Pyrene-Labeled Graft Copolymers of *N*-Vinylcaprolactam: Synthesis and Solution Properties in Water

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ABSTRACT: Thermosensitive homopolymers of *N*-vinylcaprolactam and graft copolymers of *N*-vinylcaprolactam and poly(ethylene oxide)−alkyl methacrylate macromonomer were synthesized with different amounts of hydrophobic fluorescent labels, pyrene, and naphthalene. Association of the polymers in dilute aqueous solutions was studied below and above the LCST using dynamic light scattering, microcalorimetry, and various fluorescence spectroscopy techniques, including nonradiative energy transfer (NRET) and quenching experiments. At room temperature the labeled PVCL homopolymers associate via hydrophobic interactions between labels of different chains, while the amphiphilically grafted PVCL forms mainly intrapolymeric micelles. Upon heating, the polymers aggregate in water and form stable nanosized particles. Results from quenching experiments indicate that the PEO containing grafts are able to create a hydrophilic environment in the vicinity of pyrene units, even at temperatures higher than the LCST. The absence of energy transfer from naphthalene-labeled polymers to pyrene-labeled polymers confirms that, on the contrary to labeled homopolymers, the grafted copolymers do not mix at room temperature, even after the heating cycle. The consequences of amphiphilic modification are discussed in terms of the mechanism of stabilization and of the possible applications of PVCL samples carrying both hydrophobic groups and poly(ethylene oxide) groups.

Introduction

There exists a wealth of information on the solution properties of neutral water-soluble polymers and on their applications in household fluids and cosmetic and pharmaceutical formulations as well as in performing fluids for industrial processes, such as waterborne coatings or paper treatment fluids.[1−4] This remarkable situation results from a number of factors, related both to the quest for "green" processes and to the wide range of physicochemical properties that can be achieved with this class of polymers, either as available commercially or after further modification. Unlike polyelectrolytes, nonionic water-soluble polymers in water show only mild sensitivity to the presence of salts or various classes of surfactants. Their solution properties tend to reflect in a predictable way the hydrophilic/hydrophobic balance among their constituents.[5] Chemical modification via introduction of hydrophobic grafts or end groups has profound effects on the solution properties of water-soluble polymers. Hydrophobically modified macromolecules assemble in water through inter- or intrapolymeric clustering of their hydrophobic substituents.[6] The features of aqueous neutral macromolecules solutions may be modulated further, if they possess, as a consequence of the polymer chemical composition, a sensitivity to changes in pH or temperature. For examples, polymers such as poly(*N*-vinylcaprolactam), PVCL,[7] poly(*N*-isopropylacrylamide), PNIPAM,[8] poly(vinyl methyl ether), PVME,[9,10] and poly(2-ethyl-2-oxazoline)[11,12] are soluble in water as long as their solutions are kept below a precise temperature, known as their cloud point or lower critical solution temperature (LCST), at which solutions undergo phase separation.

The object of this report concerns PVCL and a new family of PVCL derivatives grafted with amphiphilic substituents each consisting of a poly(ethylene oxide) chain linked to an undecyl chain (Figure 1). PVCL shares several properties with PNIPAM, the prototype thermally responsive polymer; most notably, its aqueous solutions undergo phase transition in the same temperature range 32−38 °C. However, several studies point to the fact that the phase transitions for solutions of PVCL and PNIPAM proceed via two different mechanisms.[13,14] It has been suggested as well that PVCL, like the structurally related poly(vinylpyrrolidone), may exhibit enhanced biocompatibility, compared to PNIPAM.[15]

Reports on the synthesis of modified PVCL are scarce, especially compared to PNIPAM which has been modified by grafting[16−18] or end-modification[19] with alkyl groups, fluorescent groups,[17,18,20] and poly(ethylene oxide), PEO, moieties[21−23] as well as via copolymerization with comonomers such as acrylic acid[24,25] and various acrylamides,[5] such as glycinyl acrylamide.[26] In the case of PVCL, there have been reports on the copolymerization of *N*-vinylcaprolactam with hydrophilic monomers such as *N*-vinylpyrrolidone,[27] methacrylic acid,[28] and *N*-vinylimidazole[29] and, more recently, on the synthesis of PVCL bearing either hydrophilic or hydrophobic side chains.[30] A preliminary study of the properties of hydrophobically modified PVCL indicates that, like their PNIPAM counterparts, they form polymeric micelles in water below the phase transition temperature.[30]

We report here a detailed investigation of the solution properties of hydrophobically modified PVCL grafted with varying amounts of alkyl groups each terminated with a poly(ethylene oxide) chain. The main tools of our study are static and dynamic light scattering (SLS and DLS), scanning microcalorimetry, and fluorescence spec-

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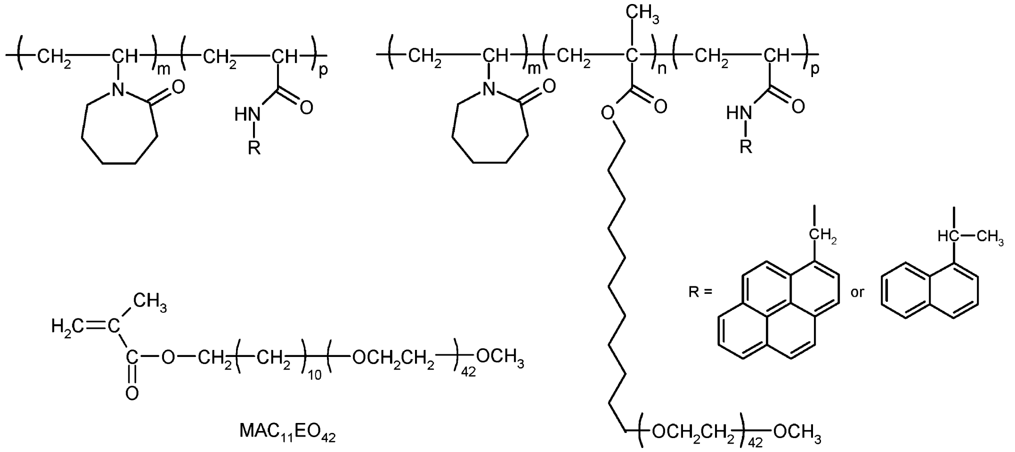

Figure 1. Chemical structures of the labeled PVCL, grafted PVCL, and the amphiphilic macromonomer $MAC_{11}EO_{42}$.

