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AMPHIPHILIC COPOLYMERS IN AQUEOUS MEDIUM : CHARACTERIZATION OF THE STRUCTURE OF THE HYDROPHOBIC MICRODOMAINS

Abdesselam Benjelloun[1], Catherine Dorion[2], Alain Brembilla[1]*, Marie-Laure Viriot[2] and Marc Adrian[3]

[1] Laboratoire de Chimie-Physique Macromoléculaire, CNRS-URA 494 and
[2] Département de Chimie-Physique des Réactions, CNRS-URA 328, ENSIC-INPL, 1 rue Grandville, BP 451, F-54001 Nancy Cedex, France
[3] Laboratoire d'Analyse Ultrastructurale, Université de Lausanne, Bât. de Biologie, CH-1015 Lausanne, Switzerland

ABSTRACT

The behaviour of new cationic amphiphilic copolymers derived from 3-vinylpyridine was studied in aqueous medium. The formation of hydrophobic microdomains was evidenced by fluorescence spectroscopy using a molecular rotor as a fluorescent probe and their structure was visualized by cryo-transmission electron microcospy (Cryo-TEM). The parameters (shape and size) obtained from Cryo-TEM were discussed in relation to the copolymers composition and to the local viscosity defined by the rotor fluorescence quantum yield.

INTRODUCTION

For the last decades, cationic amphiphilic polymers have received an increasing attention in many fields throughout their physico-chemical properties in aqueous medium. Indeed, their capacity of self-aggregation due to the alkyl side chains interactions gives rise to the formation of a micelle-like microphase structure which can reveal interesting applications in the field of dissolution, transport and reactivity of lipophilic reactants in water. Most of the studies reported in the literature evidence, essentially by means of spectroscopic methods, the formation of hydrophobic microdomains in aqueous polyamphiphiles solutions. Only a few works report on the own structure of these aggregates, and the attempts to determine various parameters such as shape, size and aggregation number, remain still a matter of discussion. A rather new technique *i.e.* cryo-transmission electron microscopy, widely used in the case of biological objects, has afforded promising results in the polyamphiphiles field (Ref. 1). In this way, we decided to apply this technique to a series of cationic amphiphilic copolymers derived from 3-vinylpyridine. The self-aggregation mode and the structure of the resulting microdomains

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depend on the copolymers composition. In this paper, we report the preliminary results concerning the direct imaging of the aggregates for all of the copolymers. The structural parameters (*e.g.* shape) will be compared to the informations (*e.g.* local viscosity) obtained from the quantum yield of a molecular rotor by fluorescence spectroscopy.

EXPERIMENTAL PART

Materials

The synthesis of the monomers, 1-methyl-3-vinylpyridinium bromide ($VPBC_1$) and 1-hexadecyl-3-vinylpyridinium bromide ($VPBC_{16}$) was described in a previous paper (Ref. 2). The polymers were prepared by homo or copolymerization of the two former monomers by using AIBN (α,α'-azoisobutyronitrile, Fluka reagent recrystallized from methanol) as initiator at 60 °C for 24 h. In the following, the copolymers are referred to as $PVPBC_1C_{16}$ (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 monomers.

Methods

Absorption spectra were recorded on a Perkin Elmer (Lambda 2) UV-visible spectrophotometer.

Fluorescence emission spectra of the rotor (1,1-dicyano-(4'-dimethylaminophenyl)-1,3-butadiene) ($4.3\ 10^{-6}$ M ; stock solution in ethanol at $1.3\ 10^{-3}$ M, *i.e.*, less than 3.5 ‰ solvent addition in volume), were recorded in the 500-600 nm range, on a SPEX Fluorolog-2 spectrometer equipped with a thermostatically controlled cell at 30 °C (slit width 1.5 mm ; $\Delta\lambda_{1/2} = 5.64$ nm). In all solvents, the excitation wavelength ($\lambda_{ex} = 490$ nm) generates only one single fluorescence peak, the position of which is medium dependent (Ref. 3). Solvents used for UV-visible absorption and fluorescence studies were spectral grade and were purified before use by distillation.

