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Thermosensitive graft copolymers of an amphiphilic macromonomer and *N*-vinylcaprolactam: synthesis and solution properties in dilute aqueous solutions below and above the LCST

Antti Laukkanen[a], Lauri Valtola[a], Françoise M. Winnik[b], Heikki Tenhu[a,*]

[a]*Laboratory of Polymer Chemistry, University of Helsinki, PB 55, FIN-00014 HY, Finland*
[b]*Department of Chemistry and Faculty of Pharmacy, Université de Montréal, CP 6128 Succursale Centre-ville, Montréal, Que., Canada H3C 3J7*

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Abstract

Thermosensitive graft copolymers of *N*-vinylcaprolactam and poly(ethylene oxide)-alkyl methacrylate macromonomer were synthesised with different grafting densities. Association of the polymers in dilute aqueous solutions was studied below and above the LCST using static and dynamic light scattering, microcalorimetry, pressure perturbation calorimetry (PPC), and fluorescence probe experiments. Owing to the amphiphilicity of the grafts, the most densely grafted polymer forms intrapolymeric structures while the less grafted polymers build up mixtures of intra- and interpolymeric associates at temperatures below LCST. These assemblies solubilise hydrophobic substances such as pyrene. Upon heating, the graft copolymers aggregate in water and form nano-sized particles, mesoglobules. Heat induced particles are exceptionally stable against further aggregation, and the dilution of the solutions has no effect on the particle size or shape. The structure of the particles can be frozen by physically crosslinking the collapsed polymers with a phenol, e.g. 1,2-benzenediol.

Keywords: Thermosensitive polymers; Poly(*N*-vinylcaprolactam); Macromonomers

1. Introduction

Water soluble associative polymers have interested scientists in both academia and industry for several decades. Neutral polymers of varying hydrophilic/hydrophobic balance show a wide range of solution properties in water. For example, hydrophobically modified water soluble macromolecules associate in water forming hydrophobic microdomains [1], and these assemblies impart both dilute and semidilute solutions of unique properties which have found numerous industrial applications [2–4]. Amphiphilic polymers can be produced, not only by modification of water soluble macromolecules, but also by introducing hydrophilic groups onto non-soluble polymers [5].

Several synthetic strategies have been applied to produce well-defined amphiphilic graft copolymers of distinct solution properties. One approach is the post-modification of a preformed polymer either with hydrophilic chains, such as poly(ethylene oxide), PEO [5,6], or with hydrophobic groups, as in the cases of hydrophobically modified cellulose ethers [7]. Another method is the direct copolymerisation of vinyl monomers with hydrophobic or hydrophilic comonomers, such as alkyl methacrylates [8] or methacrylate functionalised PEO chains [5]. Schulz et al. have used methacrylate functionalised amphiphilic macromonomers, composed of alkyl segment and PEO chain, as comonomers with acrylamide [9]. The structure of the macromonomer employed in this study was such that the polymerizable group was attached to the hydrophilic end of the amphiphile. As a consequence, in the graft copolymer, the hydrophobic anchor was separated from the polymer backbone by a hydrophilic PEO spacer. The resulting graft copolymers predominantly formed interpolymeric associates in water of polymer concentration higher than the overlap concentration C^*. The presence of such associates results in an enhancement of solution viscosity, compared to that of the unsubstituted polymer. In dilute solutions,

* Corresponding author. Tel.: +1 358 9 19150322; fax: +1 358 9 19150330.
E-mail address: heikki.tenhu@helsinki.fi (H. Tenhu).

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however, the graft copolymer adopts a contracted conformation via intramolecular interaction and the amphiphilic grafts form micellar structures within a polymer chain. Polymers that show a temperature dependent solubility in water, such as poly(*N*-isopropylacrylamide), PNIPAM, have also been grafted with hydrophobically modified PEO-chains [10]. In that study, Berlinova et al. showed that copolymers of NIPAM and macromonomers consisting of PEO bearing a terminal perfluorooctyl group form intra- or intermolecular associates in water depending on the grafting density and on the concentration of the solution.

Amphiphilic poly(ethylene oxide) macromonomers have been used also as reactive surfactants in emulsion [11,12] and dispersion [13–15] polymerisations to produce stable polymer particles, latexes. The steric stabilisation created by the non-ionic amphiphilic grafts is a way to prepare particles that have a pronounced stability against aggregation even at high electrolyte concentration [16] or after a freeze–thaw cycle [17].