Table 1. Summary of the Reaction Conditions

						composition	
sample	[VCL] (mol/L)	[$MAC_{11}EO_{42}$] (mmol/L)	[PyAAm] (mmol/L)	[NpAAm] (mmol/L)	[AIBN] (mmol/L)	$MAC_{11}EO_{42}$ (mol %)	label (mol/g)
PVCL-Py1	0.90		3.0		2.1		3.3×10^{-5}
PVCL-Py2[a]	0.90		3.0		2.1		6.7×10^{-5}
PVCL-$C_{11}EO_{42}$-Py1	0.90	11.8	3.0		2.1	1.1	2.8×10^{-5}
PVCL-$C_{11}EO_{42}$-Py2[a]	0.90	11.8	3.0		2.1	1.2	4.6×10^{-5}
PVCL-Np	0.90			3.0	2.1		3.2×10^{-5}
PVCL-$C_{11}EO_{42}$-Np	0.90	11.8		3.0	2.1	1.1	2.7×10^{-5}

[a] Polymerized in DMF.

troscopy measurements carried out with PVCL samples labeled with either pyrene or naphthalene groups. The photophysics of pyrene, in particular the relative extent of pyrene monomer and excimer emission, were monitored as a function of polymer concentration, temperature, and the presence of a quencher of fluorescence. The efficiency of nonradiative energy transfer (NRET) between naphthalene and pyrene in mixed solutions of naphthalene- and pyrene-labeled samples was determined in order to assess the extent of interpolymeric association in aqueous solutions of PVCL and grafted PVCL samples. We monitored the temperature-induced collapse of the PVCL chains and its consequences on the size and stability of the polymeric assemblies. We demonstrate that, as a consequence of their molecular architecture, the modified PVCL samples self-assemble in water below the solution LCST, forming polymeric micelles that preserve their internal structure when brought above the phase transition temperature. The consequences of this noteworthy behavior are discussed in terms of the mechanism of stabilization and of the possible applications of PVCL samples carrying both hydrophobic groups and poly(ethylene oxide) groups.

Experimental Section

Materials. 1-Pyrenylmethylamine hydrochloride, 95%, 1-(1-naphthyl)ethylamine, 98%, nitromethane, 99+%, and acryloyl chloride were purchased from Aldrich Chemicals Corp. and were used as received. Dichloromethane was distilled from calcium hydride under nitrogen. Triethylamine was dried by distillation from NaOH under N_2. 2,2′-Azobis(isobutyronitrile) (AIBN, Aldrich Chemicals) was purified by recrystallization from methanol. *N*-Vinylcaprolactam (VCL, 98% Aldrich Chemicals Corp.) was recrystallized from benzene. The amphiphilic macromonomer, $MAC_{11}EO_{42}$ ($M_w = 2110$ g/mol, Figure 1), was prepared as described previously.[31] It consists of a hydrophilic PEO chain (42 ethylene oxide units) linked to a undecyl (11 methylene units) methacrylate.

Synthesis of (1-Pyrenylmethyl)acrylamide (PyAAm). Acryloyl chloride (1.0 mL, 12.3 mmol) was added to a stirred solution of 1-pyrenylmethylamine hydrochloride (1.02 g, 3.7 mmol) and triethylamine (3.0 g, 30 mmol) in 60 mL of dichloromethane over a period of 30 min under nitrogen at 0 °C. The reaction mixture was stirred overnight at room temperature in the dark; it was diluted with chloroform (50 mL) and filtered to remove triethylamine hydrochloride formed during the reaction. The filtrate was washed with 1 M HCl, brine, 1 M $NaHCO_3$, and brine, successively. The organic layer was dried over magnesium sulfate, filtered, and evaporated under reduced pressure to give a crude product, which was recrystallized from dichloromethane three times yielding 1-pyrenylmethylacrylamide as pale yellow crystals. ^1H NMR: Bruker 300 MHz spectrometer: δ, (ppm, $CDCl_3$): 5.2 (d, 2 H), 5.7 (d, 1 H), 5.9 (s, 1 H) 6.1 (t, 1 H) 6.4 (d, 1 H), 7.5 (m, 4 H), 7.9–8.3 (m, 9 H).

Synthesis of 1-(1-Naphthyl)ethylacrylamide (NpAAm). Acryloyl chloride (0.55 g, 6.1 mmol) was added to a stirred solution of 1-(1-naphthyl)ethylamine (0.70 g, 4.1 mmol) and triethylamine (0.62 g, 6.1 mmol) in dichloromethane (24 mL) over a period of 30 min under nitrogen at 0 °C. The reaction mixture was stirred overnight at room temperature in the dark; it was diluted with chloroform (30 mL) and filtered in order to remove triethylamine hydrochloride crystals. The filtrate was washed with 1 M HCl, brine, 1 M $NaHCO_3$, and brine, successively. The organic layer was dried over magnesium sulfate and evaporated under reduced pressure to give crude product, which was recrystallized from dichloromethane three times to give(1-naphthyl)ethylacrylamide as white crystals. ^1H NMR: Bruker 300 MHz spectrometer: δ, (ppm, $CDCl_3$): 1.7 (d, 2 H), 5.6 (d, 1 H), 5.8 (s, 1 H) 6.0 (m, 2 H), 6.3 (d, 1 H), 7.5 (m, 4 H), 7.8 (d, 1 H), 7.9 (d, 1 H), 8.1 (d, 1 H).

Polymerizations. All polymerizations were conducted in solutions, choosing either benzene or dimethylformamide (DMF) as solvent, using the concentrations of monomer(s) and AIBN, the free-radical initiator, listed in Table 1. The monomers were dissolved in the solvent, and the solution was degassed with nitrogen for 30 min at room temperature. The reaction mixture was heated to 70 °C, charged at once with the initiator solution, and kept at 70 °C for 15 h. After that, the reaction mixture was cooled to room temperature, and the polymer was isolated by precipitation from benzene into hexane or from DMF into diethyl ether. The polymers were purified further by two additional precipitations from THF into hexane. The recovered dry polymers were dissolved in water and purified by dialysis against water for 7 days. They were isolated by freeze-drying. The structure and purity of the polymers were ascertained by ^{1}H NMR spectroscopy with a 200 MHz Varian Gemini 2000 spectrometer: for labeled PVCL δ (ppm, $CDCl_3$): 4.4 (1 H, $-NCH-$ at the α position), 3.2 (2H, $-NCH_2-$), 2.4 (2H, $-COCH_2-$), 1.2−2.0 (6H, $-CH_2-$ at caprolactam ring, and 2H, $-CH_2-$ at backbone). The spectrum of grafted PVCL shows a well-separated signal at 3.7 ppm, corresponding to methylene protons of the macromonomer ethylene oxide chains. The degree of grafting of the copolymers was determined on the basis of the signals at 3.7 and 4.4 ppm. The incorporation levels of pyrenyl groups and naphthyl groups were determined from the UV absorbance of polymer solutions in methanol, using as model compounds 1-pyrenylmethylamine (ϵ = 37 000 at 340 nm) and 1-(1-naphthyl)-ethylamine (ϵ = 7900 at 290 nm) for the Py and Np groups, respectively. The compositions of the copolymers are listed in Table 1.

