrene excimer emission from the monolayer was monitored as a function of surface pressure. The spectroscopic data offer evidence for the gradual formation, with increasing surface pressure, of a ground-state species emitting around 460 nm, attributed either to pyrene aggregates among chromophores of two or more PDA molecules or to PDA/4-(1-dodecylaniline) complexes.[86]

Yamazaki and co-workers reported a detailed analysis of the spectroscopy of 16-(1-pyrenyl)hexadecanoic acid (PHA, **16**, n = 15) introduced into a stearic acid monolayer by the LB two-step procedure: first, a small amount of PHA was spread on a water surface in the presence of stearic acid and compressed; then, this layer was transferred onto a solid support.[87] Several layers

Table 7. Pyrene-Labeled Fatty Acids and Phospholipids

abbreviation	no.	name*	ref(s)
PBA	16 (n = 3)	4-(1-pyrenyl)butanoic acid	101
PPA	16 (n = 4)	5-(1-pyrenyl)pentanoic acid	101
PDecA	16 (n = 9)	10-(1-pyrenyl)decanoic acid	84
PDA	16 (n = 11)	12-(1-pyrenyl)dodecanoic acid	85, 86
PHA	16 (n = 15)	16-(1-pyrenyl)hexadecanoic acid	87
PSA		pyrenesulfonic acid	102
PTAB		[4-(1-pyrenyl)butyl]trimethyl ammonium bromide	97
DPy		1-dodecylpyrene	100
PPHPC	**17**	1-palmitoyl-2-[(1-pyrenyl)hexanoyl]phosphatidylcholine	88, 89
PPDPC	**18**	1-palmitoyl-2-[(1-pyrenyl)decanoyl]phosphatidylcholine	88–90
DPDPC	**19**	bis[10-(1-pyrenyl)decanoyl]phosphatidylcholine	88, 89
PyDPPE	**20**	*N*-(1-pyrenesulfonyl)dipalmitoyl-L-α-phosphatidyl-ethanolamine, triethylammonium salt	103, 104

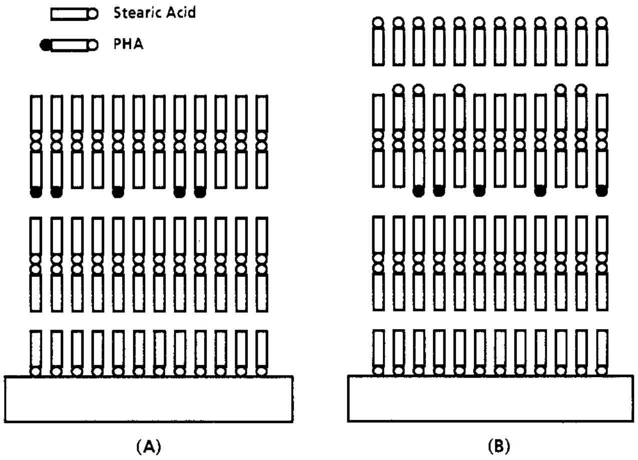


Figure 18. Schematic illustration of the multilayer structure of the LB films of PHA in stearic acid. Reprinted from ref 87. Copyright 1987 American Chemical Society.

were deposited on a quartz plate in the order depicted in Figure 18: (i) five layers of the calcium salt of stearic acid; (ii) a monolayer or a bilayer consisting of stearic acid and small amounts of PHA (0.7–26 mol %); and (iii) a monolayer of stearic acid. Under steady-state measurement conditions, these assemblies exhibit a strong excimer emission, of increasing intensity, relative to the monomer emission, with increasing PHA concentration in the stearic acid layer. Excitation spectra monitored for the monomer emission (397 nm) and for the excimer emission (474 nm) are clearly different. Bands in the excimer excitation spectrum are red-shifted and broader than those of the monomer excitation spectrum.

Yamazaki et al. report in their publication a detailed analysis of picosecond and nanosecond time-resolved spectra measured with this system.[87] These spectra were analyzed as a sum of four components originating from (1) in the short-time domain (0–200 ps), a structured emission (381, 401, 422 nm) (see Table 8) attributed to the fluorescence of an excited ground-state dimer; (2) after ca. 1 ns, a broad, featureless emission centered at 470 nm, attributed to a dynamic excimer (E_2); (3) in the 100–200-ps time domain, a structured emission (377, 397, 421 nm) attributed to the emission of locally-excited pyrene; and (4) in the 100 ps to 10 ns time frame a broad, featureless emission centered at 420 nm attributed to another excimer (E_1). This comprehensive study led to the following description of the structure of a PHA-stearic acid monolayer: within a layer, the pyrenes exist either isolated or as dimers or higher aggregates, the respective amount of each domain depending on the PHA concentration. In the aggregated domains, two types of excimers form: a sandwich-type excimer and a partial overlap excimer formed within 200 ps after excitation.

2. Pyrene-Labeled Phospholipids

Phospholipid LB films containing small amounts of a double-chain pyrenyl amphiphile (PPDPC (**17**) see Figure 17 and Table 7) were examined at the N_2/water interface by Bohorquez and Patterson,[88] and after transfer onto a solid substrate by Finnish researchers.[89] The time-resolved spectroscopy of PPDPC in a dioleoylphosphatidylcholine layer (probe to lipid molar ratios of 1:7 and 1:3) at the N_2/water interface indicated that at room temperature excimer formation is diffusion controlled, independent of the surface pressure. There was no indication of the existence of preformed pyrene dimers. This result contrasts with the situation in a monolayer of the same phospholipid doped with the pyrenyl fatty acid PDA. This single-chain compound undergoes a high degree of chromophore association among the labeled fatty acids.

LB films of dipalmitoylphosphatidylcholine containing varying proportions of PPDPC or a doubly-labeled phospholipid DPDPC (**19**, see Table 7) were examined after transfer onto glass slides. Films prepared with PPDPC exhibited properties characteristic of diffusion-controlled excimer formation at probe to lipid molar ratios as high as 0.67. The rise time of the excimer was short at this level of incorporation (ca. 2.8 ns). By contrast, in films prepared under identical conditions but doped with the dipyrenyl phospholipid DPDPC, extensive chromophore association was observed at probe to lipid molar ratios as low as 0.167. The issue of whether the chromophore aggregation is inter- or intramolecular was not addressed in this report. In a recent publication the same group reports conditions under which films containing the singly-labeled phospholipid PPDPC also show evidence for pyrene/pyrene aggregates, which upon excitation undergo either self-quenching or excimer-like emission.[90] Under these conditions aggregation must involve chromophores brought into close proximity within clusters of several PPDPC molecules.

3. Pyrene-Labeled Polymers

"Hairy-rod" polymers form molecular assemblies which mimic the architecture of LB films of surfactants and phospholipids (Figure 19).[91] Their design, synthesis, and use to prepare LB structures are an active

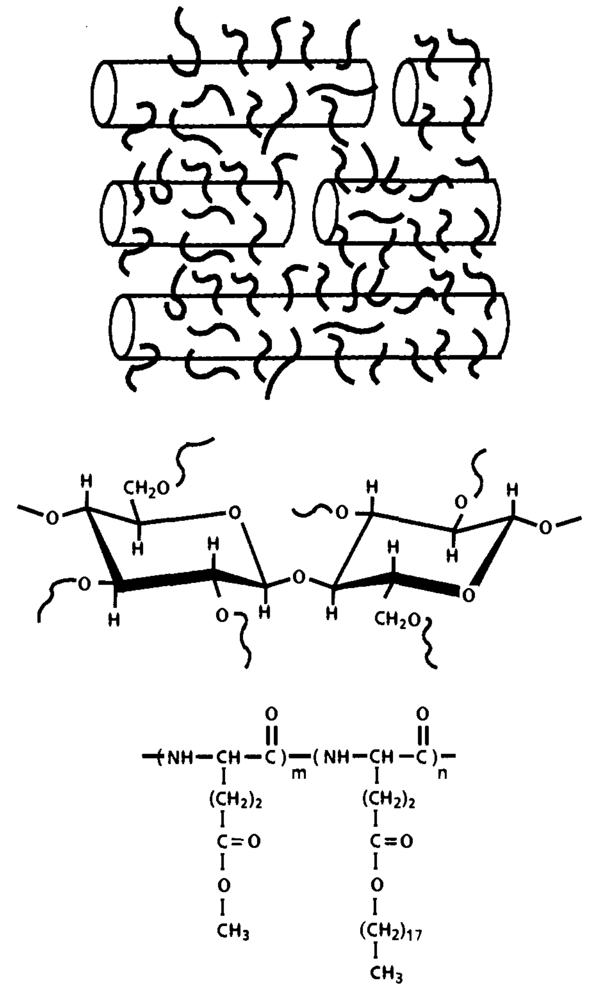
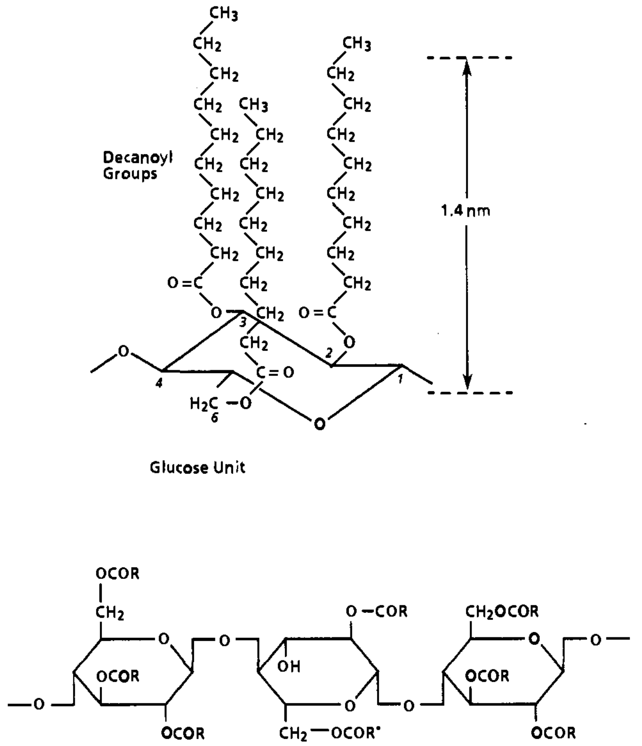