In this study, we have used an amphiphilic PEO-alkyl methacrylate as comonomer of *N*-vinylcaprolactam, to prepare amphiphilically grafted poly(*N*-vinylcaprolactam), PVCL. Contrary to previous studies with amphiphilically grafted associative polymers [9,10], we designed a macromonomer such the polymerizable group is linked to the hydrophobic end of the amphiphile, in order to favor intramolecular association of the alkyl segments, over interpolymeric association. The polymer itself, PVCL, like PNIPAM, is soluble in cold water but phase separates when the temperature exceeds a critical value, the lower critical solution temperature, LCST [18]. It has been shown that PVCL forms nano-sized aggregates when the solution is heated above the cloud point [19]. It was therefore expected that introduction of amphiphilic grafts on a polymer chain will modify the structure of these heat-induced aggregates, and possibly stabilize them against flocculation, as in the case of sterically stabilised latexes [16]. The surface of the aggregates is covered with highly hydrophilic PEO chains, which further stabilise the particles.

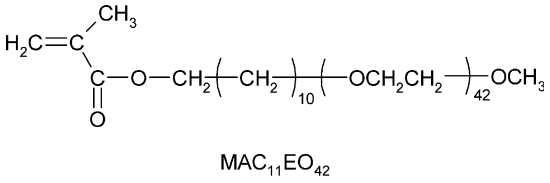
The aim of this study was to synthesize amphiphilically grafted thermally responsive polymers which are capable, on the one hand, to form associates below the LCST via interaction among grafted chains, and on the other hand, form sterically shielded colloidal particles at elevated temperatures. The micellisation of the macromonomer itself was investigated as well in order to assess how the association of this low molecular weight amphiphile is affected upon linking it to a PVCL chain. The association of the graft copolymers in solutions below and above their LCST was monitored by light scattering and by fluorescence spectroscopy, using pyrene as a probe. Moreover, the thermodynamic parameters associated with the phase transition were determined using differential scanning microcalorimetry and pressure perturbation calorimetry. Based on the understanding of the mechanism of the temperature collapse of the copolymers and on their stabilization in solution above the LCST, we devised a new method, presented in the last part of the article, to produce PVCL nanoparticles that remain stable even upon cooling to room temperature.

2. Experimental section

2.1. Materials

N-Vinylcaprolactam, VCL (98%, Aldrich Chemicals, Germany) was purified by recrystallization from benzene. The amphiphilic PEO macromonomer, $MAC_{11}EO_{42}$ (M_w = 2110 g/mol), Fig. 1, was prepared as described previously [20] using the method reported by Liu et al. [21]. First, the hydroxyl group in 11-bromoundecanol (0.09 mol) was protected with 3,4-dihydro-2*H*-pyran (0.32 mol). Then, the protected 11-bromoundecanol (0.03 mol) was connected to the hydroxyl end group of poly(ethylene oxide)$_{42}$ monomethyl ether (0.015 mol) using the Williamson reaction. The protective group was removed by acid hydrolysis, and finally, a reactive methacrylate end group was introduced to the ω-methoxy poly(ethylene oxide)$_{42}$ undecanol (0.01 mol) using methacryloyl chloride (0.05 mol). The macromonomer was purified by precipitating from chloroform with ether. The pure product was dried in a vacuum and stored at −18 °C in darkness.

2,2′-Azo-bisisobutyronitrile (AIBN, Aldrich Chemicals,



$MAC_{11}EO_{42}$

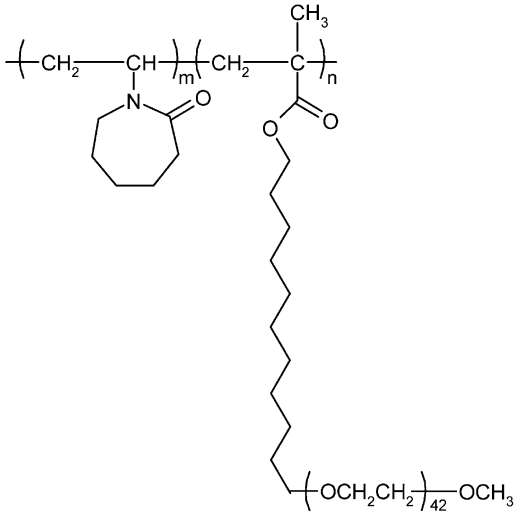


Fig. 1. Chemical structures of the graft copolymers and the PEO-alkyl macromonomer $MAC_{11}EO_{42}$.

Switzerland) was purified by recrystallization from methanol. Tetrahydrofuran (THF) and hexane (both HPLC grade from Rathburn, Scotland), and benzene (99.7%, Reidel-de-Haen, Germany) were used without further purification.

