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Detection of hydrophobic microdomains in aqueous solutions of amphiphilic polymers using fluorescent molecular rotors

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Homopolymers and random copolymers derived from 3-vinylpyridium salts form compact coils in aqueous solutions. The resulting hydrophobic microdomains were characterized by both the hypsochromic shift of the wavelength emission band and the fluorescence quantum yield increase of a molecular rotor used as a fluorescence probe.

(Keywords: amphiphilic polymers; hydrophobic microdomains; molecular rotor; fluorescence spectroscopy)

Introduction

In the last few years many physicochemical studies have been undertaken on molecular organized systems acting as hosts for hydrophobic molecules, in order to highlight the structure of these assemblies[1,2]. Among the techniques encountered in the literature, fluorescence spectroscopy with pyrene as a probe has been more particularly used in the case of amphiphilic polymers in aqueous media to provide, due to modifications of the molecular local environment of the chromophore, useful information about the change of the macromolecule conformation (e.g., formation of hydrophobic microdomains)[3–7]. Unfortunately, polymers, due to long alkyl chain quaternized pyridine rings as repeat units (like polyvinylpyridinium salts), are efficient quenchers for pyrene[8]. Our objective was to find fluorescence probes, still sensitive to polarity and viscosity changes, but with short enough excited state lifetimes in order to reduce or eliminate the quenching processes. To satisfy these requirements, fluorescent molecular rotors were selected[9]. These probes, upon electronic excitation, lead to the formation of an initial ICT (intramolecular charge transfer) state with partial electron transfer, generally followed by a twisting of the molecule to give a complete electron transfer in a TICT (twisted ICT) state, in which the donor orbital is perpendicular to the acceptor orbital. Depending on the molecular structure, both ICT and TICT states can be radiative, especially in fluid polar media, as for example 4-(*N*,*N*-dialkylamino)benzene derivatives. On the contrary, for benzylidene and cinnamylidene derivatives, the TICT state is nonluminescent.

These flexible fluorescent dye molecules have been proven to be sensitive to both the local polarity and local viscosity (rigidity) of the surroundings, in various solvents[10] as well as inside assemblies formed by conventional surfactant micelles[11], phospholipid and surfactant vesicles[12], or biological membranes[13]. For the polymers, most of the studies have been carried out in organic media or in the solid state in order to obtain information on the free volume distribution available for molecular relaxation[14,15] or to determine the glass transition temperature[16], the physical ageing[17] or even to monitor polymerization rates[18,19].

In the present paper, we report preliminary results of the use of a cinnamylidene type molecular rotor (1,1-dicyano-4-(*p*-dimethylaminophenyl)-1,3-butadiene) as a fluorescence probe in order to study the behavior, in aqueous media, of a new water-soluble copolymer series derived from the 3-vinylpyridinium salts, for example, poly(methylvinylpyridinium-*co*-hexadecylvinylpyridinium dibromide). A first approach to evaluate the influence of both the content and the length of the pendent alkyl groups (hydrophilic/hydrophobic balance) has been investigated. The results have been compared to those obtained on the one hand with the two extreme homopolymers (the polyelectrolyte (poly(methylvinylpyridinium bromide)) and the polysoap (poly(hexadecylvinylpyridinium bromide))) and on the other hand, just as a conventional surfactant, with CTAB or better, 3-ethyl-1-hexadecylpyridinium bromide, which is an analogue of the latter homopolymer repeat unit (*Table 1*).

Experimental

The fluorescent probe, 1,1-dicyano-4-(*p*-dimethylaminophenyl)-1,3-butadiene, (disubstituted (*p*-dimethylamino)cinnamylidene, DMAC), was synthesized by a Knoevenagel's reaction between 4-(*N*,*N*-dimethylamino)cinnamaldehyde and malononitrile with an 84% yield (m.p. = 147°C; literature 146–148°C[20]).

