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Fluorescence to Study Aggregation of Amphiphilic Copolymers in Aqueous Media

Céline Frochot[1], Alain Brembilla[2], Pierre Lochon[2] and Marie-Laure Viriot[1]*

[1] Département de Chimie-Physique des Réactions, UMR 7630 CNRS-INPL and
[2] Laboratoire de Chimie-Physique Macromoléculaire, UMR 7568 CNRS-INPL,
Groupe ENSIC, 1 rue Grandville, BP 451, F-54001 Nancy Cedex, France

SUMMARY : Random copolymers of 3-hexadecyl-1-vinylimidazolium bromide and N-vinyl-2-pyrrolidinone were synthesized by free radical copolymerization at various feed compositions. These copolymers, whose structure was determined by ^{13}C NMR spectrometry, self-aggregate in aqueous media. The formation of the resulting hydrophobic microdomains was analyzed by spectrofluorimetry, using pyrene and a molecular rotor as fluorescent tracers. The results are discussed in relation to the molecular structure of the copolymers, particularly for the case of the micro cohesion sensed by the molecular rotor.

Introduction

For several years, there has been a growing interest in the preparation and the behavioural study of amphiphilic polymers. Owing to their tendency to self-aggregate in an aqueous medium, due to both intra and/or intermolecular interactions of the long alkyl chains, these polymers offer outstanding properties, which can find applications in many fields (enhanced oil recovery membranes, biomedical application...). However, the physico-chemical properties of these polymers are strongly dependent on the chemical nature of both the hydrophilic and hydrophobic moieties (respectively : cationic, anionic, non-ionic... and aliphatic, cyclic, aromatic...) and most importantly, their relative ordering in the macromolecular chain1). In order to establish a relationship between the molecular structure and the behaviour of these polymers in aqueous solution, random copolymers of 3-hexadecyl-1-vinylimidazolium bromide (cationic part) with a spacing hydrophilic monomer such as N-vinyl-2-pyrrolidinone (non-ionic part) were synthesized with various monomer feed compositions at a low conversion rate. The aim of this study is to highlight the aggregation tendency. Hence, we used fluorescence spectroscopy, which is a technique well suited to give information about

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the change of the macromolecule conformation[2)]. Studies were accomplished with fluorescent tracers, such as pyrene, and a molecular rotor. In particular information, linked to the local microenvironment viscosity of the tracer and obtained from the fluorescence quantum yield of the rotor, will be correlated to the molecular structure of the copolymers and their capacity for coiling.

Experimental

Materials

The synthesis of the monomer 1-hexadecyl-3-vinylimidazolium bromide (C_{16}Vim) was described in a previous paper[3)]. N-vinyl-2-pyrrolidinone (NVP) purchased from Aldrich was purified by distillation on a condensing cold surface.

Copolymers were prepared by copolymerization of the two monomers. Various molar fractions of C_{16}Vim and NVP (total amount of monomers = 4 10^{-2} mol) and 30 mL of ethanol were introduced in a reactor (50 mL) and degassed under vacuum by application of the freeze-thaw technique. Then 4 10^{-4} mol AIBN (Fluka, recrystallized from methanol) was added under Ar and the waterproof reactor was immersed, under stirring, in a thermostated bath at 60°C for times ranging from 5 to 20 min, according to the feed composition. Copolymerizations were stopped by cooling with liquid air. The copolymers were precipitated by addition of acetone, filtered off, and then purified by a double dissolution in chloroform, followed by a precipitation by acetone. After drying under vacuum at 50°C for 24 hrs, the conversion ratios (< 10%) were determined by weighing.

Methods

UV-visible absorption and Fluorescence

Stock solutions were prepared by dissolution of the polymer in an aqueous mixture (1-propanol/water, 3/97, v/v). Absorption spectra were recorded on a Perkin Elmer (Lambda 2) UV-visible spectrophotometer.