2.2. Polymerizations

The copolymer samples were prepared by the following procedure. A solution of the monomers in benzene (see Table 1) was degassed at room temperature with nitrogen for 30 min, after which it was heated to 70 °C. As soon as the polymerization temperature was reached, a solution of AIBN was injected into the solution. After 20 h, the mixture was cooled to room temperature. The polymer was isolated by precipitation into hexane, purified further by two reprecipitations from THF into hexane, followed by extensive dialysis against water (5 days). The polymer was isolated by freeze-drying from aqueous solution. The structure and purity of the polymer was ascertained by ^{1}H NMR spectroscopy with a 200 MHz Varian Gemini 2000 spectrometer (Palo Alto, USA): δ (ppm, $CDCl_3$): 4.4 (1H, –NCH– at 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 spectra of the graft copolymers show a well separated signal at 3.7 ppm, corresponding to methylene protons of the macromonomer ethylene oxide chains. The macromonomer content in the copolymer was calculated based on the signals at 3.7 and 4.4 ppm.

2.3. Instrumentation

2.3.1. Laser light scattering

Dynamic, DLS, and static, SLS, light scattering experiments were performed using Brookhaven Instruments BI-200SM goniometer and a BI-9000AT digital correlator (Brookhaven Instruments Corporation, NY, USA). A laser (LEXEL 85, 1W, Lexel Corporation, Fremont, USA) operating at 514.5 nm wavelength in the power range of 15–30 mW was used as a light source. The SLS data were treated using Zimm's double extrapolation method. Molecular weight determinations were conducted at 20.0 °C in THF. High precision refractometer (Abbe 60/ED, Bellingham & Stanley, England) was used to measure the dn/dc values at $\lambda = 514.5$ nm. DLS measurements were performed with a scattering angle of 90°. The time correlation functions were analyzed by the Laplace inversion program (CONTIN) and with the cumulants method. The cumulant analysis was used to estimate the polydispersity, μ_2/Γ^2, of the mesoglobules at 50 °C. Intensities measured in counts of photons per second were normalised with respect to the Rayleigh ratio of toluene. Methodological aspects of DLS can be found elsewhere [22]. The measurement temperature was controlled by means of a Lauda RC 6C thermostat. Solutions were purified of dust using Millex PVDF 0.45 μm filter units.

For measurements carried out at 50 °C, aqueous polymer solutions (2 mL) were filtered into light scattering cells at room temperature and quickly heated to 50 °C by placing the cell from 21 °C into oven at 50 °C. The heating rate at the transition temperature (i.e. at 32–34 °C) was approximately 2 °C min^{-1}. The samples were allowed to stabilise at 50 °C for 1 h prior to measurement.

Table 1
Summary of the reaction conditions

Sample	[VCL] (mol/L)	[$MAC_{11}EO_{42}$] (mmol/L)	[AIBN] (mmol/L)	Yield (%)[a]
PVCL	1.08	–	2.56	60
PVCL-g-6	1.08	3.6	2.56	70
PVCL-g-13	1.08	7.1	2.56	65
PVCL-g-16	1.08	10.9	2.56	55
PVCL-g-18	1.08	14.5	2.56	70
PVCL-g-34	1.08	21.3	2.56	60

[a] Measured gravimetrically.

2.3.2. High sensitivity differential scanning calorimetry (HS DSC)

HS DSC measurements were performed on a VP-DSC microcalorimeter (MicroCal Inc., Northampton, USA) at an external pressure of ca. 180 kPa. The cell volume was 0.507 ml. Scans were performed from 10 to 100 °C at a heating rate of 60 °C h^{-1}. Prior to each scan the sample was kept at 10 °C for 15 min. Data were corrected for instrument response time and analyzed using the software supplied by the manufacturer. The polymer concentration was 1.0 mg/ml.

2.3.3. Pressure perturbation calorimetry (PPC)

Measurements were performed on a VP-DSC microcalorimeter equipped with a pressure perturbation accessory (MicroCal Inc). Pressure perturbation calorimetry measures the heat change resulting from a pressure change above a polymer solution in the DSC cell. This heat change can be used to calculate α_p, the thermal coefficient of expansion of the partial volume, $\bar{V}$, of the polymer (Eq. (1)). From the temperature dependence of α_p, one can obtain the relative volume change for transition, $\Delta V/V$, by integration of $\alpha_p(T)$ [23].

$$\alpha_p = \frac{1}{\bar{V}}\left(\frac{\partial \bar{V}}{\partial T}\right)_P \quad (1)$$

The pressure applied during the compression cycle was 500 kPa. The reference cell and sample cell volumes were identical (0.507 mL). The polymer concentration was 5.0 g L^{-1}. Data were analyzed as described elsewhere [23], using the software supplied by the manufacturer. The partial specific volumes of the polymers were determined by an increment method based on the group contribution theory developed to estimate $\bar{V}$of aqueous systems and estimated to be accurate within 2% [24]. Thus, for PVCL,

$\bar{V} = 0.834\ cm^3\ g^{-1}$. The influence of the $C_{11}EO_{42}$ graft on $\bar{V}$ of the corresponding graft copolymers was negligible; therefore the same value was used for the copolymers as well. Additional information on how the PPC technique has been applied to study thermally responsive polymers has been published elsewhere [19,25,26].