Figure 19. Examples of molecular structures of "hairy-rod" polymers (from ref 91).

area of research, since it was suggested by Orthmann and Wegner[92] that they might impart exceptional structural stability to ultrathin films. Cellulose is an intrinsically stiff macromolecule. If functionalized with alkoxy groups it becomes an amphiphilic "hairy-rod" polymer soluble in common organic solvents. Cellulose tri(*n*-alkanoates), such as cellulose (*n*-decanoate) (CDT, Figure 20), forms homogeneous monolayer films in the LB process.[93] The homogeneity of monolayered and multilayered COT LB films was investigated by fluorescence measurements. Films were prepared with pyrene-labeled cellulose (*n*-octanoates) (COT-Py, Figure 19) in which a small fraction of octadecyl groups was replaced by 4-(1-pyrenyl)butyl moieties. All the polymers had an overall degree of substitution (DS) of 3 (i.e., no free hydroxyl groups), with the number of pyrene groups per glucose (DS_{Py}) in the various samples ranging from 0.007 to 1.1.[94,95]



CDT R = R' = C_9H_{19}

COT R = R' = $C_{17}H_{35}$

COT-Py R = $C_{17}H_{35}$ R' = $(CH_2)_3$-Py

Figure 20. Molecular structure of pyrene-labeled cellulose trialkanoates and schematic molecular model of a glucose unit.

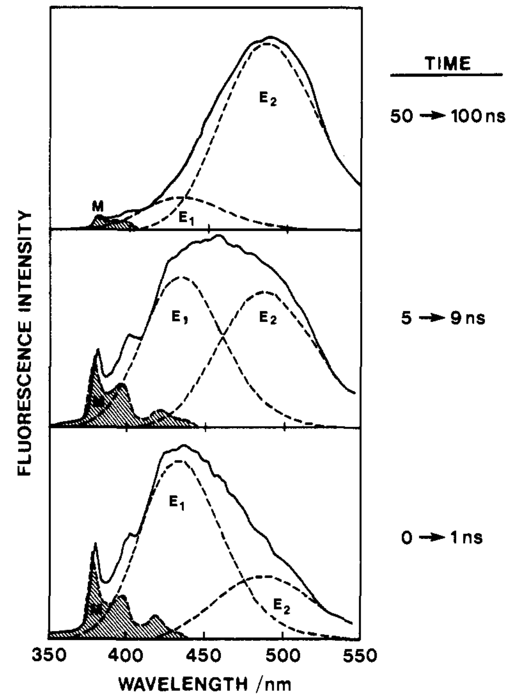


Figure 21. Time-resolved fluorescence spectra of a 2-Layer Film of COT-Py. Reprinted from ref 95. Copyright 1992 American Chemical Society.

LB films assembled with the most highly labeled polymer exhibited intense pyrene excimer emission, almost to the exclusion of monomer contribution. Steady-state emission spectra were recorded both at the air/water interface and after transfer onto a quartz support. They were identical and showed no evidence for pyrene ground-state aggregation. However analysis of films by time-resolved fluorescence spectroscopy uncovered the formation of two types of excimers (Figure 21): in the short time domain after excitation, a blue-shifted (E_1) excimer with maximum at 430 nm, and after ca. 6 ns an excimer E_2, with maximum emission at 480 nm, corresponding to the excimer emission observed under steady-state conditions. By analogy with the analysis of PHA in stearic acid monolayers, Tsujii et al.[95] assigned the emission E_2 to a sandwichtype

Table 8. **Band Positions in the Time-Resolved and Steady-State Fluorescence Spectra of 16-(1-Pyrenyl)hexadecanoic Acid (PHA, 16, n = 15) in Stearic Acid LB Films and of CTO-Py LB Films**

	PHA[a]		CTO-Py[b]	
emissive species	time domain	band positions, nm	time domain	band positions, nm
	t = 0–200 ps		t = 0–1 ns	
dimer (D)		381, 401, 422		377
excimer E_1		420		430
excimer E_2		470		480
	t > 10 ns		t > 50 ns	
monomer (M)		377, 397, 421		377
excimer E_2		470		480
	steady state		steady state	
monomer (M)		377, 397, 421		
excimer		470		470

[a] λ_{exc} = 337 nm; PHA, 3.6 mol % in PHA.[87] [b] λ_{exc} = 330 nm.[95]

excimer and E_1 to an excimer with partial overlap (Table 8). At the high level of substitution the blue excimer may result from a proximity effect rather than some specific association between the pyrenes. Note that the Kyoto research group reported the fluorescence properties of another "hairy-rod" polymer, pyrene-labeled poly(vinyloctal) but they detected no evidence for pyrene aggregation in films made with this material.[96]

E. Pyrene in Micelles and Vesicles

The pyrene chromophore is frequently used as a probe to measure properties of surfactant micelles,[97] phospholipid vesicles,[98] and surfactant/polymers aggregates,[99] For most applications it is desirable to achieve molecular solubilization of pyrene in a given system, i.e. conditions where pyrene groups do not exist as dimers or aggregates. It is not surprising therefore that there are very few reported cases of "static pyrene excimer" in micellar solutions and in liposomes. The practitioners in this field are well aware of the importance of taking into account nonrandom probe distribution in micelles and of the often unexpectedly slow kinetics associated with the dissolution process for hydrophobic probes which aggregate readily in water. Clear evidence for pyrene ground-state aggregation in micellar systems has been provided in a study of sodium dodecyl sulfate micelles doped with the hydrophobic 1-dodecylpyrene, for probes to micelle ratios lower than 0.01.[100] Even ionic pyrenyl derivatives can form aggregates in water in the presence of surfactants at concentrations lower than the critical micelle concentration. Examples of this situation are given in studies of sodium 5-(1-pyrenyl)pentanoate,[101] 1-pyrenesulfonic acid,[102] and sodium 4-(1-pyrenyl)butanoate[97] in the presence of cetyltrimethylammonium chloride, and of [4-(1-pyrenyl)butyl]methylammonium bromide and sodium dodecyl sulfate.[97] Chromophore aggregation has been noted when pyrene-substituted phospholipids are incorporated into liposomes[103,104] or cell membranes.[105]

V. Survey of Pyrene-Labeled Water-Soluble Polymers

Since the work of Tazuke[1,2] on anthryl-labeled polyionenes, there has been a growing number of reports on the spectroscopy of hydrophobic chromophores in aqueous solutions of dye-labeled polymers. This section is a compendium of pyrene-labeled polymers which, when dissolved in water, exhibit the spectral features characteristic of pyrene pre-association. The objectives here are (1) to outline the various approaches used in the preparation of pyrene-labeled polymers; (2) to survey the structures of these polymers; and (3) to relate the spectroscopy of the label to the solution properties of these polymers in water, with emphasis on the driving forces for pyrene aggregation and on the structure of the hydrophobic microdomains the chromophores generate upon association.

A. Synthesis of Labeled Polymers

There are three possible approaches to prepare labeled polymers. A dye-substituted monomer may be added as a comonomer to a polymerization mixture, to yield a randomly labeled copolymer. An unlabeled polymer may be modified by reaction with a dye-substituted reagent, either randomly along the chain or at specific sites. A specially synthesized "reactive polymer" may be treated with an appropriate derivative of a fluorescent dye. All three approaches have been employed to synthesize labeled polymers of interest in this review.

1. Copolymerization of a Dye-Substituted Monomer

Most pyrene-labeled polymers discussed in this review were prepared by copolymerization. They include polyelectrolytes, such as pyrene-labeled polyacrylic acid, prepared by free-radical copolymerization in DMF of acrylic acid and 2-[[4-(1-pyrenyl)butanoyl]amino]propenoic acid,[106] (1-pyrenyl)acrylic acid,[107] or 1-vinylpyrene,[108] as well as neutral polymers, such as pyrene-labeled hydrophobically modified poly(N-isopropylacrylamides).[109] In these syntheses the level of label incorporation is dictated by the initial monomer feeds and the reactivity ratios of the various comonomers.