Instrumentation and Analytical Techniques. Fluorescence Spectroscopy. Steady-state fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a GRAMS/32 data analysis system. Temperature control of the samples was achieved using a water-jacketed cell holder connected to a Neslab circulating bath. The temperature of the sample fluid was measured with a thermocouple immersed in a water-filled cuvette placed in one of the four cell holders. All measurements were carried out at 20 °C unless otherwise stated. Emission spectra were recorded with an excitation wavelength 334 nm. The slits were set at 1.0−2.0 mm (excitation) and 0.5−1.0 mm (emission) depending on the chromophore concentration. The excimer-to-monomer ratio (I_E/I_M) was calculated by taking the ratio of the emission intensity at 480 nm to the half-sum of the emission intensities at 380 and 400 nm.

Fluorescence lifetimes were measured using a Fluorolog-Tau-3 multifrequency phase modulation fluorometer (Jobin-Yvon Horiba Inc.). The excitation light from a 450 W xenon lamp was modulated with a Pockels cell. Phase and modulation values were determined relative to a glycogen aqueous solution. The excitation wavelength was set at 334 nm. Pyrene monomer and excimer emissions were monitored at 396 and 480 nm, respectively. The frequency of the analyzing light was chosen in the range of 0.1−100 MHz. All measurements were carried out at 20 °C. Data were analyzed with the Datamax Spectroscopy software based on GRAMS/32 from Galactic Ind. Data were fit to a multiexponential decay law, $F(t) = \sum a_i e^{-t/\tau_i}$, where a_i and τ_i are the preexponential factors and the lifetime of the ith component, respectively. The goodness of the fit was determined by the χ^2 value ($\chi^2 < 1.1$) and by examination of the residuals. The average lifetime $\langle t \rangle$ was calculated from $\langle t \rangle = \sum a_i \tau_i^2 / \sum a_i \tau_i$.

Solutions for Spectroscopic Analysis. Solutions for analysis were prepared from stock solutions (1 g/L). Solutions were kept in the dark at 5 °C for 12 h prior to measurements. Aqueous solutions were not degassed. Methanol solutions were purged with solvent-saturated argon for 5 min.

The quenching experiments were performed with aqueous solutions of PVCL-Py1 and PVCL-$C_{11}EO_{42}$-Py1 at temperatures below the phase transition temperature (20 °C) and above it (50°C). The water-soluble neutral compound, nitromethane, was employed as the quencher of pyrene fluorescence. The polymer concentration was kept constant (0.05 g L^{-1}) while the quencher concentration varied from 0 to 15 mM. Data were analyzed following the Stern−Volmer model.[32] In this treatment the fluorescence intensities I_0 and I in the absence and in the presence of quencher, respectively, are related to the quencher concentration [Q] by eq 1, where K_{SV} is the Stern−Volmer constant, k_q is the bimolecular quenching rate constant, and τ_0 is the lifetime of the fluorophore in the absence of quencher:

$$I_0/I = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q] \qquad (1)$$

For nonradiative energy transfer measurements mixed solutions of pyrene- and naphthalene-labeled polymers were prepared by mixing preformed aqueous solutions of the labeled polymers at 20 °C. The mixtures were allowed to equilibrate for 5 h before the measurements. Solutions with the same concentration of naphthalene (0.5 g L^{-1}) or pyrene (0.125 g L^{-1}) labeled polymer were prepared as well. Heating of the mixtures was accomplished by placing the mixed solutions (2 mL) in a measurement cell into a sample holder at 50 °C. After the sample was allowed to equilibrate for 1 h the solution was cooled to room temperature.

Laser Light Scattering. Static light scattering (SLS) and dynamic light scattering (DLS) measurements were performed using a Brookhaven Instruments BI-200SM goniometer and a BI-9000AT digital correlator. A laser (LEXEL 85, 1 W) operating at a wavelength of 514.5 nm in the power range of 15−50 mW was used as the light source. Time correlation functions were analyzed with a Laplace inversion program CONTIN. The SLS data were treated using Zimm's double-extrapolation method. Molecular weight determinations were conducted at 20.0 °C using solutions of the polymers in THF. The dynamic light scattering (DLS) measurements were performed with a scattering angle of 90°. The specific refractive index increment (dn/dc) was measured with a Abbe 60/ED high-precision refractometer (Bellingham and Stanley, Ltd.) at a wavelength of 514.5 nm. For measurements carried out at 50 °C, aqueous polymer solutions (2 mL, 0.10 g L^{-1}) were filtered at room temperature, quickly heated to 50 °C, and stabilized at this temperature for 1 h prior to measurement. The heating was accomplished by placing the cell from 21 °C into oven at 50 °C. The heating rate at the transition temperature (i.e., at 32 °C) was approximately 2 °C min^{-1}.

Size Exclusion Chromatography (SEC). Measurements were performed with a Waters liquid chromatography system equipped with a Waters 2410 differential refractometer and a Waters 2487 UV as detectors. Three Styragel columns (HR2, HR4, HR6) were used in series. HPLC-grade chloroform was used as eluent with a flow rate of 0.8 mL/min at 30 °C.

High Sensitivity Differential Scanning Microcalorimetry (HS DSC). HSDSC measurements were conducted with a Microcal VP-DSC instrument. Experiments were carried out with scanning rates of 10, 20, 30, 60, and 90 °C h^{-1}. Samples were scanned from 10 to 100 °C under elevated pressure (170 kPa). The polymer concentration of the aqueous solutions scanned was 1.0 g L^{-1}. Prior to each scan, the sample was kept at 10 °C for 15 min.

UV Spectroscopy. UV spectra were measured using a Hewlett-Packard 8452A photodiode array spectrometer equipped with a Hewlett-Packard 89090A temperature controller.

Results and Discussion

Synthesis and Characterization of the Polymers. Six different modified PVCL samples were prepared via free radical solution polymerization of vinylcaprolactam and one or several comonomers, together with PVCL which will serve as a reference material (Tables 1 and 2). The polymerizations were carried out either in benzene, yielding polymers of molecular weights of ~250 000 g mol^{-1}, or in DMF, resulting in polymers of lower molecular weight (~40 000 g mol^{-1}). Fluorescently labeled polymers were obtained by copolymerization of VCL with either pyrenyl- or naphthyl-substituted acrylamides in benzene. PVCLs modified with amphiphilic grafts were prepared in a similar manner using the macromonomer $MAC_{11}EO_{42}$ (Figure 1) and fluorescent monomers as comonomers in the solution polymerization of VCL. The reactivity of VCL is known to be quite low compared to other monomers, such as methacrylates,[33] and this may affect the randomness of the distribution of the labels and grafted

Table 2. Molecular Properties of the PVCL Polymers and Characteristic Solution Properties

				mean hydrodynamic radius R_h (nm)			I_E/I_M	
sample	M_w[a] (g/mol)	T_{onset}[b] (°C)	ΔH (J/g)[c]	methanol at 20 °C	water at 20 °C	water at 50 °C	water at 20 °C	methanol at 20 °C
PVCL-330	330 000	32.1	31	17	15	89		
PVCL-Py1	220 000	32.1	31	15	22	84	1.74	1.13
PVCL-Py2	47 000	38.0	30	9	79[d]	158	0.78	0.66
PVCL-$C_{11}EO_{42}$-Py1	290 000	33.1	24	25	27	110	0.33	0.45
PVCL-$C_{11}EO_{42}$-Py2	36 000	39.7	27	6	5	176	0.14	0.23
PVCL-Np	210 000	32.7	32	15	17	83		
PVCL-$C_{11}EO_{42}$-Np	250 000	33.0	26	20	22	85		