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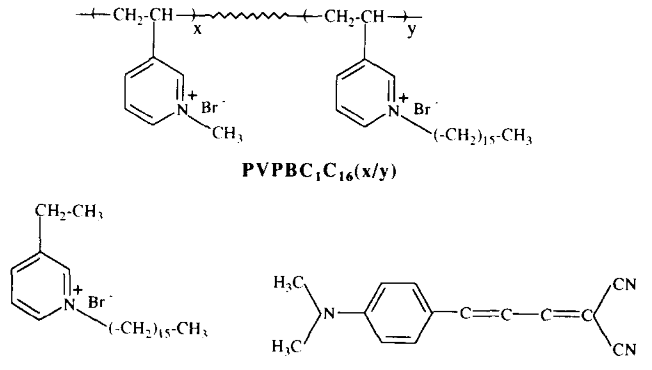
Table 1 Schematic representation of the copolymers, the model EHPB and the rotor DMAC



PVPBC$_1$C$_{16}$(x/y)

EHPB

DMAC

The syntheses of the model surfactant 3-ethyl-l-hexadecylpyridinium bromide (EHPB; $CMC = 3.7 \times 10^{-4}$ M, $T_{\text{Krafft}} = 9.8$°C) and the monomers 1-methyl-3-vinylpyridinium bromide (VPBC$_1$) and 1-hexadecyl-3-vinylpyridinium bromide (VPBC$_{16}$) have been described in a previous paper[21]. The polymers were prepared by homo or copolymerization of the two former monomers using AIBN (α,α'-azoisobutyronitrile) (Fluka reagent, recrystallized from methanol) at 60°C for 24h[21]. PVPBC$_1$ and PVPBC$_{16}$ will denote hereafter the polyelectrolyte and the polysoap with methyl and hexadecyl pendent chains, respectively; the copolymers are referred to as PVPBC$_1$C$_{16}$(x/y), where C$_1$ and C$_{16}$ denote the alkyl pendent chains and x and y are the final molar percentages of the two different units. These copolymers were characterized by *FT*i.r. (Bruker IFS 25 spectrophotometer) and ^{1}H and ^{13}C n.m.r. (Bruker AC-200P spectrometer in deuterochloroform solutions). Copolymer composition was determined by elemental analysis (obtained from the Service Central d'Analyse du CNRS).

Solvents used for u.v.–visible absorption and fluorescence studies were spectral grade, their water content not exceeding 0.1 wt%, except for the most polar methanol and ethanol (up to 0.2 wt%).

Absorption spectra were recorded on a Perkin–Elmer (Lambda 2) u.v.–visible spectrophotometer.

Fluorescence emission spectra of DMAC (4.3×10^{-6} M; stock solution in ethanol at 1.3×10^{-3} M, i.e., less than 3.5‰ solvent addition in volume), either in solution or added to the polymer solutions, were recorded at 500–600 nm on a SPEX Fluorolog-2 spectrofluorometer (slit width: 1.5 mm; bandpass = 5.64 nm), equipped with a thermostated cell compartment ($T = 30$ °C). The excitation wavelength ($\lambda_{\text{ex}} = 490$ nm) generates in all solvents only one single fluorescence emission, whose maximum wavelength is medium dependent.

For EHPB, the *CMC* value was obtained by surface-tension measurements at 30 ± 0.1°C with a Wilhelmy type surface tensiometer (Krüss K8). For this new surfactant, the Krafft point was determined by solubility measurements in distilled water.

Results and discussion

Amphiphilic polymers are known to form microdomains in aqueous media by intra- and/or intermolecular interactions between hydrophobic chains according to the studied concentration range. This peculiar environment can be evidenced by the use of fluorescent molecular rotors, either on the polarity aspect based on the fluorescence emission wavelength value or on the cohesion behaviour according to the fluorescence emission intensity.