2. Polymer Postmodification

By this technique, commercial or natural polymers can be labeled, often without significant changes in molecular weight or molecular weight distribution. In addition it is sometimes possible to link the dyes to specific sites on the polymers, for example to one or both end groups. Chemical reactions employed to prepare labeled polymers by this route include:

Ether Formation. This reaction is applicable if the polymer to be labeled has free hydroxyl groups and if it is stable to strong bases. A polymer is treated with a strong base to generate alkoxides and subsequently reacted with a dye bearing a primary alkyl halide or a tosylate substituent. The technique has been employed to label polysaccharides, such as hydroxypropyl cellulose (HPC),[23] and poly(ethylene oxide).[110]

Ester Formation. The reaction has been employed to label olefin–maleic anhydride copolymers[111] and hydroxylated polymers bearing hydroxyl end groups, such as poly(ethylene oxide).[112]

Amide Formation. This reaction has been useful to modify copolymers of maleic anhydride and alkyl vinyl ethers.[113]

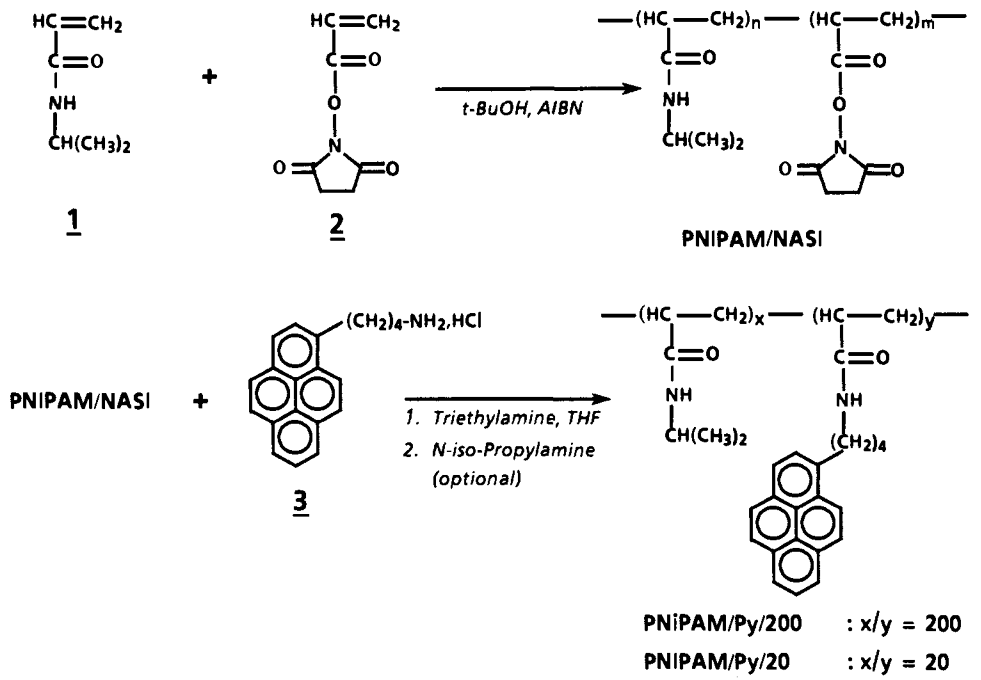
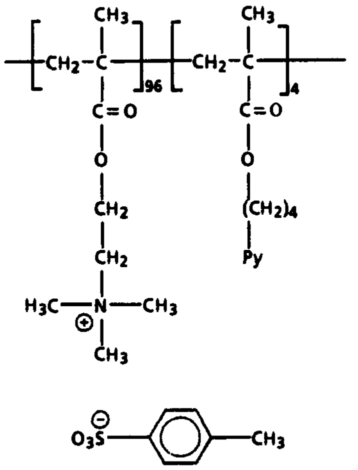


Figure 22. Synthetic scheme for the preparation of pyrene-labeled poly(*N*-isopropylacrylamide) (PNIPAM-Py) starting from a reactive polymer. Reprinted from ref 113. Copyright 1975 American Chemical Society.

Chart 1. Structures of the Pyrene-Labeled Polyelectrolytes 18 and 19 (from ref 118)



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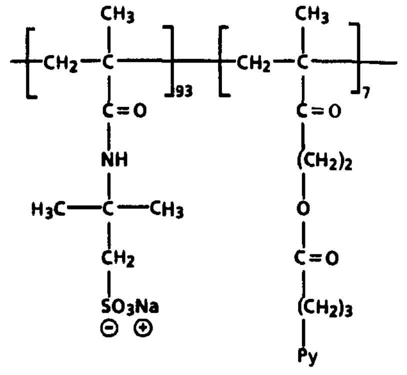

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3. Labeling of a "Reactive" Polymer

This approach allows one to label polymers having no functionality readily amenable to reacting with dye derivatives. The idea is to prepare a polymer carrying at random a small number of "reactive" functional groups. These are sites where fluorescent groups can be introduced. The reactive groups should be stable enough to allow storage of the polymer under normal conditions. The technique is illustrated here in the case of pyrene-labeled poly(*N*-isopropylacrylamide) (PNIPAM-Py, Figure 22).[114] This polymer was prepared by reaction of 4-(1-pyrenyl)butylamine with a random copolymer of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide.[115,116] By varying the initial ratio of amine to reactive polymer it was possible to synthesize PNIPAM-Py samples with different amounts of pyrene incorporation. After all the dye-labeled amine had been consumed, the polymer was treated with excess isopropyl amine to convert residual unreacted *N*-oxysuccinimide groups to *N*-isopropylamide groups.

The main advantage of the method over the direct copolymerization of dye-substituted monomers is that it eliminates possible interferences of the chromophores during the polymerization. Aromatic derivatives often act as chain-transfer agents. Also, from a single reactive polymer it is possible to prepare polymers, identical with respect to their molecular characteristics, but labeled with different chromophores or with various amounts of the same chromophore.

B. Labeled Polyelectrolytes

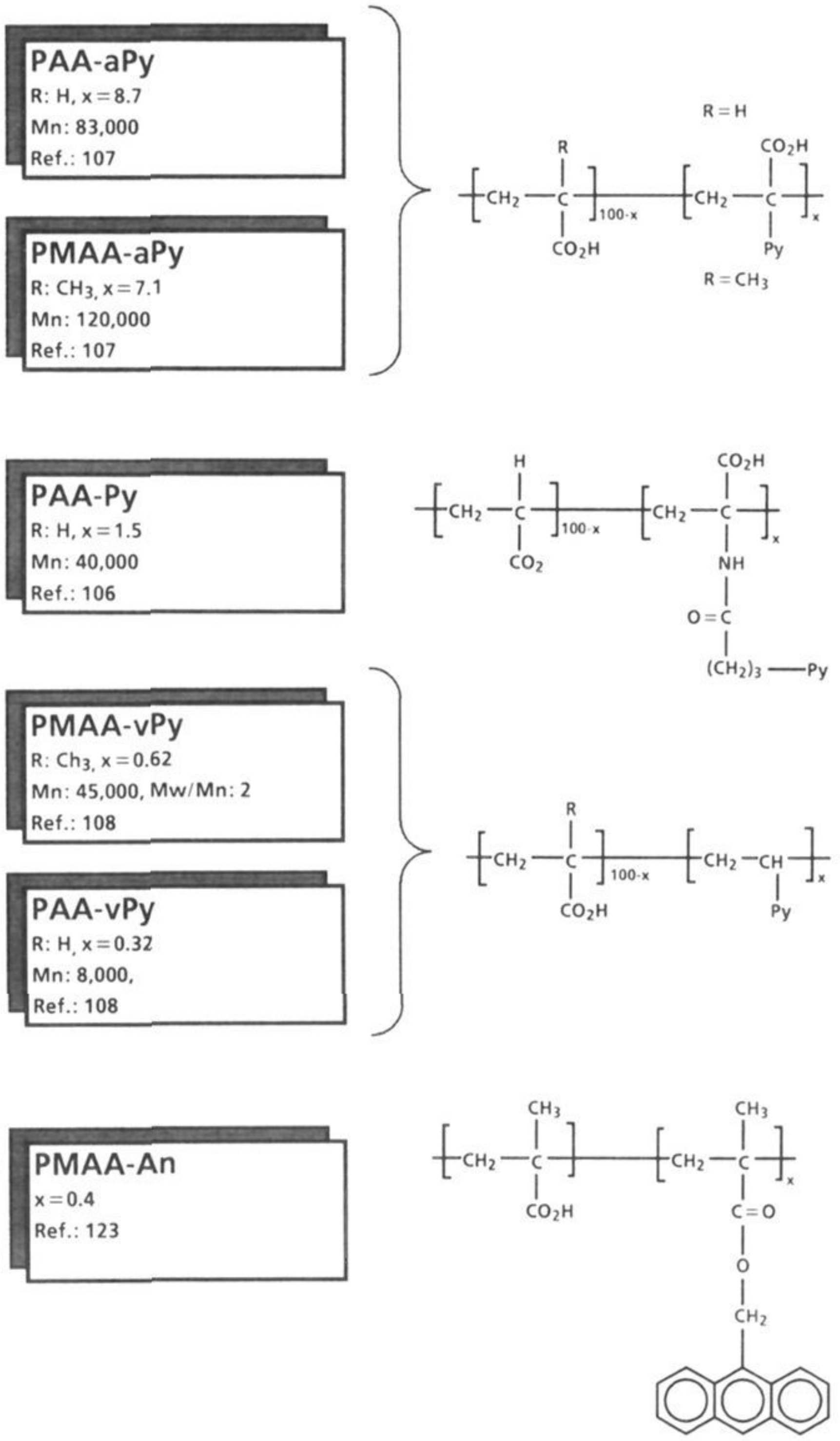
The first recognition of the unusual formation of stable pyrene aggregates in dilute polymeric solution was reported by Herkstroeter et al.[117] in a study of aqueous solutions of two pyrene-labeled polyelectrolytes (see Chart 1), a polycation and a polyanion, each carrying about 4 mol % of pyrene linked at random to the main chain via short alkyl chains. The Kodak group described in detail how pyrene aggregation caused by hydrophobic interactions among chromophores is detectable spectroscopically (1) by a loss of resolution in the absorption and excitation spectra concomitant with small red-shifts, and (2) by a strong excimer fluorescence at pyrene concentrations (ca. 1 × 10^{-5} mol L^{-1}) well below the minimum concentration required for excimer formation in solutions of free pyrene. They suggested that the ground-state interactions must be weak, since they are easily destroyed by the addition of organic solvents, such as methanol, that can provide solvation for the pyrene substituents. From concentration studies showing that the ratio of excimer to monomer emission (I_E/I_M) is insensitive to dilution with water, they concluded that the aggregation must occur among pyrenes attached to the same polyelectrolyte chain coiled upon itself. In this early study no time-dependent measurements were performed. No further data has been reported on these specific polymers.

