

Conformational Changes of Pyrene-Labeled Polyelectrolytes with pH: Effect of Hydrophobic Modifications

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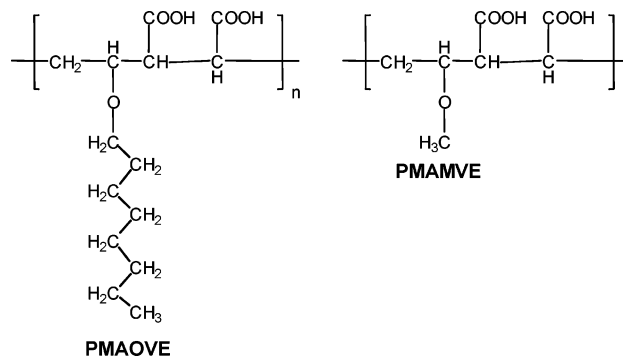
Structural changes of pyrene-labeled and unlabeled poly(maleic acid/octyl vinyl ether) (PMAOVE) and poly(maleic acid/methyl vinyl ether) (PMAMVE) with changes in pH have been investigated in this study. The changes in the photophysical properties of pyrene are interpreted to investigate uncoiling or swelling of the polymeric chains with pH. The vibrational fine structure of the pyrene fluorescence (I_3/I_1) and the ratio between excimer and monomer fluorescence (I_e/I_m) of both pyrene-labeled and unlabeled PMAMVE and PMAOVE suggest that, at pH 4, the polymers are in the coiled form and PMAOVE forms hydrophobic nanodomains. An increase in pH ionizes a number of COOH groups on both PMAMVE and PMAOVE, which leads to the stretching or swelling of the polymers.

Introduction

Macromolecules with hydrophobic groups as side chains (hydrophobically modified, HM, polymers)¹ have important applications in many industries and medicine, such as in drug delivery.^{2,3} In addition, these polymers can serve as simplified models of natural polyelectrolytes. HM polymers with a hydrophobic side groups can form intramolecular nanodomains in aqueous solutions. Unlike surfactant micelles, which are intermolecular aggregates, intramolecular nanodomains appear at all polymer concentrations, with no critical micelle concentration of polymer observed in aqueous solutions.⁴ Fluorescence probe techniques are often used to study such polymer conformations in aqueous solution.^{5–7} Many studies have employed the hydrolyzed form of the regularly alternating copolymers of maleic anhydride and alkyl vinyl ethers because of their aggregation behavior in solution.^{1,4,8–14} They form intramolecular nanodomains at low pH, but the extent of microdomain formation diminishes with increasing pH as a result of the repulsion between the ionized carboxylate groups.^{15,16} Fluorescence quenching studies have revealed that these HM polymers form large numbers of relatively small nanodomains.^{9–13} The presence of hydrophobic nanodomains in aqueous solutions enables the HM polymers also to remove organics from aqueous solutions. Because most HM polymers are sensitive to pH and ionic strength, regulation of these variables can also be used to manipulate conformation and solution rheology.^{15–18}

To explore the determining factor for HM polymer conformation in solution, an intermediate modified, poly(maleic acid/alkyl vinyl ether) homologue, namely, poly(maleic acid/octyl vinyl ether) (PMAOVE) (Chart 1), labeled with pyrene, was studied using fluorescence spectroscopy. To isolate the effect of hydrophobic substitution on the solution behavior, a homologue without the hydrophobic chain, pyrene-labeled poly(maleic acid/methyl vinyl ether) (PMAMVE) (Chart 1), was also studied.

CHART 1: Structures of the Polymers Poly(maleic acid/octyl vinyl ether), PMAOVE, and Poly(maleic acid/methyl vinyl ether), PMAMVE



Experimental Section

Materials. Polymers. The polymers, PMAOVE and PMAMVE, were provided by International Specialty Products, Inc. These polymers were synthesized using free-radical polymerization of a 1:1 mole ratio of maleic anhydride and vinyl ether in toluene with Vaso-69 (azo bis-valeryl nitrile) as the initiator. The products were purified twice by first being dissolved in acetone (5 wt %) and then being precipitated with excess tertiary butanol (40 times by volume). Residual solvent was removed by vacuum-drying at 50 °C to constant mass. The anhydride moiety of the polymer was then hydrolyzed in triply distilled water to make an approximately 5 wt % solution. The solution was stirred at 500 rpm at 70 °C for about 12 h and then freeze-dried. As determined by gel permeation chromatography, the weight-average molecular weight was 160 000 Da with a polydispersity index of 1.2 for PMAOVE and 200 000 Da with a polydispersity index of 1.6 for PMAMVE.