Fluorescence emission spectra were recorded on a SPEX Fluorolog-2 spectrometer equipped with a thermostatically controlled cell at 30°C.

For pyrene (1.1 10^{-6} M in the final solution) no excimer was observed ; the samples were excited at 332 nm and the emission spectra of pyrene showed vibronic peaks at λ_1 = 372 nm (I_1) and λ_3 = 383 nm (I_3) with a slit width = 0.5 mm ($\Delta\lambda_{1/2}$ = 1.88 nm).

For the rotor (1,1-dicyano-(4'-(N,N-dimethylamino)phenyl)-1,3-butadiene, noted DMAC) (3.3 10^{-6} M in the final solution), spectra were recorded in the 500-600 nm range (slit width 1.5 mm ; $\Delta\lambda_{1/2}$ = 5.64 nm) and the excitation wavelength (λ_{ex} = 511 nm) generates only one single fluorescence peak[4)].

Copolymers characterization

Copolymers compositions were determined by ^{13}C NMR using $CDCl_3$ as solvent. ^{13}C spectra were recorded on a Bruker AC-200P spectrometer.

Results and discussion

Composition of C_{16}Vim and NVP copolymers

NMR spectra of the copolymers exhibit a peak at 176 ppm, which is characteristic of the carbon atom of the NVP carbonyl group. The resonance peak area (noted $A_{C=O}$) was compared with the total resonance area of all the peaks (A_t) corresponding to the aliphatic carbon atoms of the two units. F_A and 1- F_A denote the C_{16}Vim and NVP molar fractions respectively, F_A is calculated from $A_{C=O}$ and A_t values according to the following equation :

$$\frac{A_t}{A_{C=O}} = \frac{5(1-F_A)+18F_A}{1-F_A}$$

Polarity studies

Pyrene as a polarity tracer

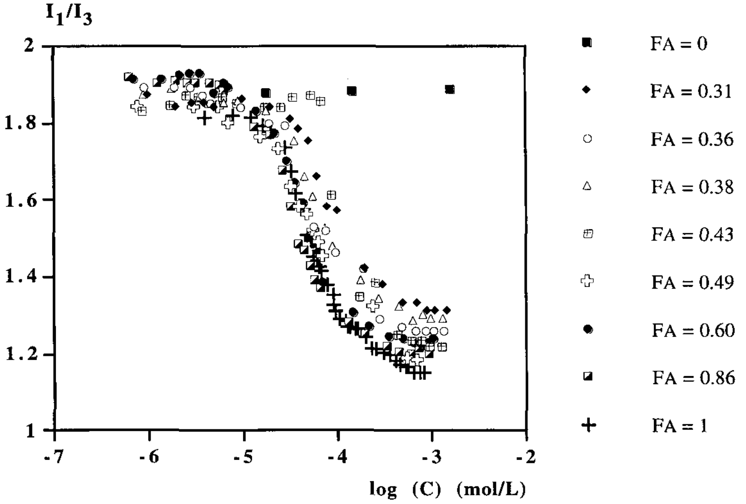


Fig. 1: Variation of I_1/I_3 *versus* the copolymer concentration (log scale).

Figure 1 shows the variations of I_1/I_3 ratio for pyrene for the concentration of the different copolymers (log scale). The decrease of I_1/I_3 extends over a range of concentrations of more than one unit. Except for the homopolymer, poly(N-vinyl-2-pyrrolidinone), I_1/I_3 decreases from its highest value (around 1.89 with our apparatus, in the mixture 1-propanol/water, 3/97, v/v) as the copolymer concentration increases, to reach a final plateau value. This value, characterizing the polarity in the surroundings of the tracer (hydrophobic microdomains), depends on the copolymer composition. As expected, the hydrophobic character of the tracer microenvironment increases as F_A increases, F_A being equal to the imidazolium units (or long chains C_{16}Vim) ratio in the copolymer. The lowest value for I_1/I_3 was observed for the homopolymer containing long chain imidazolium moieties only. From each curve, it is possible to determine the concentration Cm, which corresponds to the onset of the hydrophobic microdomains detected by the probe. It was interesting to determine the pyrene binding constant K with the microdomains in relation to the hydrophobicity of the copolymers. The determination of K gives also a first indication on the capacity of the microdomains to incorporate hydrophobic compounds. Pyrene is distributed between the bulk phase (b) and the microdomains phase (m) according K : $K = \dfrac{[Py]_m}{[Py]_b + [C - C_m]}$ (1) where $[Py]_m$ and $[Py]_b$ are the concentrations of pyrene in the microdomains and the bulk phase respectively.