1. Poly(acrylic acid) (PAA) and Poly(methacrylic acid) (PMAA)

There are reports on three structurally different samples of pyrene-labeled poly(acrylic acid) and poly(methacrylic acid). Their structures, compositions, and molecular weights are listed in Chart 2. Random distribution of the label along the chain was assumed in all cases. The conformation of polycarboxylic acids exhibits a well-documented pH dependence: at low pH, the polymers adopt a coiled conformation, and at high pH the chains expand as a result of intrapolymeric electrostatic repulsion.[118] Upon addition of base PAA expands smoothly with increasing density of ionic charges along the polymer chain. The dimensions of PMAA undergo little change in the initial stages of ionization. With continuing increase in the degree of ionization of the carboxyl groups, there is an abrupt transition to a highly expanded state, within a relatively narrow critical pH range. The resistance to chain expansion in the case of PMAA has been attributed to hydrophobic attractive interactions of the α-methyl groups which resist the uncoiling of the polymer.

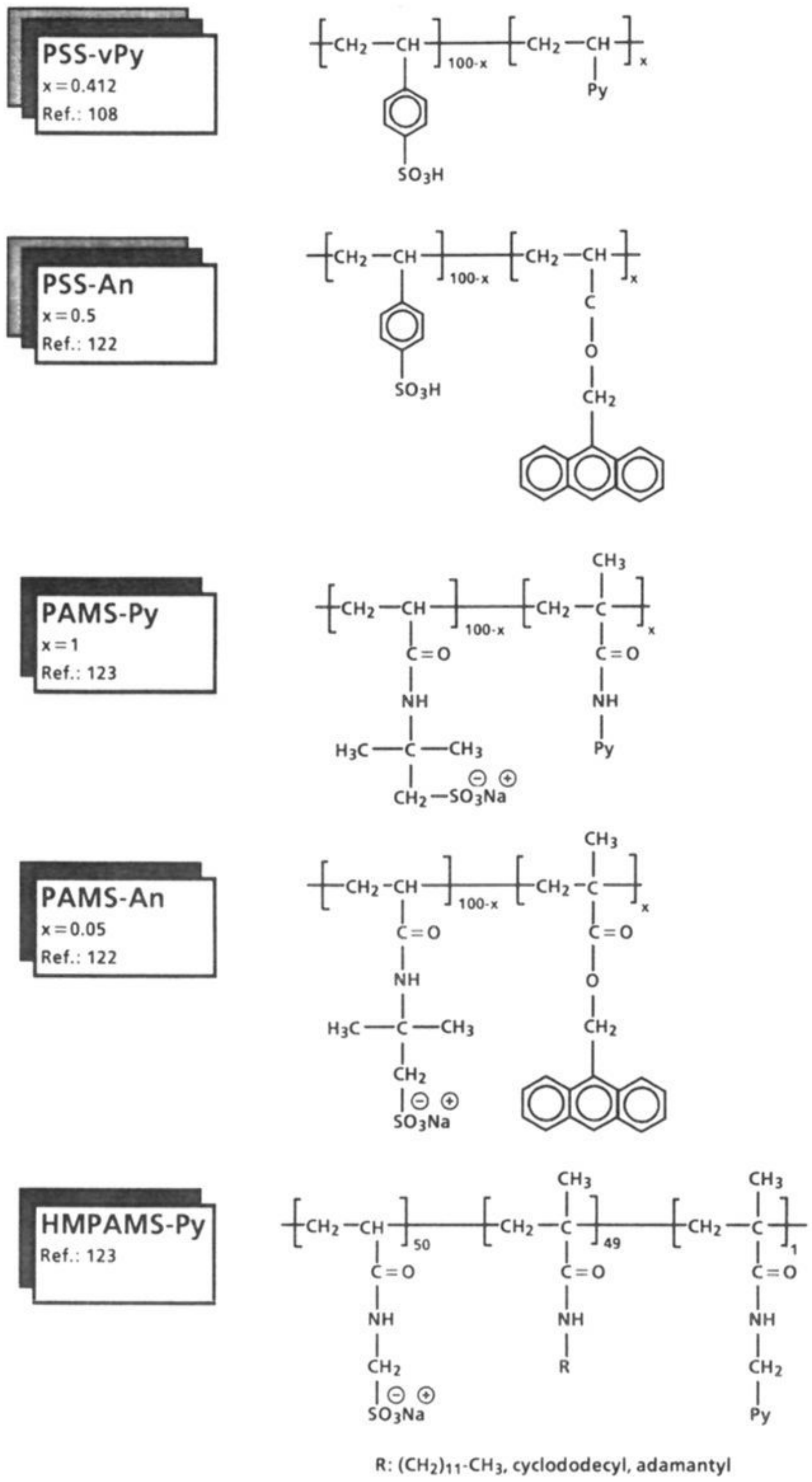
Turro and Arora observed pyrene preassociation in acidic solutions of the labeled poly(acrylic acid) PAA-Py.[106] In aqueous solution the pyrene absorption spectrum is broadened (P_A = 1.6, pH 5.3) compared to that of the polymer in methanol (P_A = 2.6). For the polymer in water the excimer emission is strong (I_E/I_M = 0.90, λ_E = 479 nm), and the excitation spectra viewed at the monomer and excimer emissions are different (P_M = 1.9, P_E = 1.37). The pyrene aggregates undergo significant dissociation upon neutralization of the polymer, as seen, for example, by the decrease in pyrene excimer emission (I_E/I_M = 0.2 at pH 10). The pH-dependent behavior of solutions of PAA-Py is in fact more complicated than one might anticipate. A plot of I_E/I_M against pH showed three distinct regions: a moderate decrease in I_E/I_M in the pH range 3–5.5, followed by a sharp decrease in I_E/I_M (pH 5.5–7.5) and finally a moderate decrease in the 7.5–10 pH domain. The residual excimer emission at high pH was attributed to the interactions among those pyrenes attached to the chain in close proximity. Further studies with the same polymer demonstrate that the pyrene–pyrene aggregates are disrupted by the addition of substances, such as polymers,[119] surfactants,[120] and colloidal alumina,[121] which interact with either the polymer or the pyrene substituents.

Chart 2. Structure, Composition, and Physical Characteristics of Labeled Poly(acrylic acids) and Poly(methacrylic acids)



Chu and Thomas prepared labeled poly(acrylic acid) (PAA-aPy, Chart 2) and poly(methacrylic acid) (PMAA-aPy, Chart 2) by copolymerization of 1-pyrenylacrylic acid with acrylic acid and methacrylic acid, respectively.[107] Solutions of PAA-aPy and of PMAA-aPy did not exhibit any pyrene excimer fluorescence over the entire pH range, in spite of the fact that the polymers carried a larger number of pyrenes (expressed in monomer unit molar fraction) than the polymer PAA-Py studied by Turro and Arora. The polymers prepared in the two laboratories are structurally different: in the Turro and Arora sample, the pyrenes are linked to the backbone via a 5-atom chain, whereas in the Chu and Thomas samples the chromophores are linked directly, by one carbon–carbon bond, to the main chain. In Webber's group pyrene-labeled poly(acrylic acid) (PAA-vPy, Chart 2) and poly(methacrylic acid) (PMMA-vPy, Chart 2) were obtained by copolymerization of 1-vinylpyrene with acrylic acid and methacrylic acid, respectively.[108] Polymers with a very low level of pyrene incorporation were studied. In water these polymers exhibited a weak excimer emission. Stramel et al.[108] ascertained that this emission arises from a dynamic process, on the basis of the similarity of the excitation

Chart 3. Structure, Composition, and Physical Characteristics of Labeled Poly(styrenesulfonate), Poly(acrylamido-2-methylpropanesulfonates), and Hydrophobically-Modified Poly(acrylamido-2-methylpropanesulfonates)



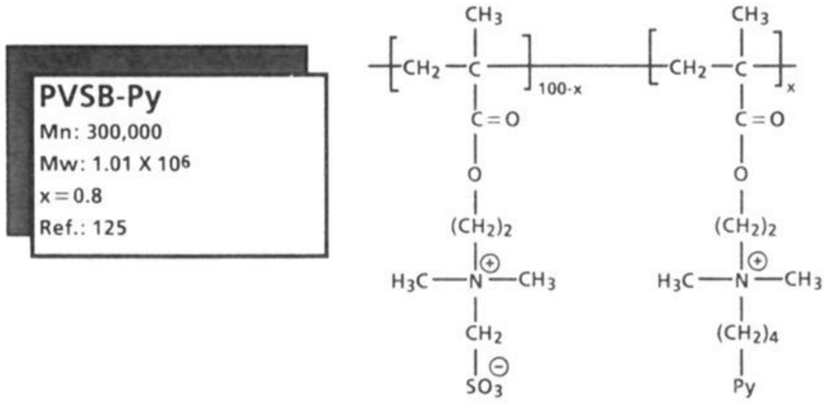
spectra at observation wavelengths of 380 (monomer) and 490 nm (excimer).