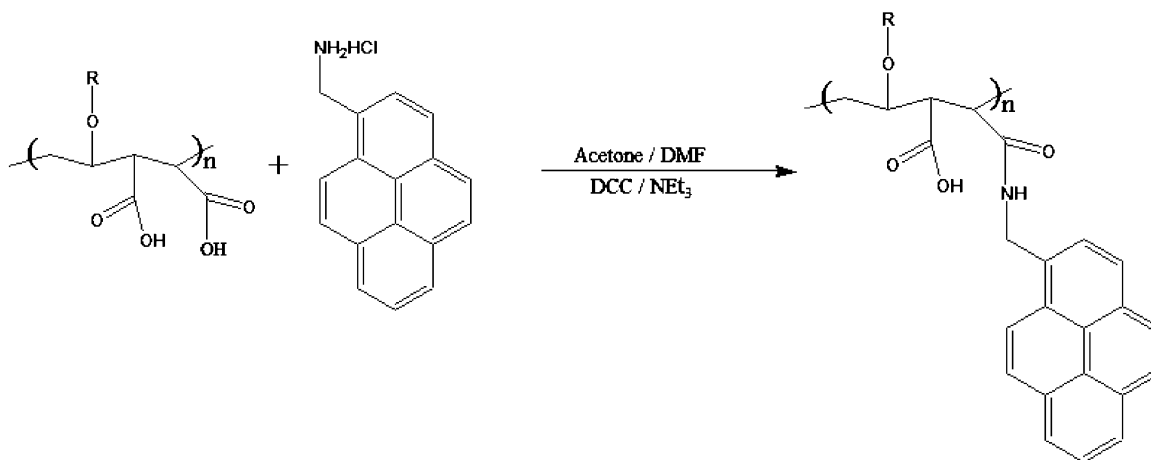
Probes and Additives. Pyrene and pyrene methylamine were purchased from Aldrich Chemical Co. and were used as received (>99% pure). Solutions of 0.1 N hydrochloric acid and 0.1 N NaOH were purchased from Fisher Scientific. NaCl was

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SCHEME 1: Labeling of Polymer with Pyrene



purchased from Fluka and was specified to be 99.5% pure. The water used was triply distilled.

Methods. Labeling of Polymer with Pyrene. The labeling procedure involves dissolving the polymer in a solvent such as acetone or DMF along with the required amounts of dicyclohexylcarbodiimide, triethylamine, and the label with an amine functional group. A brief scheme of the labeling procedure is shown below (Scheme 1).

Fluorescence Spectroscopy. Fluorescence spectroscopy has been useful for the investigation of aggregation and conformational behavior of polymers that are appropriately labeled. The high sensitivity of the fluorescence label permits small amounts of label to be used so that the properties of the label do not significantly influence the behavior of the polyelectrolyte itself.^{19–24} The ratio of the intensity of the third (I_3 at 383 nm) to the first (I_1 at 373 nm) fluorescence band of pyrene monomer (I_3/I_1) reflects the polarity experienced by the fluorescence probe.^{25–30} The peak at around 480 nm is assigned to the excimer fluorescence of pyrene, and the intensity ratio of the excimer fluorescence to the monomer fluorescence, I_e/I_m , is termed the “coiling index”.^{5,27,31}

Fluorescence spectra were recorded with a SPEX Fluorolog 3-22 spectrometer. The excitation wavelengths were 335 and 341 nm for unlabeled and labeled polymer, respectively, and the emission was monitored between 350 and 600 nm.