[a] The molecular weight distribution for all polymers was approximately 1.7. [b] The onset of aggregation determined from the onset of the endothermic peak in the DSC scan. Accuracy ±0.1 °C. [c] In joules per grams of dry polymer. Accuracy ±2 J/g. [d] A very weak signal located at 5 nm was also observed.

alkyl poly(ethylene oxide) moieties along the chain. It may be expected that the distribution of the latter along the polymer backbone be random, rather than “blocky”, given the larger size of the macromonomer, compared to VCL. Steric effects may offset the difference in the reactivity between the macromonomer methacrylate group and VCL.[34] Size exclusion chromatography was used to ascertain the absence of low-molecular-weight residues in the polymer samples. All polymers were characterized by NMR spectroscopy to establish the level of macromonomer incorporation and UV absorption to determine the fluorescent label content of the copolymers assuming that the extinction coefficient of the chromophores, pyrene or naphthalene, linked to the polymers is identical to that of the corresponding model compound. The molecular weights of all copolymers were obtained from static light scattering measurements.

Solution Properties of the Modified PVCL Samples. Microcalorimetry Studies. Aqueous solutions of PVCL exhibit a reversible heat-induced phase transition, changing from homogeneous solutions to milky, phase-separated fluids, as the temperature reaches a value between 32 and 38 °C. A series of DSC measurements were carried out to ascertain that labeling PVCL with small levels of Py or Np does not affect significantly the phase transition temperature of the corresponding solutions and to determine the LCST of the PEO-alkyl grafted PVCL solutions. They yielded the temperature of the onset of the transition (T_{onset}), the temperature of the endotherm maximum (T_m), and the heat of the transition (ΔH) (Table 2). We have shown previously that T_{onset} corresponds more closely to the cloud point temperature and can be taken as the onset of the aggregation process as well.[35]

The onset temperatures of the transitions for solutions of grafted PVCL samples are higher by ~1 °C compared to those of PVCL of comparable molecular weight (Figure 2 and Table 2). In general, introducing hydrophobic substituents along a thermosensitive water-soluble polymer results in a decrease of the phase transition temperature,[5] whereas incorporation of hydrophilic groups triggers an increase of this temperature. In the modified PVCL samples described here, an equal number of hydrophobic and hydrophilic moieties are linked to the PVCL chain. The fact that the transition temperature is higher for solutions of the modified polymers implies an enhancement of the *hydrophilicity* of the polymer. This trend is consistent with the fact that the hydrophilic ethylene oxide chain is longer (42 EO units) than the hydrophobic chain (11 methylene units). We note that the onset temperature of the transition is not affected significantly by the introduction of the fluorescent labels, with values of ~32.5 ± 0.1 °C for samples of high molecular weight and ~38 ± 0.1 °C for samples of lower molecular weights. This effect of molecular weight on the phase transition temperature of PVCL aqueous solutions is a distinctive feature of this polymer,[7] and the low level of fluorescent groups incorporation does not affect it.

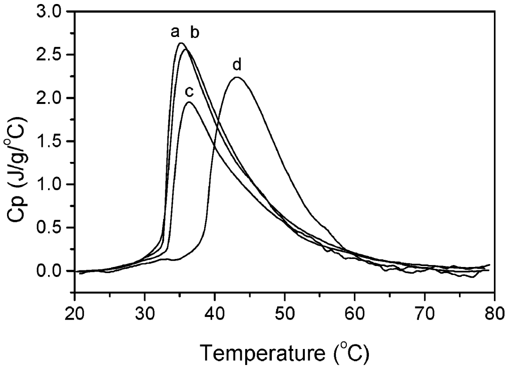

Figure 2. Microcalorimetric endotherms for aqueous solutions of PVCL-330 (a), PVCL-Py1 (b), PVCL-$C_{11}EO_{42}$-Py (c), and PVCL-Py2 (d). Heating rate: 60 °C h^{-1}; polymer concentration: 1 g L^{-1}.

The experimental heat of the transition, ΔH, was proportional, within experimental error, to the VCL content of each modified PVCL (Table 2). Neither grafting nor fluorescence labeling had any significant effect on the enthalpy of transition, which is dominated by the thermodynamics of the PVCL chains and its interactions with water molecules. This observation is in agreement with a previous report of the hydrophilically modified PVCL phase transition.[36] In summary, the microcalorimetry studies lead us to conclude that the presence of grafted amphiphilic groups has a mild effect on the thermodynamic characteristics of the phase transition of aqueous PVCL, as expected on the basis of previous studies, and that the low level of fluorescent groups incorporation remains undetected, from a thermodynamic viewpoint.

Light Scattering Studies. We set out next to assess by dynamic light scattering measurements the effects of the fluorescent labels and the amphiphilic grafts on the association of PVCL in water, below the phase transition temperature (20 °C) and above it (50 °C). Measurements were carried out also on solutions of the copolymers in methanol, a good solvent for PVCL, and the amphiphilic chains grafted onto PVCL to estimate the hydrodynamic radius (R_h) of the copolymers under conditions for which interpolymeric association does not occur. If one compares the hydrodynamic radii of PVCL and amphiphilically grafted PVCL, one notes that the

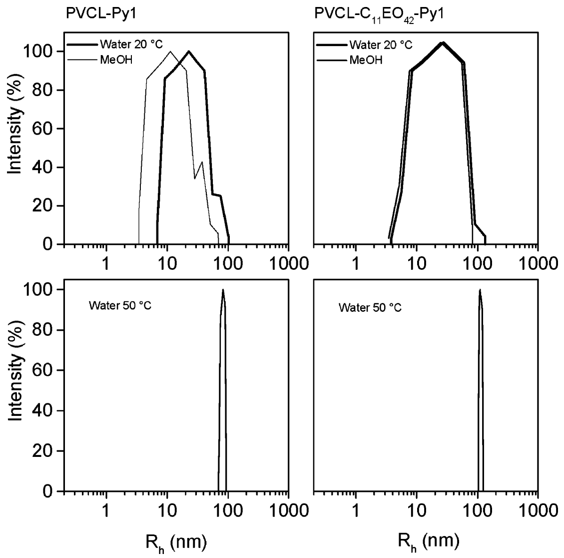

Figure 3. Apparent hydrodynamic radii, R_h, for PVCL-Py1 (left column) and PVCL-$C_{11}EO_{42}$-Py1 (right column) in methanol at 20 °C, in water at 20 °C, and in water at 50 °C. Polymer concentration was 1 g L^{-1}, except at 50 °C where the concentration was 0.1 g L^{-1}.

hydrodynamic radius of grafted copolymer is consistently slightly larger than those of unmodified PVCL (Table 2). Nonetheless, the differences are small enough to be attributed to the increase in the molecular weight of individual polymer chains due to grafting and not to interchain aggregation. Representative size distributions are depicted in Figure 3, top, which present the size distribution of solutions of the PVCL samples of higher molecular weight.