Microdomain polarity. In order to correlate the polarity influence on the fluorescence emission wavelength of the rotor DMAC to the nature of amphiphilic polymer microdomains, it was first necessary to study its behaviour in solvents of different polarities.

Two types of solvent were examined, aprotic solvents (esters, ethers, hydrocarbons) and protic solvents (water, alcohols). In order to establish a polarity scale, we used the parameters which expressed at best the local interactions between solute and solvent, i.e., the $E_T(30)$ values defined by Dimroth and Reichardt[22]. As shown in *Figure 1*, the representative points for the $\lambda_{f\,\text{max}}$ values for DMAC *versus* the solvent polarity parameters are distributed on two straight lines: the first one *1* correlates well the aprotic solvents (polar or non) for which the equation can be expressed by $\lambda_{f\,\text{max}} = 4.88\, E_T(30) + 373.4$ ($r^2 = 0.933$), and the second line *2* includes the protic solvents (water and alcohols) with a fairly good relationship: $\lambda_{f\,\text{max}} = 1.06\, E_T(30) + 522.4$ ($r^2 = 0.999$). In the case of amphiphilic polymers solubilized in aqueous media, the scale defined by line *2* will be preferred to evaluate the polarity of the hydrophobic microdomains formed.

Returning to the polymers, the maximum emission wavelength $\lambda_{f\,\text{max}}$ of DMAC was measured as a function of their concentration (given by the total monomeric units) and the results are reported in *Figure 2*. For each polymer, except polyelectrolyte (PVPBC$_1$), λ_{fmax} decreases from its highest value (590 nm, value also observed in neat water) as the concentration of the polymer increases, to reach a final plateau value. Moreover, this final value diminishes as the hydrophobicity increases (from polyelectrolyte PVPBC$_1$ to polysoap PVPBC$_{16}$). Then, the largest hypsochromic shift ($\Delta\lambda_{\text{fmax}} = 14$ nm) was measured for the homopolymer PVPBC$_{16}$ (λ_{fmax} final = 576 nm, a value similar to that obtained in ethanol). With regard to polarity, the progressive replacement of a methyl by a hexadecyl chain is analogous to a shift from water to ethanol on line *2*.

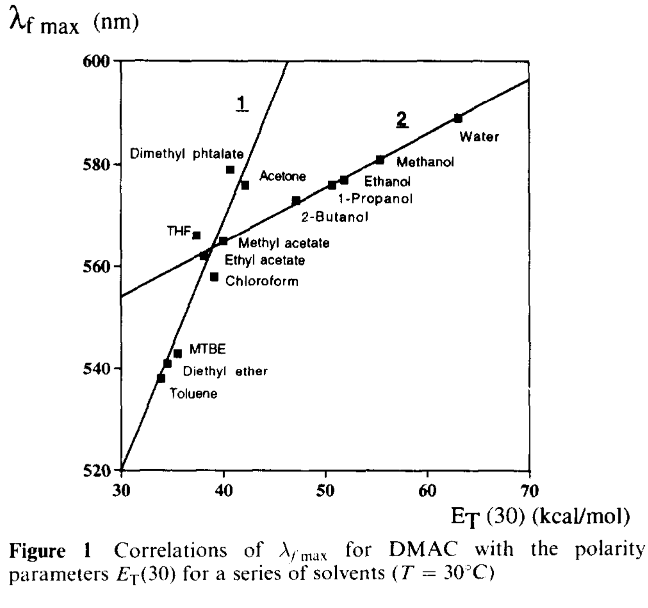


Figure 1 Correlations of $\lambda_{f\,\text{max}}$ for DMAC with the polarity parameters $E_T(30)$ for a series of solvents ($T = 30$°C)