2. Polysulfonates

Webber's group reported the synthesis and solution properties of a pyrene-labeled poly(styrene sulfonate) (PSS-vPy, Chart 3).[108] No pyrene excimer was detected. Anthryl-labeled polysulfonates were examined recently by Itoh et al.[122] They prepared labeled samples of poly(styrene sulfonate) (PSS-An, Chart 3), poly(methacrylic acid) (PMAA-An, Chart 2), and poly(acrylamidopropanesulfonic acid) (PAPMS-An, Chart 3) carrying, at random, along the chain low levels (<0.5 mol %) of 9-anthryl groups. Under no circumstances did these polymers exhibit excimer fluorescence.

Morishima et al.[123] prepared a series of pyrene-labeled amphiphilic copolymers of 2-acrylamido-2-methylpropanesulfonic acid (AMPS). Terpolymers of 50 mol % AMPS, 1 mol % 1-(1-pyrenylmethyl)methacrylamide, and 49 mol % *n*-dodecyl-, or cyclodecyl-, or adamantylmethacrylamide were synthesized (HMPAMS-Py, Chart 3). A copolymer of 99 mol % AMPS and 1 mol % (1-pyrenylmethyl)methacrylamide (PAMS-Py, Chart 3) was prepared also to serve as a reference. All the polymers were soluble in water, as well as in a few polar organic solvents, such as methanol, *N*,*N*-dimethylformamide, and dimethyl sulfoxide. The emission spectra of the polymers in water exhibit pyrene monomer emission to the exclusion of excimer emission. The absence of excimer emission in solutions of the terpolymers was taken as an indication that the chromophores are isolated from each other in the hydrophobic microdomains formed by the hydrophobic substituents. The authors cite the low degree of label incorporation to account for the absence of excimer emission from aqueous solutions of the reference polymer, PAMS-Py. They report spectroscopic differences between this polymer and the amphiphilic terpolymers: the pyrene fluorescence intensity is lower in PAMS-Py; the monomer emission fine structure indicates a more polar environment for pyrene attached to PAMS-Py; and the band maxima are blue-shifted by 2–3 nm in the absorption spectrum of PAMPS-Py, compared to the spectra of pyrene in the amphiphilic copolymers.

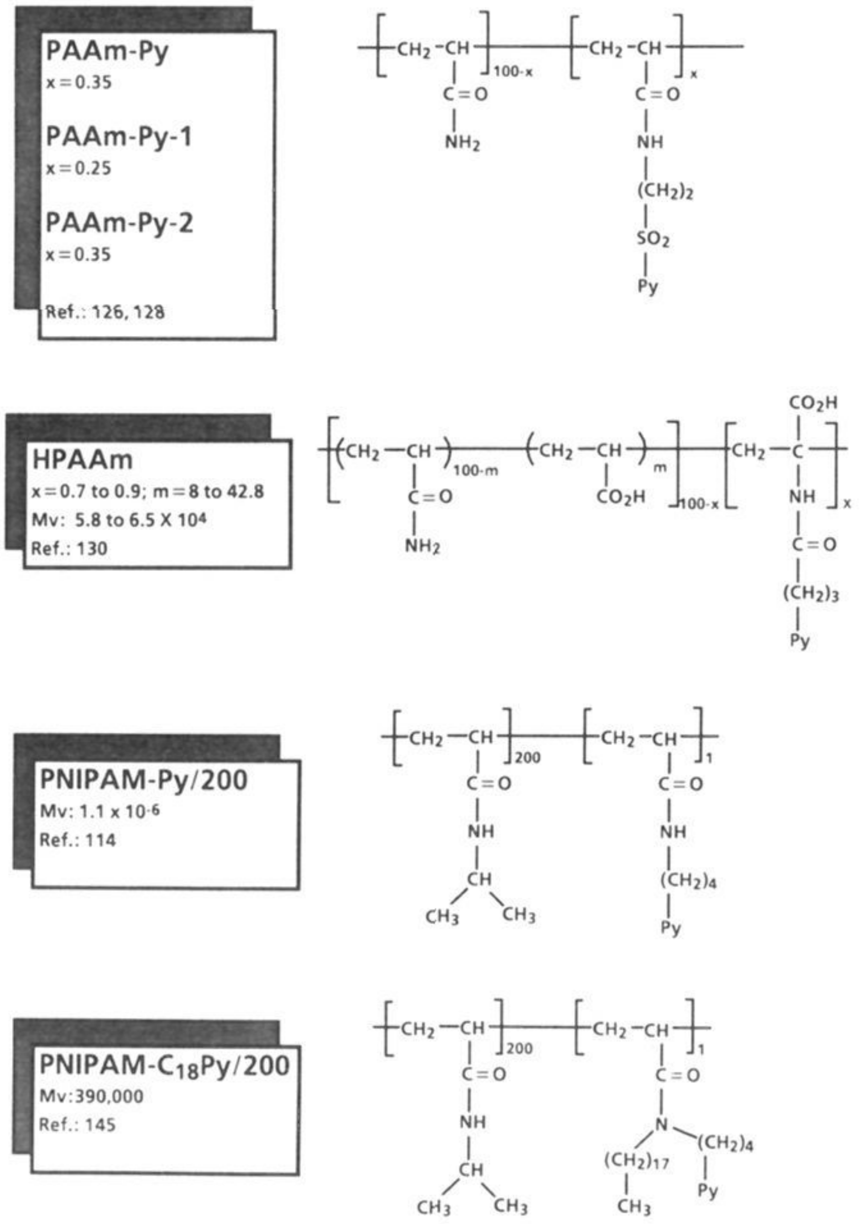
Chart 4. Structure, Composition, and Physical Characteristics of Pyrene-Labeled Poly(vinylsulfobetaines)



3. Poly(vinylsulfobetaines)

Poly(vinylsulfobetaines) are insoluble in water as a result of strong intra- and intermolecular Coulombic interactions.[124] The polymers, however, are soluble in salt solutions, as a consequence, it is believed, of chain expansion. To probe this phenomenon, Wielema and Engberts prepared a pyrene-labeled sulfobetaine (PVSB-Py, Chart 4). The polymer was obtained by free-radical copolymerization of *N*,*N*-dimethyl[2-(methacryloxy)ethyl][1-(2-sulfoethyl)ammonium betaine and *N*,*N*-dimethyl[2-(methacryloxy)ethyl][4-(1-pyrenyl)butyl]ammonium betaine.[125] The level of pyrene incorporation on the polymer was kept low (ca. 0.8 mol %) in an attempt not to perturb substantially the solution behavior of the polymer. Surprisingly, though, the labeled polymer was soluble in water and, upon addition of incremental amounts of salt, it first precipitated, then at a sufficiently high salt concentration, it went into solution again. The emission of the polymer was monitored during this process. In a solution of the polymer in water, the fluorescence was dominated by the pyrene monomer contribution ($I_E/I_M = 0.08$). The ratio I_E/I_M exhibited a mild dependence on salt concentration, but Wielema and Engberts were not able to correlate the small changes in relative monomer and excimer emissions to the macroscopic

Chart 5. Structure, Composition, and Physical Characteristics of Pyrene-Labeled Poly(acrylamides), Partially-Hydrolyzed Poly(acrylamides), and Poly(*N*-isopropylacrylamides)



behavior of the polymer in solution. Also in this publication no evidence is given to suggest that the excimer may originate from preassociated pyrenes.

4. Polyacrylamide and Partially-Hydrolyzed Polyacrylamides

McCormick, Hoyle, and co-workers have carried out extensive studies on pyrene-labeled polyacrylamides (PAAm-Py, see Chart 5), prepared by free-radical copolymerizations of acrylamide and *N*-(1-pyrenylsulfonamido)ethylacrylamide.[126] One copolymer, PAAm-Py-1, labeled with 0.25 mol % pyrene, was prepared by a "micellar" technique[127] in the presence of sodium dodecyl sulfate. A second sample, PAAm-Py-2, containing 0.35 mol % pyrene, was obtained under homogeneous conditions in a water/DMF solution. The emission spectra of both samples in water present an excimer contribution, in addition to a strong pyrene monomer fluorescence.[128] For both polymers the excimer emission originates from pyrene aggregates, as established from measurements of excitation spectra and time-dependent emission profiles: i.e. excitation spectra viewed for the monomer and excimer emission were different and the excimer time-dependent profiles showed no rising component, on the nanosecond time scale. There are however significant differences between the two samples. Most noteworthy is the substantial enhancement of the excimer emission relative to monomer emission in PAAm-Py-1 (I_E/I_M = 0.54, polymer concentration: 2.2×10^{-4} g L^{-1}) compared to PAAm-Py-2 ($I_E/I_M = 0.22$, polymer concentration 1.9×10^{-4} g L^{-1}). The authors attributed this difference to a greater local concentration of pyrene in an aqueous solution of PAAm-Py-1, relative to a solution of PAAm-Py-2 of equal chromophore concentration.[128] They concluded that the copolymer prepared in a micellar system has a "blocky" microstructure with short sequences enriched in pyrene-substituted monomer, while in the copolymer prepared under homogeneous conditions the distribution of labels along the polymer chain is more uniform. Ezzell et al. also conducted a series of rheological measurements with solutions of the two polymers.[126] The study revealed major differences between the macroscopic properties of the two polymers. Associative thickening behavior was observed in the copolymer obtained in the surfactant-polymerized sample, but not in PAAm-Py-2.