Also presented in Figure 3, top, are the size distribution recorded at 20 °C for aqueous solutions of the same polymers. Nearly identical distributions were obtained for solutions of $C_{11}EO_{42}$-grafted PVCL dissolved in water and in methanol. Thus, we must conclude that the conformation of the polymers in the two solvents are analogous and, more importantly, that the presence of PEO grafts effectively prevents interchain association, even for polymers carrying hydrophobic Py groups. We note, however, that the size distribution of the pyrene-labeled polymer PVCL-Py1 in water is shifted slightly to higher sizes, compared to the profile recorded for a solution in methanol, (Figure 2, top). This observation is taken as an indication that the Py-labeled polymers may undergo some interchain association, presumably via hydrophobic interactions between the chromophores, as observed often in aqueous solutions of pyrene-labeled neutral polymers.[20,37] Moreover, interchain association is enhanced in PVCL-Py2 compared to PVCL-Py1 (Table 2). The lower molecular weight and higher degree of labeling of PVCL-Py2, compared to PVCL-Py1, may account for this trend.

The dynamic light scattering measurements were carried out also with aqueous solutions of the polymers kept at 50 °C, a temperature higher than the phase transition temperature of all polymers in water (Table 2). Polymer solutions prepared at 20 °C were heated rapidly to 50 °C and kept at this temperature for 1 h prior to measurement. Under these conditions, the solutions (0.1 g L^{-1}) turned faintly opaque to the eye. The size distribuitons of the solutions reveal the existence of polymer associates with hydrodynamic radii from 78 to 176 nm, depending on the sample (Table 2). Remarkably narrow size distributions were observed for the aggregates (Figure 3). One might expect that the grafted PEO chains act as steric stabilizers of the PVCL aggregates. It is noteworthy, however, that even the unmodified labeled PVCL samples form aggregates of narrow size distribution as well, resisting macroscopic aggregation and phase separation under the dilute conditions chosen to carry out the dynamic light scattering measurements (Figure 3). This behavior agrees well with previous reports on PVCL[35]and PNIPAM[38,39] solutions maintained at a temperature higher than their respective LCST. The hydrodynamic radius of the aggregates formed by higher molecular weight polymers are slightly smaller than those of the corresponding polymers of lower mass (Table 2) and that, for polymers of comparable molar masses, the $C_{11}EO_{42}$-grafted copolymers form slightly larger aggregates, compared to the unmodified polymers. It is well recognized that the heat-induced phase transition of aqueous solutions of neutral polymers, such as PVCL, coincides with the breaking of hydrogen bonds between units of the polymer chains and water molecules and, consequently, with a dehydration of the polymer chains. It is possible that a fraction of hydrated PEO chains are buried within the aggregated PVCL chains, leading to larger aggregates than in the case of unmodified PVCL chains.

Dynamic light scattering measurements reveal that the solution properties of PVCL in water are affected by the incorporation of pyrene groups, which act as hydrophobic modifiers and somewhat enhance interpolymeric association, at least in solutions kept below their cloud point. This effect of pyrene has been reported previously.[20] However, our studies did not uncover significant differences in the solution properties of the pyrene-labeled $C_{11}EO_{42}$-grafted PVCL samples under the conditions employed for the microcalorimetry and light scattering measurements. In this case, the hydrophilic/lipophilic balance among the polymer various units appears not to be affected significantly by the addition of a small number of hydrophobic fluorescent groups. There is no doubt that the size of the aggregated particles depends critically on a number of experimental variables, such as solution concentration and heating rate, and not only on the molecular weight and composition of the polymers. A detailed investigation of the particle formation of various PVCL copolymers has been carried out and will be reported elsewhere. This study is limited to experimental conditions amenable to fluorescence measurements without interference of undesirable side effects due to excessive fluorophore concentration or turbidity of the samples.

Fluorescence Studies. The photophysics of labeled PVCL were investigated under various conditions to address issues related to (1) the architecture of the polymer chains, that is the distribution of labels along macromolecules, (2) the association in cold water of labeled PVCL and labeled $C_{11}EO_{42}$-grafted PVCL, (3) the composition of the phase-separated aggregates formed in phase-separated fluids formed by PVCL-Py and PVCL-$C_{11}EO_{42}$-Py above the phase transition temperature, and (4) the occurrence of interpolymeric associations in solutions below and above their phase transition temperature.

Architecture of the Polymer Chains. We have indicated in the section describing the preparation of the PVCL copolymers that, due to the difference in the reactivity of vinylcaprolactam and either methacrylates

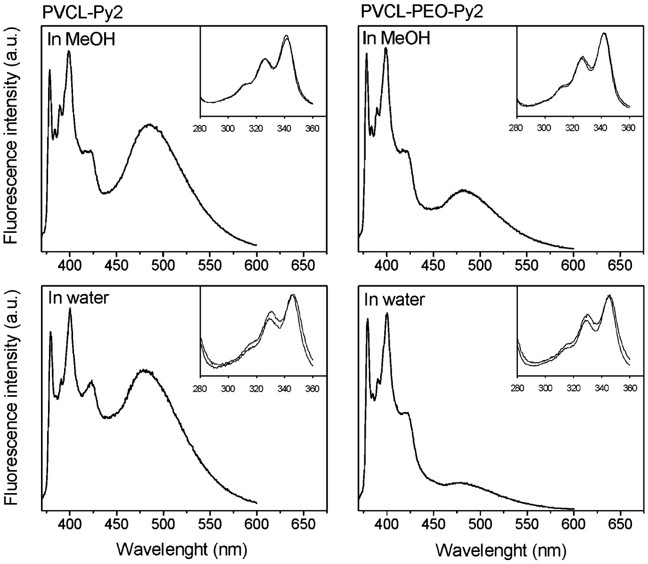

Figure 4. Emission spectrum of 0.05 g/L solutions of PVCL-Py2 (left column) and PVCL-$C_{11}EO_{42}$-Py2 (right column) in methanol at 20 °C and in water at 20 °C. Excitation spectra are presented in the inset.