Preparation of the vitrified-hydrated unstained specimens

The thin layer of a vitrified suspension was prepared as follows. A 5 µL drop of the polymer solution (concentration = 1.5 mg/mL) was deposited on a 200 mesh carbon-coated copper grid. Then the grid was held by a tweezers mounted on a guillotine-like frame and partially dried by pressing between blotting paper. The guillotine was quickly released for plunging the grid in a liquid ethane container, cooled at 99 K by liquid nitrogen. Care was taken to prevent any formation of either ice crystals (hexagonal, cubic ice layers) and/or vitreous form. The vitrified sample, kept under liquid nitrogen, was mounted on a GATAN 626 cryo-specimen holder and then rapidly introduced into the microscope (Philips CM 20). For all transfers, the specimens temperature was maintained at around 100 K. Because of the great sensitiveness of the objects, the specimens were examinated by operating in the low dose mode at an electron acceleration voltage of 120 kV so as to reduce the irradiation damages.

RESULTS AND DISCUSSION

Polarity study

The formation of hydrophobic microdomains involving intra- and/or inter-molecular alkyl side chains interactions are evidenced by the hypsochromic shift of the molecular rotor wavelength emission band as a function of the copolymer concentration (the concentration is given in molar units). The variation of $\bar{\nu}$ *vs* log[C] is characterized by an early and progressive transition which reflects the gradual formation of hydrophobic microdomains as compared with the conventional surfactants. The results summarized in Figure 1, show that for the copolymers and the homopolymer PVPBC$_{16}$, $\bar{\nu}$ increases from 16950 cm^{-1} (value in water) up to a final plateau, the value of which depends on the polymer hydrophobic character. Indeed, the shift magnitude increases with the decreasing of the hexadecyl group content. The largest shift ($\Delta\bar{\nu}$ = 412 cm^{-1}) was measured for the homopolymer PVPBC$_{16}$ (final $\bar{\nu}$ = 17360 cm^{-1}). Conversely and as expected, no variation was observed for the polyelectrolyte PVPBC$_1$.

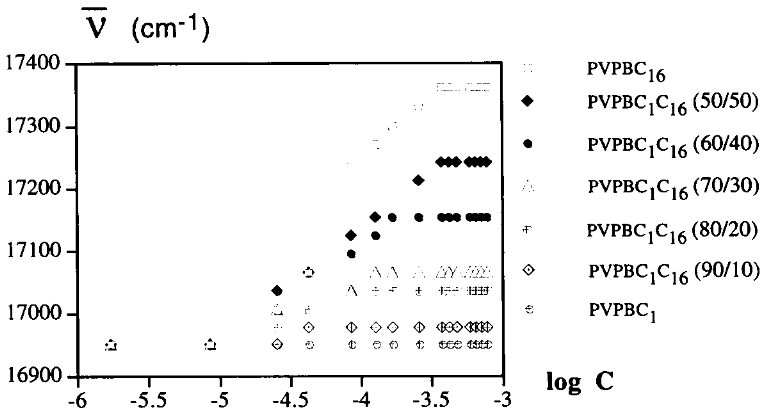


Fig. 1. Fluorescent wavenumber ($\bar{\nu}$, cm^{-1}) of the molecular rotor plotted against the polymers concentration (log. scale).

Microdomains cohesion

As mentioned previously, the molecular rotors have been also proved to be sensitive to the molecular environment local viscosity (Ref. 4). Generally, an increase of the local viscosity involves a diminution of the medium free-volume with a slowing down of the rotor motion. This leads, in our case, to a diminution of the nonradiative relaxation processes with an increase of the quantum yield Φ_f (in fact, we have considered the quantum yield variation by the ratio Φ_f/Φ_{f0}, where Φ_{f0} denotes the fluorescence quantum yield in the absence of polymer, *i.e.*, in neat water and Φ_f the fluorescence quantum yield in the polymer solution).

Figure 2 represents this ratio as a function of the polymer concentration. For all of the copolymers and the homopolymer PVPBC$_{16}$, the quantum yield increases strongly, starting at the concentration Cm which corresponds to the onset of the hydrophobic microdomains formation, to finally reach a plateau value. This behaviour is due to the lower mobility of the molecular rotor, while the medium becomes organized, which corresponds to a more viscous microenvironment.

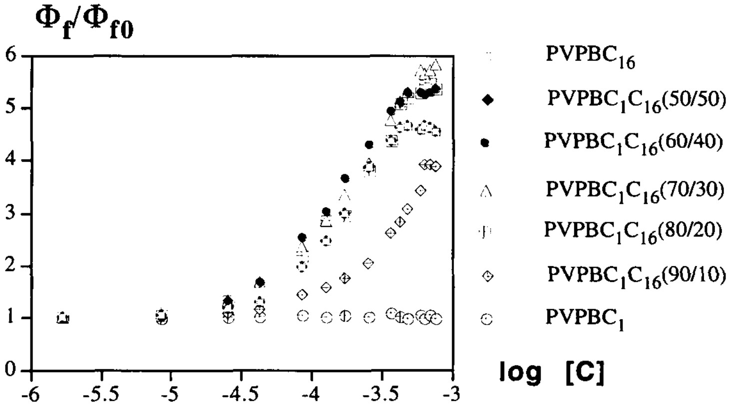


Fig. 2. Fluorescence quantum yield ratio Φ_f/Φ_{f0} as a function of the polymers concentration (log. scale).