Results and Discussion

Pyrene fluorescence was measured in aqueous solutions of unlabeled PMAOVE and PMAMVE, and the results are presented in Figure 1. Even at high concentrations, the polymer without the hydrophobic side chains, PMAMVE, shows a polarity parameter (I_3/I_1) of 0.6, which is the value we observed for pyrene in water. In contrast, a microenvironment that is substantially hydrophobic was detected in aqueous solutions of PMAOVE, the polymer with hydrophobic side chains. The micropolarity parameter increases with PMAOVE concentration, rising to a plateau at around 500 ppm. At sufficiently low PMAOVE concentrations, the amount of pyrene in the polymer hydrophobic microenvironment is probably comparable to that remaining in the water, and the observed micropolarity parameter is an average for the two environments. As the polymer concentration is increased, more pyrene is partitioned into the hydrophobic nanodomains, until the free pyrene concentration in the water becomes negligible and the polarity parameter reaches a constant plateau value.

The intramolecular chain association in PMAOVE is clearly altered by variations in the pH of the solution. Figure 1 shows

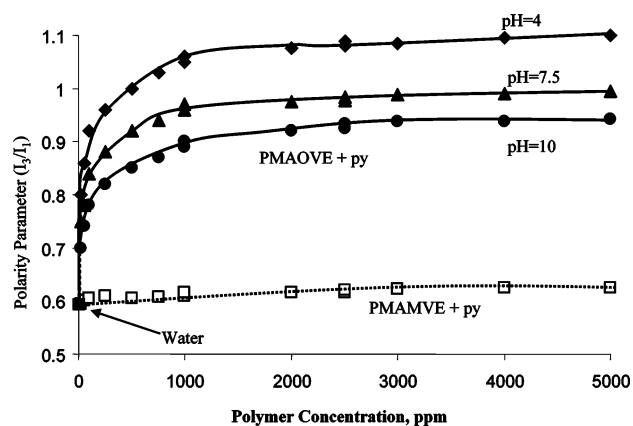


Figure 1. Change in the polarity parameter (I_3/I_1) of pyrene (5×10^{-6} M) fluorescence in aqueous solutions of poly(maleic acid/octyl vinyl ether), PMAOVE, at pH values of 4 (◆), 7.5 (▲), and 10 (●) and poly(maleic acid/methyl vinyl ether), PMAMVE, at pH values of 4, 7.5, and 10 (□).

that, with increasing pH, the polarity parameter (I_3/I_1) of PMAOVE solutions decreases because of increasing ionization of carboxylic groups, which causes electrostatic repulsion between adjacent segments. This results in a more stretched conformation of the polymer and, hence, reduced hydrophobic clustering.

The excimer/monomer ratio (I_e/I_m) for pyrene at constant concentration increases to a maximum in the presence of 500 ppm of PMAOVE, above which a continuous decrease is seen (Figure 2). The excimer/monomer ratio reflects the local concentration of pyrene in the microenvironment. If two or more pyrene molecules are located in one microenvironment, pyrene excimers can be formed. This implies that the polymer concentration at which the maximum ratio is reached (500 ppm of PMAOVE) corresponds to the concentration needed to dissolve the added pyrene. A further increase in polymer concentration reduces the number of pyrene molecules per polymer molecule, leading to local dilution of the pyrene and, consequently, to a decrease of I_e/I_m .

Interestingly, very different fluorescence results were obtained with pyrene-labeled PMAOVE (py-PMAOVE; Figure 3). The conformational state of each PMAOVE and PMAMVE in aqueous solution depends on pH. At low pH, when the carboxylate groups are protonated (uncharged), PMAOVE and PMAMVE adopt a random coil conformation. PMAOVE is tightly coiled because of the presence of hydrophobic groups. Addition of base increases the degree of ionization and produces

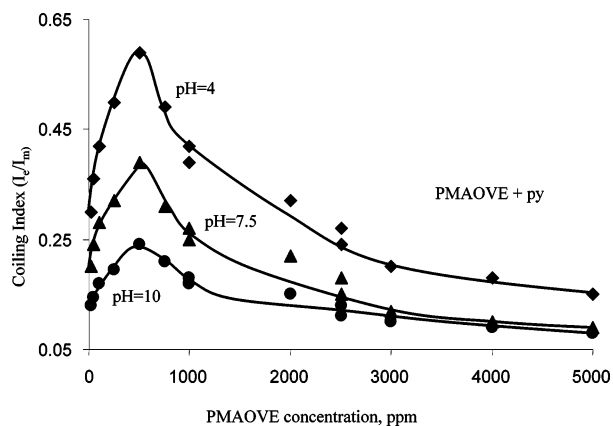


Figure 2. Change in the coiling index (I_e/I_m) for pyrene (5×10^{-6} M) fluorescence in aqueous solutions of poly(maleic acid/octyl vinyl ether), PMAOVE, at pH 4 (◆), 7.5 (▲), and 10 (●).