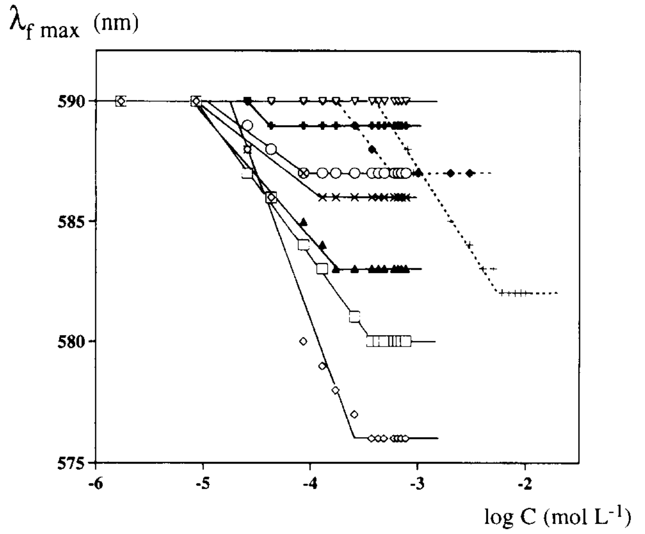


Figure 2 Shifts of the emission maximum of DMAC as a function of polymers, EHPB and CTAB concentration (logarithmic scale). C is given in molar units, T = 30 °C: ◇, PVPBC$_{16}$; □, PVPBC$_1$C$_{16}$ (50/50); ▲, PVPBC$_1$C$_{16}$ (60/40); ×, PVPBC$_1$C$_{16}$ (70/30); ○, PVPBC$_1$C$_{16}$ (80/20); +, PVPBC$_1$C$_{16}$ (90/10); ▽, PVPBC$_1$; ◆, EHPB; +, CTAB

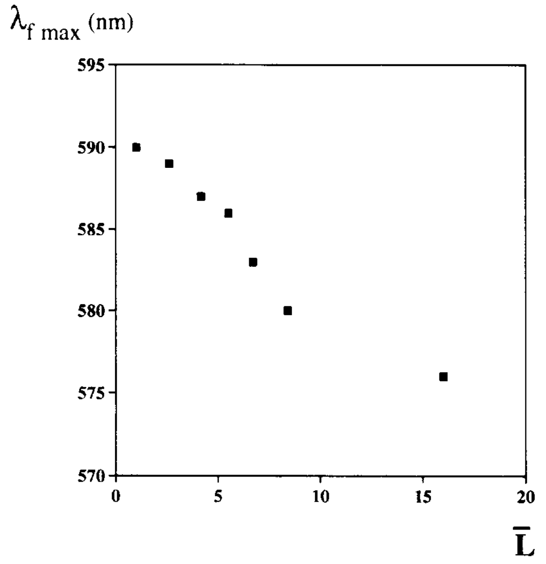


Figure 3 Correlation between the final plateau value of $\lambda_{f\,max}$ and the average side chain length $\bar{L}$

In order to take into account the comonomer content and the chain length of the alkyl groups, it would be useful to define a generalized parameter which describes the relative contribution of the pendent alkyl groups to the copolymer structure (polarity, cohesion, etc.). As described for quaternized poly(4-vinylpyridium) salts, Shinkai *et al.*[23] defined a new parameter $\bar{L}$ which is given by $\bar{L} = (ax + by)/100$, where x and y are the molar percent contents of each monomer, and a and b denote the carbon atom number of the pendent alkyl chains ($a = 1$; $b = 16$). In our case, x and y are the molar percent contents of VPBC$_1$ and VPBC$_{16}$, respectively, and $\bar{L} = (x + 16y)/100$; this value corresponds to the average length of the pendent alkyl groups per pyridine unit. Then it is possible to examine the influence of the copolymer polarity, expressed by the fluorescence emission wavelength of the rotor, as a function of $\bar{L}$. As shown in *Figure 3*, the plot of final plateau value of $\lambda_{f\,max}$ *versus* $\bar{L}$ shows that the microdomain polarity (expressed by $\lambda_{f\,max}$) decreases as expected with an increase in the hydrophobic character of the polymer and that the variation is less noticeable from $\bar{L} \approx 9 (x/y < 1)$.