The influence of the copolymer composition, (hydrophilic/hydrophobic balance) on the structure of the aggregates, was studied throughout the variations of the quantum yield (at the plateau value) as a function of a generalized parameter $\overline{L}$ (Ref. 5) which describes the relative contribution of the alkyl side chains to the copolymer structure. $\overline{L}$ is defined by $\overline{L} = (ax + by)/100$, where x and y are the molar percent contents of each monomer and a and b denote the carbon atom number of the alkyl side chains (a = 1 ; b = 16). In this case, x and y are the molar percent contents of $VPVC_1$ and $VPBC_{16}$ respectively, and $\overline{L} = (x + 16y)/100$ corresponds to the average length alkyl side chains per pyridine unit. As shown in Figure 3, the compactness of the aggregates characterized by the maximum value of the quantum yield ratio, increases with the copolymer hydrophobicity, *i.e.* from the polyelectrolyte $PVPBC_1$ until the copolymer containing 30 % of hydrophobic units. Above this composition, the increase of the hydrophobic character does not generate a significant variation of the quantum yield ratio even when the polarity is still decreasing (Fig. 1).

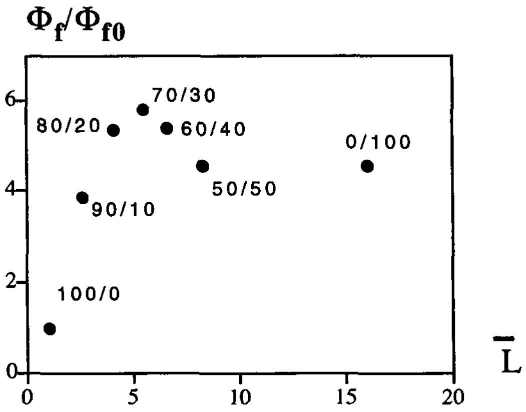
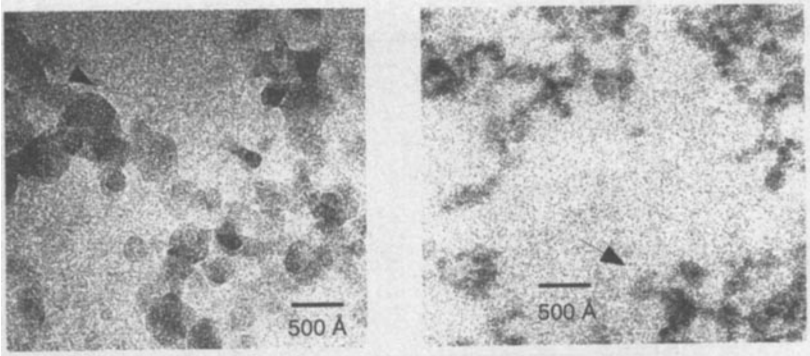


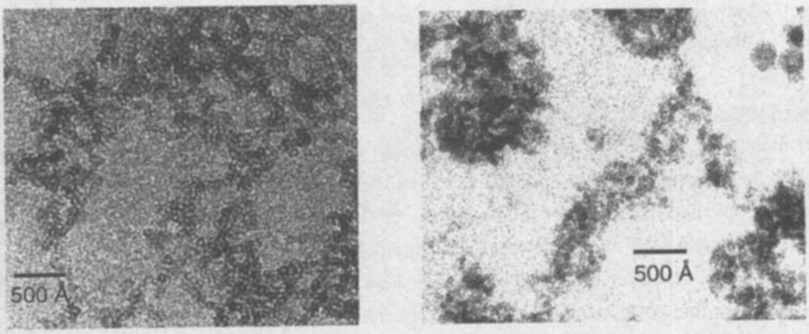
Fig. 3. Variation of the maximum value of the Φ_f/Φ_{f0} ratio and the average side chain length $\overline{L}$.