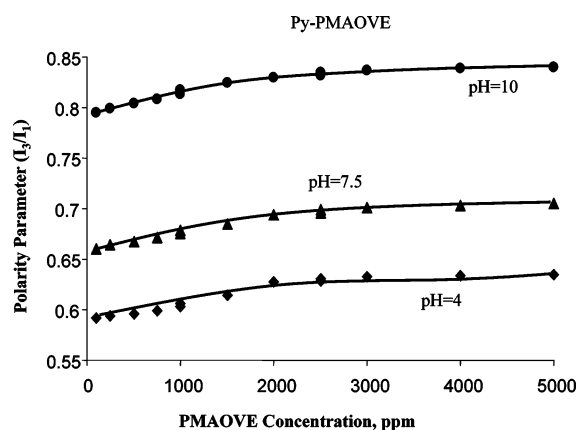


Figure 3. Change in the polarity parameter (I_3/I_1) of pyrene-labeled PMAOVE (py-PMAOVE) at pH 4 (◆), 7.5 (▲), and 10 (●).

chain expansion. The fluorescence measurements revealed that, at lower pH, the pyrene probe is solubilized within the PMAOVE coil. Figure 3 shows the association behavior of py-PMAOVE as a function of concentration and pH. In contrast to the unlabeled polymer, the I_3/I_1 ratio of the pyrene-labeled polymer increases with increasing pH. At pH 4, the I_3/I_1 ratio of py-PMAOVE is about 0.6 (Figure 3), whereas the I_3/I_1 ratio of unlabeled PMAOVE is about 1.07 (Figure 1). With increasing pH, the I_3/I_1 ratio of the pyrene-labeled polymer increases. This unusual behavior suggests that, at low pH, PMAOVE forms hydrophobic nanodomains with the carboxylic groups sticking out into solution. The hydrophobic nanodomains are probably very tightly packed, as indicated by the fluorescence results (Figure 1), so that the pyrene molecules, which are attached to the carboxylic groups in the polymer backbone, are sticking outward. With increasing pH, more carboxylic groups are ionized, and the resultant repulsion among the carboxylic groups leads to some loosening of the hydrophobic nanodomains; this, in turn, should permit the pyrene molecules to come into contact with the hydrophobic nanodomains, which increases the polarity parameter. No change of the I_3/I_1 ratio at different pH for covalently labeled or free pyrene in PMAMVE was observed, because PMAMVE does not form any hydrophobic nanodomains by itself.

Figure 4 shows the I_e/I_m values of py-PMAOVE as a function of its concentration and pH. Usually with increasing pH, the I_e/I_m ratio decreases in the case of unlabeled polymer, because of the repulsion among carboxylic groups, which results in the swelling of hydrophobic nanodomains. Yet, quite the opposite

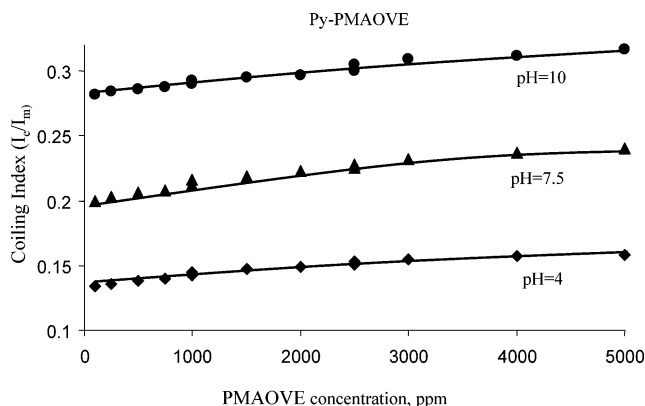


Figure 4. Change in the coiling index (I_e/I_m) of pyrene-labeled PMAOVE (py-PMAOVE) at pH 4 (◆), 7.5 (▲), and 10 (●).