or acrylamides during free radical polymerization, the distribution of the labels and grafts was not expected to be random, but rather that the various copolymers may present a “blocky” architecture. One can address this question with respect to the distribution of the fluorescent dyes along the macromolecules, using the propensity of pyrene groups to form excited-state dimers (“excimers”) under conditions of high label local concentration. If a polymer carries a small number of pyrene groups distributed along the chain, the Py*/Py encounter probability will be low; hence, excimer emission will be weak compared to the monomer emission.[20] Conversely, if the copolymer presents blocks of high pyrenylacrylamide density, the contribution of pyrene excimers to the overall emission will be high as a result of the presence of high pyrene local concentration domains For this spectroscopic tool to be used effectively, it is imperative to dissolve the labeled polymer in a good solvent to avoid solvent-induced clustering of the labeled. Here, we measured the emission of the pyrene-labeled PVCLs dissolved in methanol, a good solvent for all PVCL samples prepared. Under such conditions, the emission of all pyrene-labeled PVCL samples showed the broad featureless emission (intensity I_E), centered between 475 and 480 nm, characteristic of pyrene excimer emission, in addition to the structured emission, with the [0,0] band at 380 nm, due to spatially isolated excited pyrenes (monomer emission, intensity I_M) (Figure 4a). The intensity of the excimer emission, relative to monomer emission, was high in all cases. A qualitative assessment of the distribution of pyrene-containing monomer units along the macromolecule may be drawn from the values of the ratio I_E/I_M computed from the various spectra (Table 2). Two trends are apparent: (1) the ratio I_E/I_M is higher for polymers prepared in benzene, independently of the presence of grafts and of the level of label incorporation, and (2) in a given series the ratio I_E/I_M is significantly lower for solutions of the grafted labeled polymers, compared to PVCL-Py, implying that blocks of pyrenylacrylamide groups exist to a lesser extent when the VCL/pyrenylacrylamide copolymerization is carried out in the presence of the macromonomer.

Excitation spectra recorded by monitoring the monomer and the excimer emissions were identical (Figure 4 top, insets), indicating that the excimers are formed by a dynamic mechanism which requires diffusion-controlled encounter of a ground-state pyrene and a pyrene in its singlet excited state.[40] Time-dependent measurements also support this conclusion: the excimer profile exhibits a growing-in component ($\tau \sim 26$ ns, in the case of PVCL-$C_{11}EO_{42}$-Py1) and a decay component (τ 55−70 ns, depending on the polymer structure). The pyrene monomer decay profiles, in contrast, were more complex, and a three-component model was required for acceptable fits ($\chi^2 < 1.07$) of the data, with average lifetimes $<\tau>$ ranging from 80 to 100 ns. These values agree well with previous reports on the photophysics of pyrene-labeled polymers.[17,41]

Association of the Polymers in Water. The emission spectra of aqueous solutions of the pyrene-labeled copolymers present the same overall features as the spectra recorded with solutions in methanol, consisting of contributions from Py monomer and excimer emissions. However, in each case, slightly different excitation spectra were obtained when monitoring the monomer and the excimer emissions (insets in Figure 4, bottom). Moreover, in time-dependent measurements with a time resolution of ~1 ns, it was not possible to detect a growing-in component for the excimer profiles. These two observations indicate that, unlike the situation described in the previous section, the excimer formation mechanism is not dynamic but that excimers originate predominantly from preformed ground-state Py dimers or higher aggregates.[42]

There are other differences between the emission spectra recorded with aqueous and methanolic polymer solutions. Most noticeably, the relative magnitude of the excimer emission intensity is significantly higher in the case of aqueous solutions, as revealed by a comparison of the I_E/I_M values recorded for the same polymers in different solvents (Table 2). The enhancement of the excimer emission, compared to the monomer emission, indicates that water induces further association of the hydrophobic pyrene groups, consistent with the detection of polymeric micelles via dynamic light scattering. We carried out a set of measurements with polymer solutions ranging in concentration from 4×10^{-3} to 1.0 g L^{-1} and observed that the Py excimer contribution to the total emission remained constant for all concentrations, in the case of grafted copolymers, but that it showed a mild concentration dependence in the case of PVCL-Py1 and PVCL-Py2, increasing with increasing concentrations. These trends are apparent in plots of the concentration dependence of the ratio I_E/I_M (Figure 5).Thus, the grafted copolymers seem to form mainly intrapolymeric associations, and the increase in concentration does not trigger interpolymeric aggregation, at least within the concentration rang scanned here, whereas in the case of labeled homopolymers interpolymeric Py/Py associations take place to some extent. Similar observations were reported for pyrene labeled PNIPAM.[20]

Temperature Dependence of the Photophysics of the Pyrene-Labeled PVCL Samples in Water. Dilute aqueous solutions of all Py-labeled PVCLs were heated from 10 to 60 °C, and their fluorescence spectra were monitored as a function of temperature. In the case of PVCL-Py1 and PVCL-Py2, the ratio I_E/I_M remained constant until the temperature reached 32 and 38 °C,

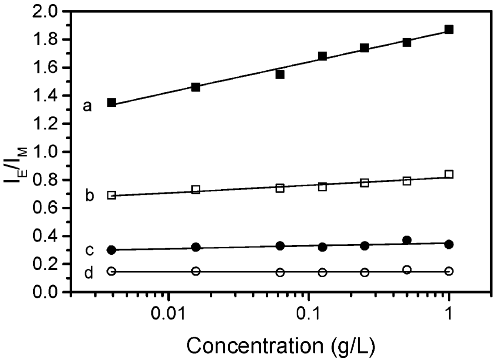

Figure 5. Concentration dependence of the changes of the ratio of the pyrene excimer emission intensity (I_E) to the pyrene monomer emission intensity (I_M) in aqueous solutions of different labeled polymers at 20 °C. PVCL-Py1 (a), PVCL-Py2 (b), PVCL-$C_{11}EO_{42}$-Py1 (c), and PVCL-$C_{11}EO_{42}$-Py2 (d). λ_{exc} = 345 nm.

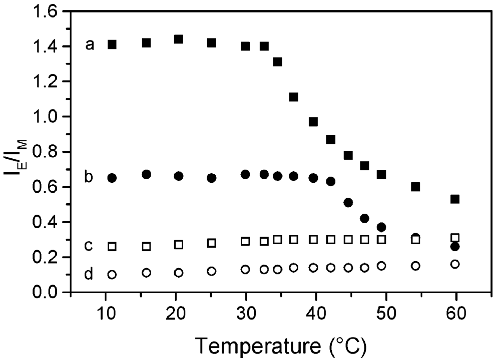

Figure 6. Plots of the changes of the ratio of the pyrene excimer emission intensity (I_E) to the pyrene monomer emission intensity (I_M) as a function of temperature in aqueous solutions of different labeled polymers (0.05 g/L). PVCL-Py1 (a), PVCL-Py2 (b), PVCL-$C_{11}EO_{42}$-Py1 (c), and PVCL-$C_{11}EO_{42}$-Py2 (d). Heating rate = 10 K h^{-1}, λ_{exc} = 345 nm.