It would be useful to make a comparison between the structure of the microdomains of amphiphilic polymers with the simpler well-established micellar organization. Thus, a fluorescence study using DMAC as a probe was developed for micellar solutions of the corresponding conventional surfactants (see *Figure 2*). The model EHPB has a final $\lambda_{f\,max}$ value (587 nm) higher than those for both the corresponding homopolymers PVPBC$_{16}$ and CTAB, indicating a more polar environment. This observation suggests that EHPB has a less tight structure than CTAB and *a fortiori* than PVPBC$_{16}$. A similar behaviour was previously found using pyrene as a fluorescent probe for poly(3-hexadecyl-1-vinylimidazolium bromide) compared to its repetitive unit model (3-hexadecyl-1-methylimidazolium bromide) in their respective microorganizations[6]. For each type of amphiphilic derivative, the chosen fluorescent probe senses the same basic molecular structure (C$_{16}$-pyridinium or C$_{16}$-imidazolium), either free or included in the polymer chain. Then the different responses relative to polarity are probably due to the fact that compared to the isolated micelles, the polymer coiling confines its hydrophobic microdomains in a more entangled structure and diminishes the water penetration, as previously described[6]. Now, compared to the copolymers, the organization of the model EHPB presents a final polarity equal to that observed for the copolymer containing only 20% molar content of hydrophobic chains, i.e., PVPBC$_1$C$_{16}$ (80/20) and *a fortiori* lower than the value for the copolymer PVPBC$_1$C$_{16}$ (90/10). The presence of a large proportion of C$_1$ chains in these copolymers can explain the ease of water penetration.

From the plots $\lambda_{f\,max}$ *versus* log C, in addition to the polarity information, it is also possible to determine the concentration corresponding to the beginning of microdomain formation for the polymers and the critical micellar concentration (*CMC*) in the case of the conventional surfactants CTAB and EHPB. The results show that the association phenomenon is detected at 2.5×10^{-5} M for PVBC$_{16}$, whereas for the copolymers this concentration is increased, as expected, up to 1×10^{-4} M. As already observed for other studies on polymers, for example, poly(N-isopropylacrylamide)[5], copolymer maleic acid/alkylvinylether[4] or polyalkylvinylimidazolium bromides[6,7] and contrarily for the classical surfactants, the association transition appears broad and stretches over a 100-fold range in polymer concentration. For the model EHPB, the *CMC* (estimated at 3.9×10^{-4} M) is in good agreement with interfacial tension measurements (*CMC* = 3.7×10^{-4} M) and is lower than the *CMC* value of 7.95×10^{-4} for CTAB (literature[24] = 8.7×10^{-4} M).

Microdomain cohesion. As previously mentioned, molecular fluorescent rotor emission is also viscosity dependent. Indeed, generally an increase of the viscosity induces a diminution of the free-volume of the medium with a subsequent slowing down of the motion of the rotor, if any. This leads to a diminution of the nonradiative relaxation processes with a consequent increase of the fluorescence quantum yield Φ_f. In order to gain further information on microdomain cohesion, variation of the fluorescence quantum yield was measured as a function of the concentration of either polymers or surfactants. In fact, *Figure 4* represents the variation of the ratio Φ_f/Φ_{f_0} (where Φ_{f_0} is the fluorescence quantum yield without polymer or surfactant, i.e., in water) *versus* concentration. In all cases, a strong increase of the Φ_f/Φ_{f_0} ratio is observed as the concentration C_m (onset of the formation of the hydrophobic microdomains) for the polymers or the *CMC* for the model EHPB is reached (note that this method leads to similar results for C_m or *CMC*). This phenomenon is due to a lower mobility of the molecular rotor when the environmental medium is increasingly organized, which corresponds to a more viscous microenvironment. By comparison with the model EHPB, a higher final value for the Φ_f/Φ_{f_0} ratio is observed for all the polymers (except the polyelectrolyte PVPBC$_1$), indicating a lower mobility of the rotor in these systems (whatever the C_1 chain proportion) than in EHPB micelles. Then, it does not seem possible to directly compare the polarity information in terms of water penetration due to a loose structure and the efficiency of the fluorescence emission as a result of the motion capacity.