Then : $I_1 / I_3 = \dfrac{[Py]_m}{[Py]}(I_1 / I_3)_m + \dfrac{[Py]_b}{[Py]}(I_1 / I_3)_b$ (2)

By combining the equations (1) and (2) :

$$(I_1 / I_3)_b - I_1 / I_3 = \frac{K[C - C_m]}{1 + K[C - C_m]}[(I_1 / I_3)_b - (I_1 / I_3)_m] \quad (3)$$

The fit of equation (3) to the experimental results was performed by using $(I_1/I_3)_m$, K and Cm as adjustable parameters$^{5)}$. From these calculations and, as expected, the binding constant K increases with the long chain C_{16}Vim content in the copolymers. The representative points for the K values *versus* F_A are distributed on a straight line : K = 64000 F_A with a good linear relationship ($r^2 = 0.93$).

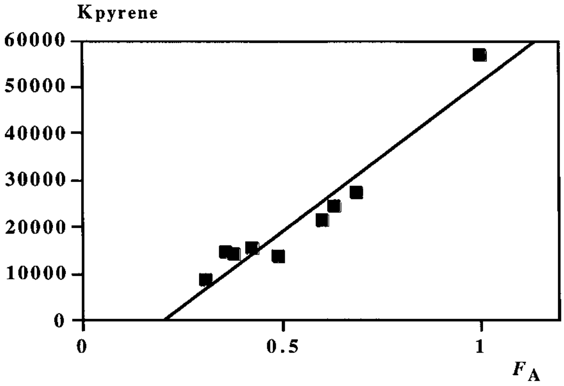


Fig. 2: Variation of the binding constant K *versus* the C_{16}Vim molar fraction F_A.

Fluorescent molecular rotor DMAC as a tracer

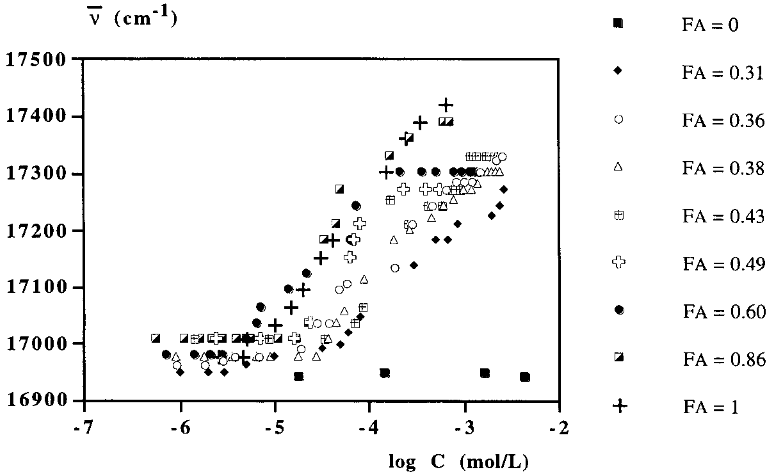


Fig. 3: Variation of the fluorescent wavenumber ($\bar{\nu}$, cm^{-1}) of the molecular rotor *versus* the copolymer concentration (log scale).