respectively, and then it decreased sharply, reaching a value of 0.4 and 0.2, respectively, for solutions at 60 °C (Figure 6). The temperature range over which the changes in I_E/I_M occur is wide, spanning nearly 30 °C. It is in the same range as the temperature over which the endothermic transition was recorded via DSC measurements, and the onset of the decrease in I_E/I_M corresponds to T_{onset} for the two polymers (Figure 2, Table 2). The changes were reversible: upon cooling the solutions, the ratio I_E/I_M increased as the temperature was lowered to attain the same value as before the heating cycle at 20 °C. The excitation spectra recorded for Py monomer and excimer emissions were affected as well by changes in solution temperature. As noted earlier, the excitation spectra did not superimpose for solutions kept below their cloud point. The differences between them vanished, however, for solutions of the polymers heated above their cloud points. From the overall spectral changes with temperature, we conclude that as the solutions reach their respective phase transition temperature, the assembly of the labeled polymers is affected: the pyrene ground-state dimers or higher aggregates that exist in cold solutions are disrupted and pyrene groups are accommodated as isolated entities within the hydrophobic environment of the colloidal PVCL particles formed above the LCST of the solutions. Interestingly, the ratio I_E/I_M for labeled homopolymers solutions at 60 °C in water takes a value lower than the value recorded for solutions of the same polymers in methanol. This observation may be taken as an indication of the higher local viscosity experienced by the pyrene groups in the collapsed polymer phase, compared to the viscosity of methanol, since in both cases the excimer is formed by a dynamic process. The increase in viscosity of phase-separated PVCL has been previously observed for spin-labeled PVCL: the segmental mobility of the spin-label is strongly decreased in the phase-separated polymer.[43]

Aqueous solutions of the pyrene-labeled graft copolymers were subjected to the same heat treatment as PVCL-Py1 and PVCL-Py2. The ratio I_E/I_M was monitored during the heating and cooling process. It remained nearly unchanged over the entire temperature range scanned in the experiment (Figure 6). A very slight decrease in monomer emission was detected as the solution temperature exceeded the respective cloud points of the two copolymers. This observation implies that the microenvironment of the Py groups is hardly affected during the phase transition despite changes in the hydration state of the polymer. It may be an indication that the hydrophilic poly(ethylene oxide) chains of the copolymer remain hydrated even in solutions heated above the cloud point of PVCL.

Quenching of Pyrene Emission. Further support for this interpretation of the photophysical properties of the Py-labeled graft copolymers as their solution undergoes temperature-induced phase separation was gained from a series of fluorescence quenching measurements using nitromethane, a neutral water-soluble quencher of Py fluorescence. Nitromethane quenches pyrene emission with a diffusion-controlled rate by an electron-transfer mechanism.[32] We ascertained first that the phase transition temperature of PVCL solutions was not affected by added nitromethane up to a concentration of 15 mmol L^{-1}, the maximum concentration used in the quenching experiments. Increasing amounts of nitromethane were added to aqueous solutions of the various PVCL samples kept either at room temperature or at 50 °C while keeping constant the polymer concentration (0.05 g L^{-1}). The decrease of pyrene monomer and excimer emission intensities with increasing quencher concentration was recorded and computed in terms of the Stern−Volmer model (see Experimental Section). Linear Stern−Volmer plots were obtained for the quenching of pyrene monomer and excimer emissions recorded for polymer solutions below or above their cloud point. Nitromethane proved to be an effective quencher of Py monomer emission, but its presence in solution hardly affected the Py excimer emission.

The Stern−Volmer plots drawn for nitromethane quenching of Py monomer are presented in Figure 7 for the cases of PVCL-Py1 (top) and PVCL-$C_{11}EO_{42}$-Py1 solutions below and above their cloud points. The Stern−Volmer constants K_{SV} depend on the polymer structure and in the case of PVCL-Py1 on the temperature. K_{SV} for PVCL-Py1 at 20 °C was 1220 ± 60 mol^{-1} L and at 50 °C 680 ± 35 mol^{-1} L. In polymer solutions kept below the cloud point, Py quenching by nitromethane is slightly more effective in the case of the grafted PVCL sample, compared to homopolymer, which indicates that the diffusion rate of nitromethane within homopolymer micelles is somewhat reduced compared to that in micelles of PEO grafted copolymers. K_{SV} for PVCL-$C_{11}EO_{42}$-Py1 at 20 °C was 1680 ± 80 mol^{-1} L and at 50 °C 1730 ± 90 mol^{-1} L.

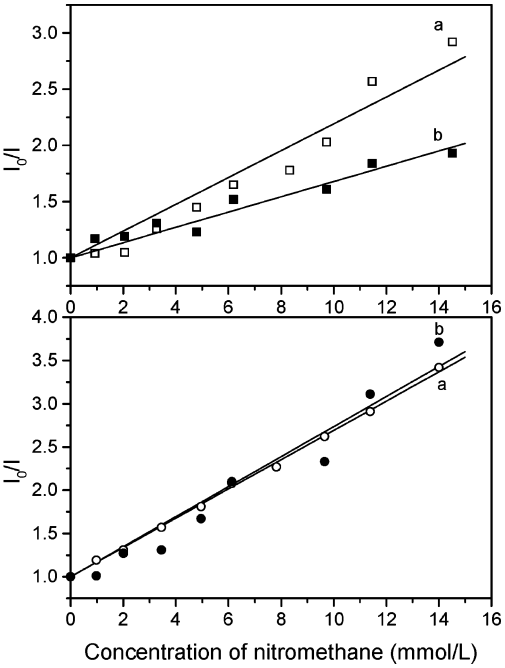

Figure 7. (top) Ratio I_0/I of pyrene monomer emission intensity in the absence and in the presence of a quencher in aqueous solutions of PVCL-Py1 (0.05 g/L) as a function of quencher concentration: (a) 20 °C, open square; (b) 50 °C, full square. (bottom) Ratio I_0/I of pyrene monomer emission intensity in the absence and in the presence of a quencher in aqueous solutions of PVCL-$C_{11}EO_{42}$-Py1 (0.05 g/L) as a function of quencher concentration: (a) 20 °C, open circle; (b) 50 °C, full circle.

Larger differences among the two classes of polymers are seen in their response to the quencher at higher temperature. First, in the case of PVCL-Py1 solutions, the quenching effect of nitromethane on PVCL-Py1 is reduced significantly at 50 °C, compared to 20 °C, at least as judged by comparing the respective Stern–Volmer constants. At 50 °C, the pyrene groups are buried within the polymer-rich phase formed via aggregation of collapsed polymer chains. It is expected that under these circumstances the chromophores are less accessible to the quencher than in the fully hydrated micelles formed at 20 °C and that, consequently, their emission is quenched to a lesser extent. A similar effect has been reported earlier for Py quenching of Py-labeled PNIPAM aqueous solutions.[44] In contrast, in the case of PVCL-$C_{11}EO_{42}$-Py1 aqueous solutions, the quenching efficiency of nitromethane is not affected by an increase of temperature (Figure 7, bottom). The fact that the diffusion of nitromethane is not hindered within aggregated PVCL-$C_{11}EO_{42}$-Py1 micelles strengthen the picture of the grafted copolymers aggregates put forward on the basis of the changes in pyrene emission with temperature, namely that the hydrophilic PEO segments remain hydrated even when the polymer backbone itself has collapsed, expelling bound water molecules. The water molecules trapped inside the phase-separated particles form a low-viscosity environment where nitromethane is free to diffuse and can come in close proximity to the pyrene groups.