In the same way, for the polymers, the maximum value of the Φ_f/Φ_{f_0} ratio tends to increase until the number of long C_{16} chains reaches a 30% molar content; after this proportion, a nonsignificant variation is observed (see *Figure 5* representing Φ_f/Φ_{f_0} ratio *versus* $\bar{L}$, where $\bar{L}$ is considered here as a structure parameter), even when the polarity is still decreasing.

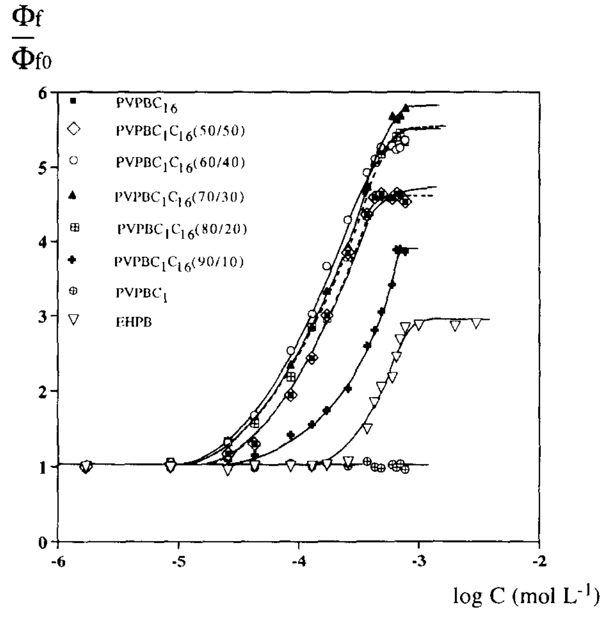


Figure 4 Variations of the quantum yield ratio Φ_f/Φ_{f_0} as a function of the polymers and EHPB concentration (logarithmic scale). C is given in molar units. T = 30°C

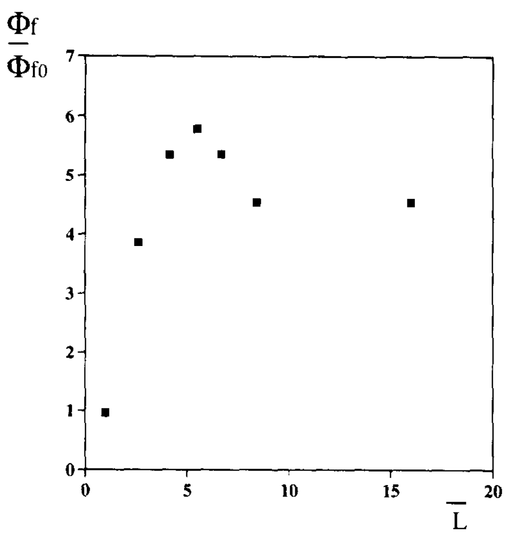


Figure 5 Correlation between the maximum value of the Φ_f/Φ_{f_0} ratio and the average side chain length $\bar{L}$

Conclusion

This present preliminary work evidences the potentialities of molecular rotors as fluorescent probes to detect the formation of hydrophobic microdomains for amphiphilic polymers, not only on the polarity aspect, associated with water penetration in the more or less tight structure, but also on the local viscosity related to the restricted motion of the probe in these organized assemblies. Then, fluorescent rotors can be advantageously used in the cases where pyrene is excluded due to quenching of its excited state. For a series of amphiphilic polymers, for which the use of the two types of probes (pyrene and rotors) is feasible, studies have been developed to compare the data obtained and especially to put trust in these methods.

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