Overall, the measured emission band wavelength $\lambda_{f\,max}$ of the rotor, shifted hypsochromically as the copolymer concentration increases. As shown in Figure 3, $\bar{\nu}$ *versus* log C, $\bar{\nu}$ increases from around 16970 cm^{-1} (in the mixture 1-propanol/water, 3/97, v/v) to a final plateau, the value of which depends on the C_{16}Vim units ratio (given by F_A). As expected, $\bar{\nu}$ (plateau value) increases as F_A increases and the largest deviation was observed for the homopolymer

(F_A = 1). In contrast, no variation was observed for the poly(N-vinyl-2-pyrrolidinone) (F_A = 0).

Microdomains cohesion

Owing to their dependence on fluorescence quantum yield Φ_f/Φ_{f_0} (where Φ_{f_0} is the fluorescence quantum yield without polymer in the medium), molecular rotors can give useful information as cohesion fluorescent tracers[2]. Generally, an increase of the local viscosity involves a diminution of the medium free-volume which restricts the free rotation of the molecular rotor.

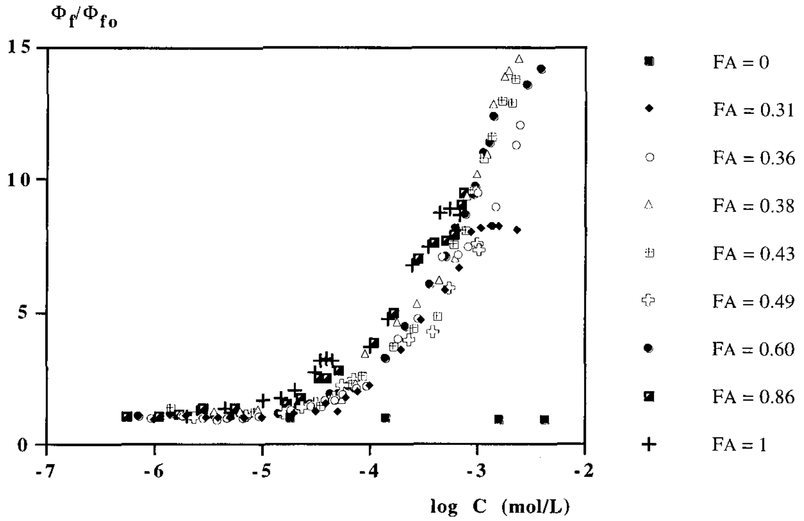


Fig. 4: Fluorescence quantum yield Φ_f/Φ_{f_0} ratio *versus* the copolymer concentration (log scale).

Figure 4 shows the variation of Φ_f/Φ_{f_0} as a function of the polymer concentration. For all of the copolymers and the homopolymer (F_A = 1) the quantum yield increases strongly with the concentration to reach finally a plateau value.

Micro cohesion and molecular structure

The probabilities $P_{sequence}$ to find particular monomer sequences at low conversion ratio (< 10%) yield information on the ordering of C_{16}Vim and NVP moieties in the principal chain.

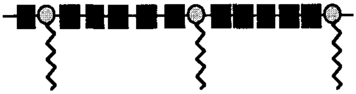
However, a better image of the sequence distribution is given by the number-average lengths of C_{16}Vim and NVP runs respectively[6] :

$l_{C16Vim} = 1 + r_{C16Vim}x$ and $l_{NVP} = 1 + (r_{C16Vim}/x)$

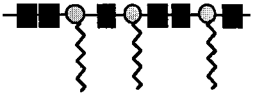
with $r_{C16Vim} = 0.5$ and $x = \dfrac{f_{C16Vim}}{1 - f_{C16Vim}}$

where f_{C16Vim} is the molar fraction in the feed charge.

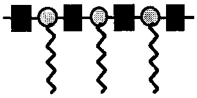
From l_{C16Vim}/l_{NVP} value, we can propose a schematic representation of the principal chain for the different copolymers.