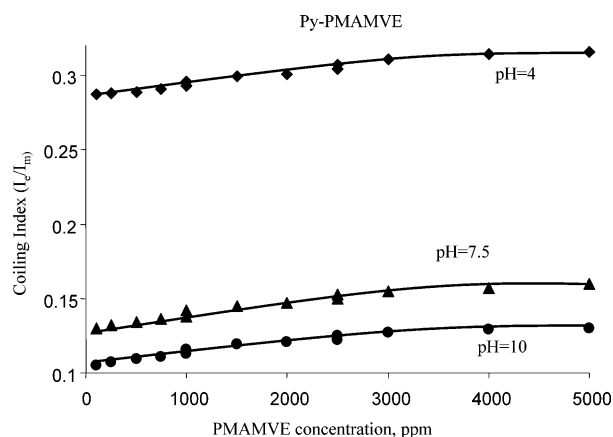


Figure 5. Change in the coiling index (I_e/I_m) of pyrene-labeled PMAMVE (py-PMAMVE) at pH 4 (◆), 7.5 (▲), and 10 (●).

coiling behavior is observed in the case of py-PMAOVE. We attribute this difference to the constraints of the pyrene bound to the polymer in comparison to the free pyrene. In this case, the coiling index increases with increasing pH. This polymer forms hydrophobic nanodomains at all concentrations; the pyrene molecules that are attached to the carboxylic groups are probably constrained in getting into the nanodomains on the polymer, particularly at lower pH when the domains are tightly packed. With increasing pH, there is an increase in the repulsion among carboxylic groups, and this, in turn, makes the nanodomains swell or open up. The hydrophobic pyrene can then enter the swollen hydrophobic nanodomains of the polymer, causing an increase in the measured coiling index value with increasing pH. Interestingly, the opposite coiling index changes are observed in the case of py-PMAMVE (Figure 5). Hydrophobic groups are absent in PMAMVE, and the bound pyrene molecules act as hydrophobic groups in the py-PMAMVE. At the lower pH of 4, only a few carboxylic groups are ionized, and py-PMAMVE orients itself in such a way that the pyrene molecules are inside the polymer core, resulting in a higher I_e/I_m value at pH 4. With increasing pH, more carboxylic groups become ionized, increasing repulsion among the ionized carboxylic groups and consequently stretching of the polymer. Such stretching of the polymer chain makes it difficult for the pyrene molecules to come into contact with each other to form excimers, and this, in turn, results in a decrease in the I_e/I_m values, as seen in Figure 5.

As per the above hypothesis, addition of free pyrene to the pyrene-labeled PMAOVE solution should give fluorescence behavior similar to that of unlabeled PMAOVE at different pH

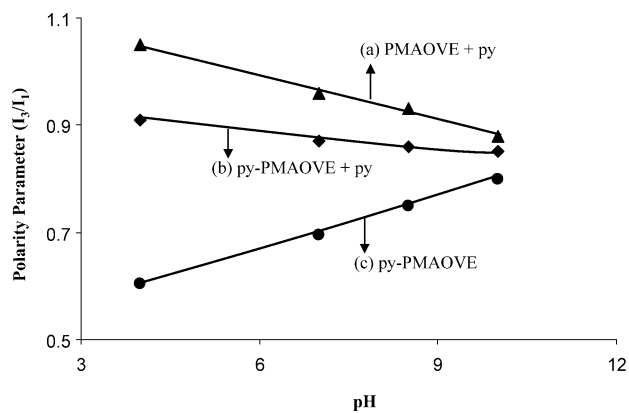


Figure 6. Change in the polarity parameter (I_3/I_1) of (a) unlabeled PMAOVE (1000 ppm) (\blacktriangle), (c) pyrene-labeled PMAOVE (1000 ppm) (\bullet), and (b) a mixture of pyrene-labeled PMAOVE (1000 ppm) and pyrene (\blacklozenge) with pH. (Free pyrene = 5×10^{-6} M.)