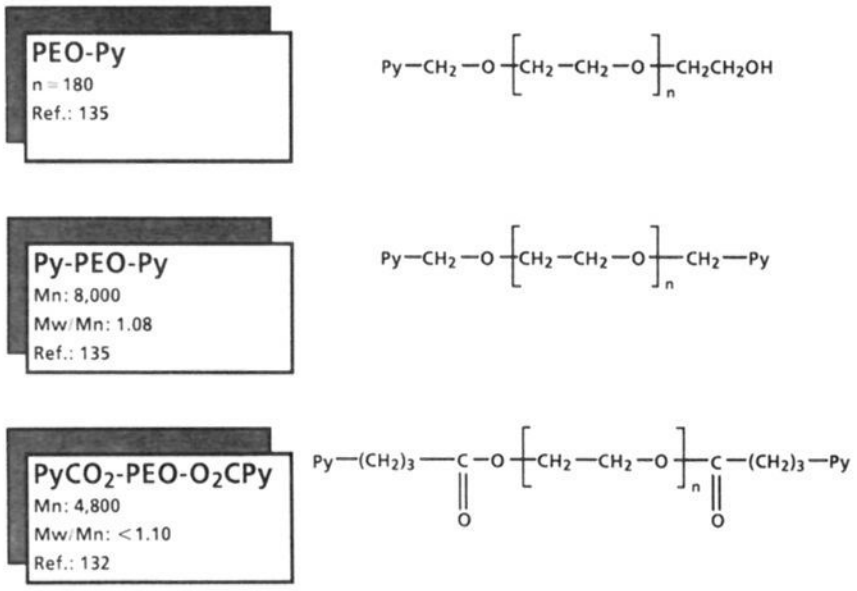
Poly(acrylamide) in water behaves as a neutral polymer, as long as the pH is kept below 10. In stronger alkaline medium the amide groups undergo hydrolysis and the polymer behaves as a polyelectrolyte. In fact, hydrolysis of a few amide groups along the chain is difficult to prevent. Most commercial samples contain a small number of carboxyl substituents (up to 4%).[129] These groups endow technical PAAm with the properties of a polyelectrolyte. Partially hydrolyzed PAAm (HPAAm, Chart 5) has solution properties different from those of "virgin" PAAm. Sivadasan et al. have reported a study of the consequences of this partial hydrolysis on the fluorescence properties of a pyrene-labeled PAAm (HPAAm-Py, Chart 5).[130] They prepared this polymer by solution copolymerization of acrylamide and a few mol % (ca. 0.2 mol %) of 2-[4-(1-pyrenyl)butanoyl]amino]propenoic acid. The polymer in water exhibited a small excimer emission in its fluorescence spectrum (I_E/I_M ca. 0.15 at pH 4). While the authors in their publications acknowledge the fact that excimer emission from pyrene in aqueous solutions may originate from pyrene aggregates and/or be formed by the classic Birks mechanism, they do not probe this aspect of the excimer emission of PAAm-Py. Changes in the ratio I_E/I_M are reported as a function of pH and of polymeric additives for solutions of PAAm-Py as well as several samples of partially hydrolyzed poly(acrylamides).

C. Pyrene-Labeled Nonionic Water-Soluble Polymers

1. Poly(ethylene oxide)

End-labeled poly(ethylene oxide) samples carrying a pyrene at both chain ends or specifically at one end only have been prepared, either via esterification of the terminal hydroxyl groups with 1-pyrenylbutanoic acid ($PyCO_2$-PEO-O_2CPy, Chart 6)[131-133] or via etherification of the hydroxyl groups with 1-pyrenylmethyl groups (PEO-Py, Py-PEO-Py, Chart 6).[110] Polymers of various molecular weights (M_n 200–9200) were used as starting materials, and samples of narrow molecular weight distributions were selected in all cases. The study of labeled poly(ethylene oxides) in water was prompted by the observation of an anomalous end-to-end cyclization behavior of this polymer in water and in methanol. Researchers in the group of M. Winnik

Chart 6. Structure, Composition, and Physical Characteristics of Pyrene-Labeled Poly(ethylene oxides)



measured the ratio I_E/I_M of a sample of Py-CO_2-PEO-O_2C-Py.[134] They were able to correlate the ratio with the inverse solvent viscosity for the polymer in many solvents, as expected for a diffusion-controlled process. For solutions of the polymer in water and methanol unexpectedly *high* I_E/I_M values were recorded.

The unusual end-to-end cyclization process of pyrene end-capped poly(ethylene oxide) in water was examined subsequently by Frank and co-workers.[112] They determined that in water a fraction of the pyrene excimer emission originates from preformed aggregates.[112] Excitation spectra monitored for the monomer and excimer emission were clearly different. The excimer time-dependent profile exhibited a rising component (τ ca. 40 ns, $R = 7$), implying that a fraction (ca. 15%) of the excimers were formed via the classic Birks mechanism. A more exact method for determining the fraction of pyrene excimer originating from preformed aggregates was described by Duhamel et al. in a recent study of a dilabeled poly(ethylene oxide) Py-PEO-Py and a singly-labeled polymer PEO-Py.[135] The technique relies on the different sensitivity to fluorescence quenching of the free and preassociated pyrenes. This analysis of steady-state quenching experiments, using sodium iodide as a quencher, gave a value of 7% for the fraction of preassociated pyrene pairs in the case of Py-PEO-Py, M_n 8200.

Char and Frank[136] compared their cyclization fluorescence data to a "capture model" (Figure 23), which assumes that when the two pyrenes attached to the chain ends are sufficiently close, within twice the capture radius, R_c, they will form a dimer, yielding static excimer emission. This attraction is superimposed upon a Gaussian distribution of the chain ends, which is not exactly correct for PEO in a good solvent like water. The predictions of the model could be fitted to experimental data for three PyCO_2-PEO-O_2CPy samples of different molecular weights. They suggest that the range of the hydrophobic interaction is about 40 Å for the pyrene end groups. This distance is comparable to that of hydrophobic attractions observed for linear hydrocarbon chains, measured by a surface force apparatus.[137] For the interaction to span such a large distance it must involve the propagation of water restructuring by hydrophobic groups rather than a direct (e.g. van der Waals) attraction between the hydrophobic substituents.

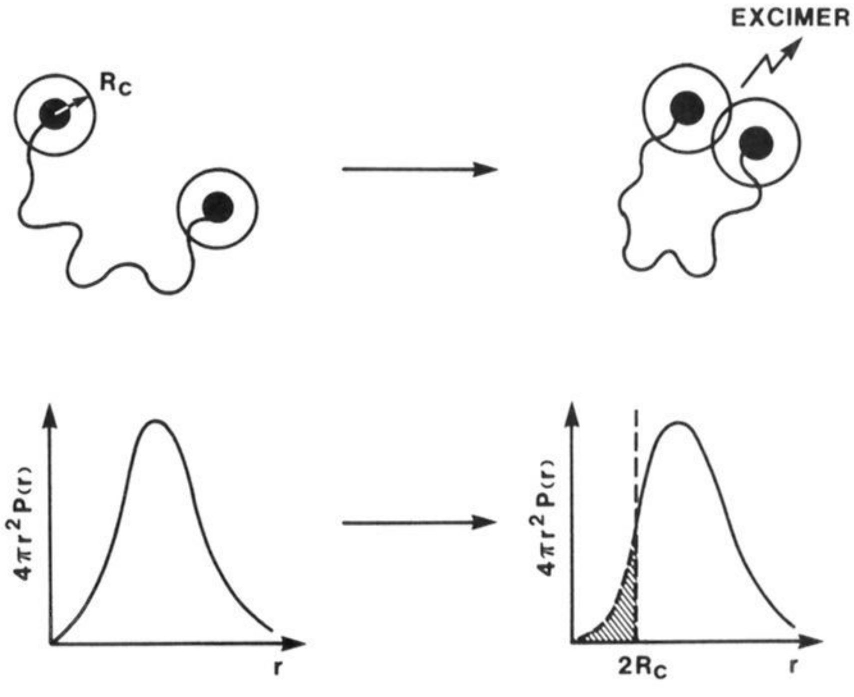


Figure 23. Schematic diagram of the "capture process" in the model of the Py-PEO-Py end-to-end cyclization process in water and the modified probability distribution function of the end-to-end distance. Reprinted from ref 136. Copyright 1989 American Chemical Society.

2. Poly(ethylene oxide)-Based Associative Thickeners

Poly(ethylene oxide) derivatives carrying hydrophobic substituents find industrial applications as associative thickeners, for example to control the rheology of a paint.[138] Usually the hydrophobic group is an alkyl or an alkylphenol chain linked to the PEO chain via a urethane group. A "model" associative thickener, PAT, in which pyrene groups serve as the terminal hydrophobic groups has been synthesized by a Rohm and Haas group.[139] It is comprised of PEO oligomers coupled through the reaction of PEO with a diisocyanate and end-capped with 1-pyrenylbutanol. Aqueous fluids containing this thickener have rheological properties similar to those of fluids modified with the corresponding commercial thickeners. The emission from the PAT pyrene end groups was monitored in several environments. Pyrene excimer emission increases with PAT concentration in water. The excimer emission grows in above a PAT concentration of 5×10^{-4} weight % and dominates the emission at a PAT concentration of 0.5 weight %. Details on the mechanism of pyrene excimer formation were not reported.