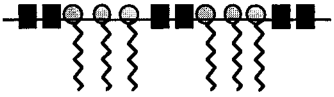
R = 0.203 ; F_A = 0.31



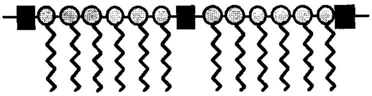
R = 0.468 ; F_A = 0.38



R = 1.02 ; F_A = 0.49



R = 1.5 ; F_A = 0.60



R = 6.7 ; F_A = 0.86

$$\boxed{R = l_{C16Vim}/l_{NVP}}$$

At low C_{16}Vim content (F_A = 0.31), the most plausible structure for the corresponding copolymer exhibits average sequences containing 5 NVP units per one C_{16}Vim unit. For this copolymer, the micro cohesion at the surroundings of the tracer, defined by Φ_f/Φ_{fo}, allows us to consider that this copolymer will not adopt a very organized structure in an aqueous medium (Figure 5). In contrast, when F_A increases (for F_A = 0.49), l_{C16Vim}/l_{NVP} becomes close to 1 and diads C_{16}Vim-NVP sequences are clearly predominant. This result is in agreement with the alternation tendency predicted from the low reactivity ratios and a higher microdomains cohesion is observed. When the sequence of C_{16}Vim moieties increases further (R = 6.7), the ratio Φ_f/Φ_{fo} decreases, indicating a lower compacity for the hydrophobic microdomains, in agreement with previous observations[7]. Indeed, a too high content of long linked alkyl chains seems to be unfavourable for compact arrangement of the microdomains.

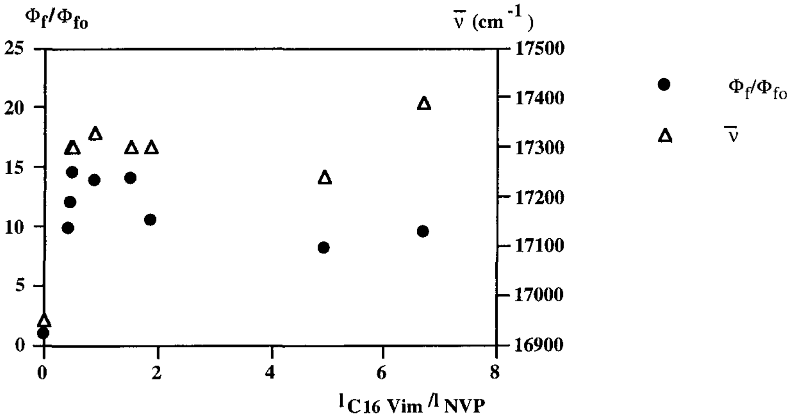


Fig. 5: Variations of $\bar{\nu}$ and Φ_f/Φ_{f_0} (plateau values) *versus* the parameter l_{C16Vim}/l_{NVP}.

The profile curve Φ_f/Φ_{f_0} *versus* l_{C16Vim}/l_{NVP} is similar to the one observed for the variations of the ratio Φ_f/Φ_{f_0} *versus* $\bar{L}$, where $\bar{L}$ is a parameter characterizing the hydrophobicity of copolymers [poly(methylvinylpyridinium-*co*-hexadecylvinylpyridinium dibromide)]. In this case, studies by cryo-Transmission-Electron-Microscopy (TEM) revealed a "string of beads" structure for the copolymers at the maximum of compactness, *i.e.* a copolymer containing about 70% of hydrophilic units. Above this value, Φ_f/Φ_{f_0} slightly decreases and then remains constant as the long alkyl chain content increases. These copolymers must adopt a fingerprint-like structure as was observed for the homopolymer poly(3-hexadecyl-1-vinylimidazolium bromide)[8].

Conclusion

This work shows the potential of fluorescent molecular rotors as tracers to characterize the hydrophobic microdomains in aqueous medium, for a series of amphiphilic copolymers in relation to their chemical structure. The information covers both the polarity aspect, and also the local viscosity of the tracer microenvironment characterizing the cohesion of the copolymer. The most compact structures are observed for copolymers presenting a high tendency to alternation.

References

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