Nonradiative Energy Transfer (NRET) Experiments. The process of NRET originates from dipole–dipole interactions between an energy donor in its singlet excited state, naphthalene in this study, and an energy acceptor (Py) in its ground state.[32] The probability of energy transfer between the two chromophores depends sensitively on their separation distance. For the Np/Py pair of chromophore the characteristic distance of the process is 28.9 Å.[45] Thus, the occurrence of NRET between Np and Py in dilute mixed solutions of polymers carrying either Np or Py signals the existence of interpolymeric association. By assessing the extent of energy transfer under various circumstances, it becomes possible to monitor closely the interactions between polymer chains and their evolution upon application of external stimuli such as changes in temperature.

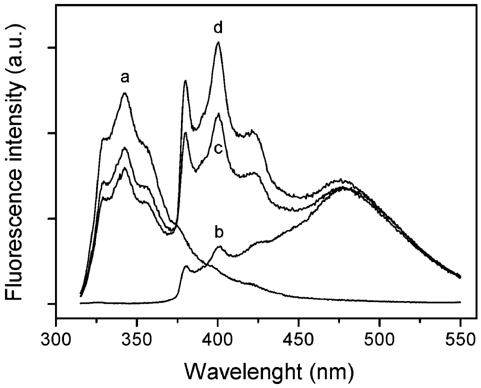

Figure 8. Fluorescence spectra of aqueous solutions labeled homopolymers: (a) PVCL-Py1 (0.125 g/L), (b) PVCL-Np (0.50 g/L) at 20 °C and a mixture of the two copolymers (total concentration 0.625 g/L) at 20 °C immediately after the mixing (c), and after the heating cycle (d).

To carry out successful NRET experiments it is necessary to find experimental conditions such that the energy donor only, or at least predominantly, absorbs the irradiation light. For the Np/Py this condition is met if one selects an excitation wavelength of 290 nm. The emission of an aqueous solution of PVCL-Np, upon excitation at 290 nm, presents a structured band with a maximum at 343 nm characteristic of Np* emission (Figure 8, spectrum a). The spectrum exhibits also a tail at longer wavelength attributable to a small contribution of Np excimer emission. Excitation of aqueous PVCL-Py1 at 290 nm results in a weak emission with contributions from both Py monomer and Py excimer (Figure 8, spectrum b). This contribution from pyrene directly excited at 290 nm needs to be taken into account in the analysis of spectra obtained from a 290 nm excitation of mixed Np- and Py-labeled polymers.

The NRET experiments were carried out first with solutions of the labeled homopolymers, PVCL-Np and PVCL- Py1. Solutions for NRET experiments were prepared by mixing at room temperature aqueous solutions of the singly labeled polymers. Their emission upon excitation at 290 nm was recorded at 20 °C immediately after mixing (Figure 8, spectrum c). The spectrum presents a strong contribution from pyrene emission in addition to the Np emission. By comparing this emission to the spectra of the solutions of each polymer of identical concentration (PVCL-Np, 0.125 g/L; PVCL-Py1, 0.50 g/L), one observes a significant quenching of the naphthalene emission together with an increase of the pyrene emission. The two concurrent effects signal the occurrence of NRET between the two chromophores and hence indicate the existence of interpolymeric association in solutions of labeled PVCL kept at room temperature. The mixed solution was heated to 50 °C, and its spectrum was recorded. The heat treatment triggers a strong enhancement of the extent of NRET, as evidenced by the large increase in

Py emission. In the separated polymer-rich phase chromophores linked to different polymer chains are brought into close proximity, allowing NRET to take place with higher efficiency than at room temperature, where only few interpolymeric contacts exist between the highly hydrated chains. The heated sample was allowed to cool to 20 °C over a period of 30 min. The spectrum of the sample was measured again at this temperature (Figure 8, spectrum d). The intensity of the emission due to Py is reduced significantly in the spectrum of the cooled solution, compared to the spectrum recorded at 50 °C, but it is stronger than in the spectrum recorded from the freshly mixed solution (Figure 8c). Thus, the level of interpolymeric association reflects the thermal history of the sample. The cooled sample was measured also after 12 h of the heating cycle, and a similar spectrum was measured as Figure 8d. The treatment described here results in a mild enhancement of interpolymer association compared to the situation immediately after mixing solutions of the two polymers.

Mixed solutions were prepared as well from preformed solutions of the grafted copolymers PVCL-$C_{11}EO_{42}$-Py1 and PVCL-$C_{11}EO_{42}$-Np. No energy transfer took place between Np and Py immediately after the mixing. The lack of NRET implies that pyrene and naphthalene labels keep separated at 20 °C, giving further support on the assumption of the intrapolymeric association of the graft copolymers. Heating the solution to 50 °C does not cause any detectable energy transfer either. This indicates that Py and Np labels have no close contacts even when the polymer chains are forced to aggregate into the same polymeric particle. The separation of the labels owes to the presence of grafted PEO chains that remain hydrated under thermal conditions where the PVCL chains collapse and phase separate from water. The labels keep separated also upon cooling the mixed solution from 50 to 20 °C, and no NRET-generated emission of Py in the fluorescence spectrum of the cold mixed solution was observed.

Conclusions

As a consequence of labeling, the polymers under study carry very few hydrophobic pyrene groups along the chain. Nonetheless, at room temperature, the properties of aqueous solutions of the labeled PVCL homopolymers are strongly influenced by the presence of the label: the polymers associate via hydrophobic interactions between labels of different chains. The resulting associates are stable at room temperature, but NRET experiments demonstrate that they are disturbed by heating−cooling cycles encompassing their phase transition temperature. Quenching experiments reveal that pyrene units are effectively protected against water-soluble quenchers at elevated temperatures due to the formation of a densely packed polymer phase within heated colloidal particles.

Linking amphiphilic grafts to labeled PVCL chains remarkably affects their solution properties. In aqueous solutions kept at room temperature, the formation of pyrene excimer is independent of polymer concentration. Also, the hydrodynamic radii of the polymers are the same, whether they are dissolved in water or in methanol, implying that the graft copolymers exist as separate entities in aqueous solutions and that the pyrene units are associated mainly intramolecularly. Results from quenching experiments indicate that the PEO containing grafts are able to create a hydrophilic environment in the vicinity of pyrene units, even at temperatures higher than the LCST. The absence of energy transfer from naphthalene-labeled polymers to pyrene labeled polymers confirms that the grafted copolymers do not mix at room temperature, even after the heating cycle. Stable intrachain micelles that are covered with poly(ethylene oxide) might meet various biotechnology needs, such as enzyme stabilization or controlled drug release, especially in view of the biocompatibility of PVCL.[46] Amphiphilic grafts in the structure stabilize the micelles and may simultaneously act as solubilization sites for a hydrophobic substance.

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