3. Cellulose Ethers

Hydroxypropyl cellulose (HPC) has been labeled with pyrene via etherification of hydroxyl groups with 4-(1-pyrenyl)butyl tosylate.[23] The label was attached randomly along the polymer backbone. This technique was applied to synthesize HPC-Py samples of different chromophore contents and to label other cellulose ethers, such as methyl cellulose and hydroxypropyl methyl cellulose (Tylose).[140] The spectroscopic properties of HPC-Py in water are discussed in detail in section II of this review for solutions kept at or below room temperature.

One of the most fascinating properties exhibited by many water-soluble polymers is a reverse temperature-dependent phase behavior. They form isotropic, one-phase systems in water at or below room temperature,

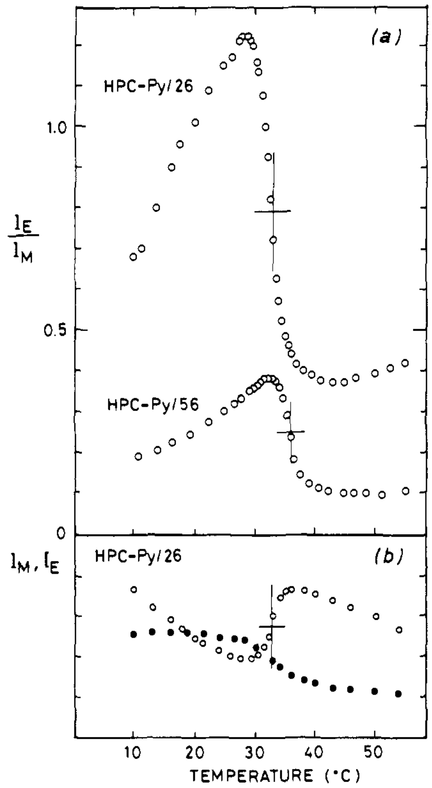


Figure 24. Temperature dependence of the fluorescence properties of aqueous solutions (26.4 ppm) of HPC-Py/26 and HPC-Py/56: (a) plots of the excimer (λ_{em} 470 nm) to monomer (λ_{em} 378 nm) emission intensity ratios (I_E/I_M); (b) plots of the changes in the monomer emission intensity (I_M, open circles) and excimer emission intensity (I_E, solid circles). Reprinted from ref 144. Copyright 1992 American Chemical Society.

but when heated above a critical temperature, often called the cloud point, they separate into two phases, as indicated visually by a sudden transition from clear solutions to opaque suspensions. The phase transition is reversible: cooling the suspension below the cloud point results in a sudden clarification of the two-phase system.[4b] Hydroxypropyl cellulose in water exhibits this phenomenon.

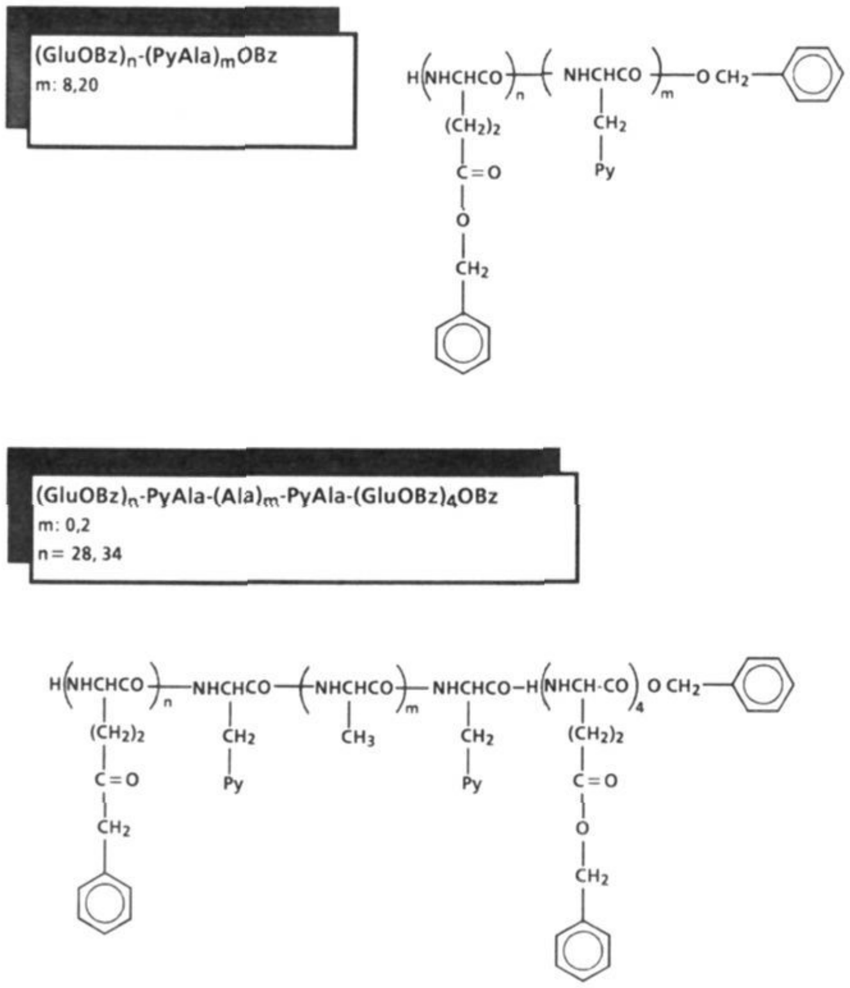
Attaching pyrene groups to HPC, even at a small level of substitution, affects the temperature at which phase transition occurs.[141] The lower critical solution temperature (LCST) of HPC-Py is lower by about 2 °C than that recorded for a solution of HPC of identical concentration (45 °C for HPC < 0.5 g L^{-1}).[142] These results are consistent with the general rule put forward by Taylor and Cerankowski that the LCST should decrease with increasing hydrophobicity of a polymer.[143] The spectroscopy of the pyrene excimer for HPC-Py in water is extremely temperature dependent. For example in the case of HPC-Py/26 the contribution of the excimer emission increases significantly from 0 to 29 °C, further heating results in an abrupt decrease of this emission which reaches a constant intensity for temperature higher than 46 °C.[143] A transition temperature can be obtained from the changes in I_E/I_M with temperature (Figure 24a). This value taken as the midpoint of the sigmoidal portion of the curve is almost identical to that obtained by turbidity determinations. Note that the sharp decrease in I_E/I_M reflects an increase in the absolute pyrene monomer emission at the expense of the excimer fluorescence intensity (Figure 24b). Above the phase-transition temperature the excitation spectra recorded for the monomer and the excimer emission are nearly identical: the maxima of the excimer excitation spectra are hardly shifted compared to those of the monomer excitation spectra. Hence, the steady-state data imply that the pyrene ground-state aggregates dissociate at the LCST. Cooling the solutions from 50 to 25 °C results in a recovery, after 24 h at 25 °C, of the properties exhibited by the solution prior to heat treatment, although the I_E/I_M ratios measured during the cooling scan are consistently smaller than those recorded during a heating scan.

Time-resolved fluorescence measurements from HPC-Py solutions below the LCST uncovered the contribution of two components, E_1 and E_2, to the excimer emission.[144] Each component exhibited a different response to the heat treatment, as shown in a series of time resolved spectra measured at 45 °C for solutions of HPC-Py/26. Most noteworthy was the observation that the spectra of samples above the LCST are strongly dominated in the short time scale (0–1 ns) by the emission of the monomer (M). The absence of emission from D and E_1 implies that at 45 °C the pyrene labels do not form aggregates susceptible to fast conversion into emissive species. However they are kept close enough to allow the formation of excimers by a dynamic process, as evidenced by the increase with time of the excimer E_2. Decay times for the monomer and the excimer emission do not show significant sensitivity to temperature. The excimer rise times, in contrast, vary markedly: they increase from ca. 250 ns at 25 °C to ca. 500 ns at 45 °C. This slowing-down of the excimer formation process may be ascribed either to an increase of the average separation distances between interacting pyrenes or to an increase in the microviscosity of the pyrene environment.

4. Poly(N-isopropylacrylamide)

Pyrene-labeled poly(*N*-isopropylacrylamides) (PNIPAM-Py, see Chart 5) with ca. 2 mol % (PNIPAM-Py/200) and ca. 20 mol % (PNIPAM-Py/20) of pyrene were prepared by reaction of [4-(1-pyrenyl)butyl]amine with a copolymer of *N*-isopropylacrylamide and *N*-(acryloxy)succinimide.[113] The emission of both polymers in water at room temperature was characterized by a strong excimer emission attributed mostly to excited ground-state pyrene aggregates. "Hydrophobically-modified" poly(*N*-isopropylacrylamides) were prepared by copolymerization of *N*-isopropylacrylamide and *N*-[4-(1-pyrenyl)butyl]-*N*-octadecylacrylamide.[145] In these polymers the pyrene label is linked to a tertiary amide nitrogen that carries also a long alkyl chain. These polymers have very different photophysical properties than PNIPAM-Py. Particularly relevant here is the spectroscopy of PNIPAM-C_{18}-Py/200 (see Chart 5), a polymer in which the level of pyrene incorporation is identical to that of PNIPAM-Py/200. In water this polymer has a fluorescence characterized by a very strong excimer emission (I_E/I_M 1.1, compared to 0.38 for PNIPAM-Py/200). The excimer emission offers all the spectroscopic characteristics of a diffusion-controlled mechanism. Neither steady-state nor time-resolved[146] measurements give any indication of a contribution from the an excimer originating from

Chart 7. Structure, Composition, and Physical Characteristics of Pyrene-Labeled Polynucleotides



preassociated pyrenes. It was concluded that the pyrene groups were located in the hydrophobic environment provided by assembled octadecyl groups either within a single polymer chain or among several polymer chains organized in larger interpolymeric micellar structures. Under these circumstances hydrophobic interactions between pyrene and alkyl groups are favored over pyrene–pyrene interactions, thus the driving force for pyrene association is lost.