values, as the free pyrene molecules will prefer to go into the hydrophobic nanodomains of PMAOVE than into the solution. Therefore, the behavior of the polarity parameter (I_3/I_1) should be similar to that of unlabeled polymer (Figure 6a). Figure 6b shows the I_3/I_1 values for the mixture of free pyrene and pyrene-labeled PMAOVE (1000 ppm) at different pH's. At pH 4, the I_3/I_1 value of the mixture is 0.93, which is less than that of unlabeled polymer ($I_3/I_1 = 1.07$, Figure 6a) but much higher than the 0.6 value for the labeled polymer (Figure 6c). Pyrene molecules attached to the polymer stick out into bulk water, and the measured I_3/I_1 value is the average of the values for the attached pyrene molecules sticking out into the water and free pyrene molecules inside the hydrophobic nanodomains of polymer. With increasing pH, the I_3/I_1 values of the mixture of unlabeled polymer with pyrene added externally decrease, whereas the I_3/I_1 ratio of the mixture of free pyrene and pyrene-labeled PMAOVE does not follow the same trend at all pH values. With increasing pH, more carboxylic groups are ionized, so there is more repulsion among the carboxylic groups, which results in the swelling of hydrophobic nanodomains. With the loosening of the hydrophobic nanodomains, the pyrene molecules that are attached to the polymer can come into contact with the swelled nanodomains. Only a few of the pyrene molecules attached to the polymer will be sticking out into the water. For this reason, the I_3/I_1 values of the above mixture do not follow the same trend with increasing pH. No change in the I_3/I_1 ratio at different pH's was observed for free pyrene and py-PMAMVE mixtures, because PMAMVE does not form any hydrophobic nanodomains by itself. The I_3/I_1 value of PMAMVE is similar at all pH values. The coiling index (I_c/I_m) of the py-PMAOVE + pyrene mixture follows a trend similar to that of polarity parameter (I_3/I_1) values. These results thus confirm the presence of stable hydrophobic nanodomains in PMAOVE in a wide range of pH.

A schematic representation of the uncoiling of the py-PMAOVE domain due to pH changes is presented in Figure 7. At pH 4, PMAOVE protects the hydrophobic groups from hostile water medium by putting them in the inner part of the coil to form very nonpolar hydrophobic nanodomains, as the carboxylic groups of PMAOVE are orientated toward the bulk water and the pyrene molecules attached to the carboxylic groups of the polymer are also orientated toward the bulk water. As the nanodomains formed by PMAOVE are very tightly packed, it is quite difficult for pyrene molecules to penetrate into the nanodomains of the polymer. With the addition of base, the degree of carboxylic ionization increases, which results in

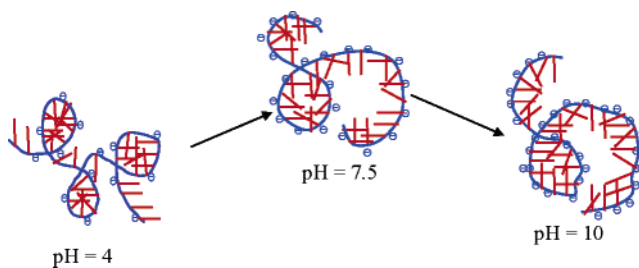


Figure 7. Schematic representation of changes in the structure of PMAOVE in aqueous solution with increasing pH.

chain expansion due to electrostatic repulsion. This leads to the swelling or loosening of the hydrophobic nanodomains of PMAOVE, allowing the pyrene molecules to come into contact with the nanodomains and, consequently, increasing the I_3/I_1 ratio.

Conclusion

Hydrophobically modified polyelectrolytes (HM polymers) undergo self-organization in aqueous environments to form well-defined assemblies. The association behaviors of poly(maleic acid/octyl vinyl ether) (PMAOVE) and poly(maleic acid/methyl vinyl ether) (PMAMVE) were studied using fluorescence techniques, employing pyrene as a probe (covalently linked to the polymers and free pyrene). Fluorescence results of unlabeled PMAOVE with added pyrene indicate the existence of hydrophobic intramolecular nanodomains formed by the octyl side groups. Under acidic conditions (pH = 4), the polymer is tightly coiled. Increasing pH ionizes COOH groups on PMAOVE, which leads to stretching or swelling of the polymer nanodomains. This structural reformation is supported by the fluorescence results of pyrene-labeled PMAOVE. Under acidic conditions (pH = 4), the hydrophobic nanodomains are tightly packed and probably not accessible for pyrene that is covalently attached to the polymer backbone. However, with increased ionization of the carboxylic groups of the polymer backbone and chain expansion, the hydrophobic nanodomains become accessible to the covalently labeled pyrene. In contrast to PMAOVE, the equivalent polymer without hydrophobic side chains, PMAMVE, does not form hydrophobic nanodomains.

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