Cryo-Transmission Electron Microscopy

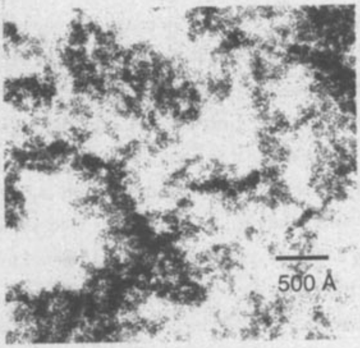
Our main objective was to get further insight into the polymer aggregates parameters (size, shape), in order to establish a relation between these parameters and both the fluorescence spectroscopy data (local order, compactness of the hydrophobic microdomains) and the chemical composition of the polymers. For this purpose, the widely used technique for the visualization of organized biological objects is the cryo-transmission electron microscopy which has been applied to aqueous solutions of cationic amphiphilic polymers. Figure 4a is a homopolymer $PVPBC_{16}$ micrograph (magnification 184,000), in which, we observe a set of dense objects, characterized by a spherical shape and a variable size, going from 200 to 700 Å. A more careful observation of these structures shows that they are built up of striations which illustrate the macromolecular chains regularly spaced out. This striations layout resembles very much to a lipidic phase image (Ref. 6) with the water layer located between the membranes. A quantitative analysis, by laser diffraction, of the tobacco mosaic virus used as a standard, has allowed the determination of the striations spacing. The periodical structure was estimated at 3.4 nm. On the copolymer $PVPBC_1C_{16}$ (50/50) micrograph (Fig. 4b), structures with regular striations are still present in addition with many untidy areas. In both cases, the same aggregation mode (striations) is in agreement with the constant value experimentally found for the quantum yield ratio. As the hydrophilic character increases, we observe, for the copolymer $PVPBC_1C_{16}$ (60/40) (Fig. 4c), an entangled worm-like structure. Indeed, the observation of the trickles outer layer reveals a succession of small circles very close to each other. These circles are likened to the hydrophobic microdomains and this type of organization is to be compared to that observed for poly(vinylbenzylhexadecyldimethylammonium chloride) (Ref. 1). In this case, the proposed "string of beads" structure is made up of a series of hydrophobic microdomains connected by short segments. From the micrograph (Fig. 4c), it is possible to evaluate roughly the hydrophobic microdomains size (diameter = 8 ± 1 nm).



(a) PVPBC$_{16}$ (b) PVPBC$_1$C$_{16}$ (50 /50)



(c) PVPBC$_1$C$_{16}$ (60 /40) (d) PVPBC$_1$C$_{16}$ (70 /30)



(e) PVPBC$_1$C$_{16}$ (90 /10)

Fig. 4. Cryo -transmission electron microscopy micrographs of vitrified solutions of the copolymers and the homopolymer.

Examples of striations are designed by arrows (Figures 4a and 4b)

However, the valuation of the alkyl chains number (methyl and hexadecyl) per microdomain is not possible because of the random distribution of the two units in the macromolecular chain. As shown in Figure 3, the slight increase of Φ_f/Φ_{f0} expresses the fact that the "string of beads" structure is more compact than that involving regular striations. When the C_1 units content in the copolymer increases until 70 % (PVPBC_1C_{16} (70/30) (Fig. 4d), the "string of beads" structure is maintained, but with smaller size microdomains (diameter ≈ 5 nm). The overall structure is yet tight according to the quantum yield ratio value. In the case of (PVPBC_1C_{16} (90/10) (Fig. 4e), the "string of beads" structure does not clearly appear, and moreover, we observe dark areas corresponding to little organized but very tight aggregates (from the quantum yield value, their compactness is slightly lower than that of the homopolymer (PVPBC_{16}). This copolymer, having only 10 % of hydrophobic units, has a particular behaviour in aqueous medium. Taking into account its higher viscosity at high concentration in water (Ref. 7), it behaves like the associative polymers where the aggregation phenomenon is then essentially due to intermolecular interactions.

CONCLUSION

Fluorescence spectroscopy using fluorescent molecular rotor as a probe and cryo-TEM are well-adapted complementary techniques for polymers microdomains characterization in aqueous solution. While cryo-TEM allows a direct imaging of the aggregates, the quantum yield ratio of the rotor is diagnostic for their compactness. In the particular case of the studied copolymers, their microdomains structure depends strongly upon their composition (hydrophilic/hydrophobic balance). For high hexadecyl units content (going from 100 to 50 %), the aggregates are well-organized in regular striations, while the so-called structure "string of beads" appears under a 50 % composition.

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