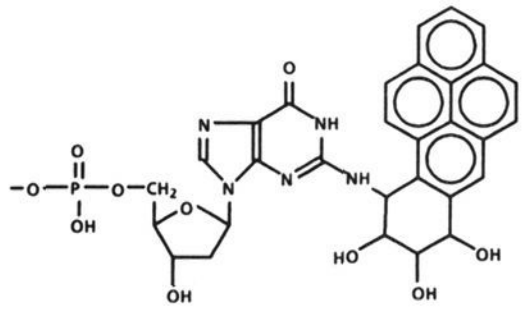
5. Polypeptides

Pyrene-labeled polypeptides were synthesized in the form of AB block copolymers, where A is a poly(δ-benzyl-DL-glutamate) chain and B a poly(1-pyrenylalanine) chain (see Chart 7).[147] Copolymers of both L and D,L-1-pyrenylalanine were examined. A significant hypochromicity in the absorption spectrum and strong circular dichroism (CD) Cotton bands for solutions of the copolymers in dimethylformamide (DMF) indicated ground-state interaction between the pyrene groups. The fluorescence spectra showed monomer and excimer emissions. The intensity of the excimer emission was dependent on the chirality of the 1-pyrenylalanine employed in the synthesis: the excimer emission was significantly lower in solutions of the copolymer prepared with 1-pyrenyl-L-alanine, compared to a solution of the copolymer obtained with D- and L-1-pyrenylalanine. Circularly polarized fluorescence spectra (CPF)[148] showed negative and positive dissymmetries in the excimer emission wavelength range. Dissymmetric CPF spectra are diagnostic of the presence of more than one emissive species.[149] Egusa et al.[147] attributed the dissymmetry observed in the CPF spectra of pyrene-labeled polyalanines to the presence of two kinds of excimer emissions: a nonpolar excimer (λ_E = 460 nm) of well-defined geometry and a polar excimer emitting at longer wavelengths (λ_E = 520 nm) which may exist in a variety of configurations and where the interchromophoric distance is longer than for the nonpolar excimer. The solvent dependence of the excimer emission of [2,2](1,3)pyrenophane (see ref 69, section IV) was quoted by Egusa et al. in support of their interpretation. Temperature-dependent measurements in the 3 to 60 °C range indicated that the total excimer quantum yield increases with temperature and that the relative contribution of the component emitting at 460 nm increases with temperature (Figure 25).

Sisido and Imanishi performed empirical energy calculations on helical poly(1-pyrenylalanine) to determine stable conformations.[150] They predicted the CD spectra of these conformations using the exciton model. Comparison of the profiles of the calculated CD spectra with the experimental spectrum suggested that the most probable helical conformation of poly(1-pyrenylalanine) is that of a left-handed α-helix-like chain. A polypeptide labeled with a pair of pyrenes linked to specific sites along the chain was synthesized by Inai et al. (see structure, Chart 7).[151] Fluorescence spectra of solutions of this polypeptide in DMF showed only a weak excimer emission, confirming that the helical polypeptide chain is rigid enough to fix large side-chain groups. No ground-state pyrene–pyrene interactions were detected in solutions of this polymer.[152]

6. Pyrene–DNA Adducts

An interesting case of pyrene preassociation was reported recently in a study of the adduct of benzo[*a*]pyrene-7,8-diol 9,10-epoxide (BPDE) to DNA.[153] The study was aimed at probing the mechanism of the mutagenicity and tumorigenic potency of the (+) enantiomer of *anti*-BPDE. The covalent adduct of (+)-BPDE with poly(dG-dC) (see structure **20**) exhibits fluorescence properties strikingly similar to those of pyrene attached as a label to synthetic water-soluble polymers. It is known that anti (+)-BPDE binds highly selectively to the exocyclic amino group of guanine.[154] Extensive formation of excimers therefore indicates favored covalent binding to bases closely to already modified guanines. Fluorescence excitation spectra and lifetime measurements revealed two populations of adducts: one that can form excimers and one that cannot. Isolated pyrenes exhibit a modest depolarization of their fluorescence, indicating the limited or slow mobility of chromophores linked to the DNA chain. Pyrene-labeled DNA oligomers and duplexes have been synthesized using chemically-modified bases and standard automated synthesis procedures.[155] Pyrene ground-state dimers were detected in these materials.



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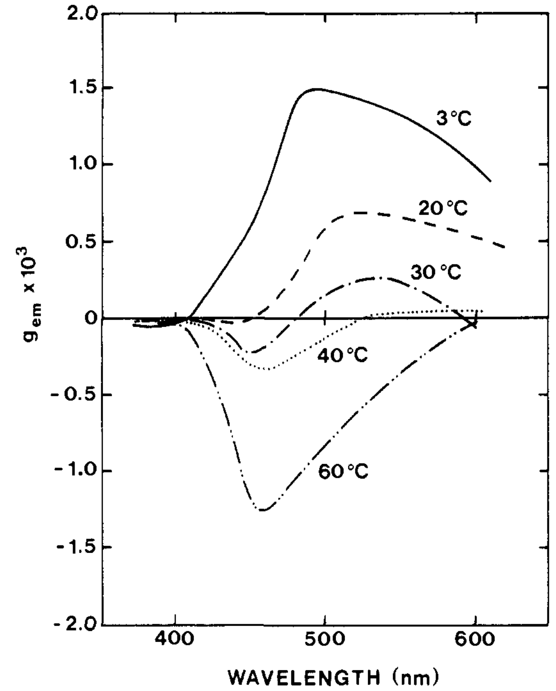

Figure 25. Circularly polarized fluorescence spectra of poly(1-PyAla)$_8$ in DMF at several temperatures; [Py] = 6 × 10^{-5}; λ_{exc} = 280 nm. Reprinted from ref 147. Copyright 1985 American Chemical Society.

VI. Summary and Outlook

The objective of this review has been to characterize the photophysics of the excimer originating from pyrene in organized systems. From this survey emerges a unified description of the spectroscopy of associated pyrenes, whether the association occurs in a pyrene crystal, in a single molecule, such as a pyrenophane or a bis-alkylpyrenyl derivative, in pyrene-doped monolayers, or in aqueous solutions of pyrene-labeled polymers. Key photophysical parameters useful in detecting pyrene preassociation were defined. Their application was illustrated with examples taken from the field of pyrene-labeled water-soluble polymers.

The emphasis of the review was placed on pyrene, but in an organized environment other chromophores, anthracene or naphthalene for example, exhibit similar spectroscopic features. Although this is a clear restriction, it is justified by the current widespread use of pyrene fluorescence as a tool in polymer science. Ground-state association of pyrene in dilute solutions of labeled polymers was perceived as an unusual occurrence less than 10 years ago. The number and variety of examples cited in this review are witnesses to the fact that the phenomenon is in fact quite common. Given the practical importance of hydrophobically-modified polymers, one can anticipate further activity in the use of pyrene as an aid to understand the properties of these polymeric systems.

VII. Abbreviations

Not included here are the photochemical symbols listed in Tables 1 and 2, the spectroscopic parameters defined in Table 3, and the abbreviations presented in Table 7.

Abbreviations

BPDE	benzo[*a*]pyrene-7,8-diol 9,10-epoxide
B2PEE	bis[1-(2-pyrenyl)ethyl] ether
CD	circular dichroism
CDT	cellulose *n*-decanoate
COT	cellulose *n*-octanoate
CPF	circularly polarized fluorescence
2DPP	2,4-di-2-pyrenylpentane
DS	degree of substitution (number of substituted hydroxyl groups of the sugar unit of polysaccharides)
HPC	hydroxypropyl cellulose
LB	Langmuir–Blodgett
LCST	lower critical solution temperature
MMA	methyl methacrylate
PAA	poly(acrylic acid)
PAMS	poly[2-(acrylamido)-2-methylpropanesulfonic acid]
PAPS	poly[(acrylamido)propanesulfonic acid]
PAT	pyrene-labeled associative thickener
2PC(n)2PC	bis(2-pyrenylcarboxy)alkanes
PEO	poly(ethylene oxide)
PMAA	poly(methacrylic acid)
PMMA	poly(methyl methacrylate)
PNIPAM	poly(*N*-isopropylacrylamide)
PS	poly(styrene)
PSS	poly(styrenesulfonic acid)
PVB	poly(vinylbutyral)
PVP	poly(vinylpyrene)
PVSB	poly(vinylsulfobetaine)
TIR	total internal reflectance

VIII. Acknowledgement

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