2.3.4. Isothermal titration calorimetry (ITC)

The titrations were performed using a VP-ITC titration microcalorimeter (Microcal Inc., Northampton, MA). The sample cell had a volume of 1.43 mL. The solution to be titrated into the continuously stirred sample was placed in a 300 μL syringe (300 rpm). Aliquots (56×5 μL) were injected into the sample cell in intervals of 300 s. In ITC experiments, one measures directly the enthalpy changes associated with processes occurring at constant temperature. Experiments were carried out by titrating micellar $MAC_{11}EO_{42}$ into water (8.62 mM solution). After each addition, the heat released or absorbed as a result of the various processes occurring in the solution is monitored by the calorimeter. We plot the results of the ITC experiments in terms of the enthalpy change per injection (ΔH_i) as a function of surfactant, i.e. $MAC_{11}EO_{42}$, concentration in the cell.

2.3.5. Fluorescence spectroscopy

Steady state fluorescence spectra were recorded with 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. Samples for analysis were prepared by dissolving the freeze-dried polymer, or the macromonomer, in water saturated with pyrene (6×10^{-7} M pyrene). Solutions were prepared by dilution of stock solutions (10 g/L) and were kept in the dark at 5 °C for 12 h prior to measurements.

2.3.6. Size exclusion chromatography

The molecular weight distributions of the polymers were determined by size exclusion chromatography (SEC) using a Waters liquid chromatography system (Milford, USA) equipped with a Waters 2410 differential refractometer and three Styragel columms (HR2, HR4, HR6) kept at 30 °C and eluted with chloroform with a 0.8 ml min^{-1} flow rate. The instrument was calibrated with polystyrene molecular weight standards (Polymer Laboratories, Amherst, USA).

2.3.7. Cloud point determinations

The cloud points of the polymer solutions were obtained by spectrophotometric detection (Shimadzu UV-1601PC spectrophotometer) of the changes in turbidity at a wavelength of 514.5 nm for polymer solutions (1 g L^{-1}) heated at a constant rate (30 °C h^{-1}) in the UV cell compartment. The cloud point temperature T_{Cp}, was taken as the temperature of the onset of turbidity of the sample.

2.3.8. Sample preparation

Aqueous solutions were prepared from dry samples in de-ionised distilled water. Water was de-ionized with an ELGASTAT UHQ-PS water purification system. The solutions were kept at room temperature for 12 h to reach equilibrium. For light scattering measurements, the solutions were purified of dust using filter units of 0.45 μm pore size (Millex, PVDF filter).

3. Results and discussion

3.1. Synthesis and characterisation of the polymers

The graft copolymers were obtained by free radical polymerisation of VCL and $MAC_{11}EO_{42}$ carried out in benzene at 70 °C. The molecular weights of the polymers, evaluated by SLS, and their composition, obtained by analysis of their ^1H NMR spectra, are listed in Table 2. The molecular weight distribution for all copolymers was approximately 1.7. The reactivity of VCL is known to be much lower than that of methacrylates [27]. Such a difference in reactivity usually leads to a non-random distribution of the grafts along the chain. However, in the case of the VCL/$MAC_{11}EO_{42}$ copolymerization, this effect is opposed by a steric effect due to the bulkiness of the grafted chains, such that the distribution of grafts along the polymer backbone will be more random than expected from the reactivity ratios [28].

3.2. Association of the amphiphilic macromonomers in water

We assessed first the association of the macromonomer in water, using two complementary techniques: a fluorescence probe method and isothermal titration calorimetry, which allow one to determine the critical micellization concentration and the enthalpy of micellization of the macromonomer. These parameters are needed as control values to complement data gathered in the study of the amphiphilically modified PVCL samples, as described in the following section.

The fluorescence measurements rely on the photophysics of pyrene, a hydrophobic probe which reports changes in its microenvironment by changes in its emission fine structure [29]. Pyrene is a strongly hydrophobic probe that has a very low solubility in water. In the presence of micelles, Py is preferentially solubilised in the hydrophobic core of the micellar assemblies. The change in the microenvironment of pyrene, from hydrophilic to hydrophobic can be detected by monitoring the ratio, I_1/I_3, of the intensities of the [0,0]

Table 2
Molecular characteristics of the polymers and summary of the microcalorimetry studies

Sample	M_w[a] (g/mol)	$MAC_{11}EO_{42}$ content (wt%)	Grafting density[b]	T_{Cp}[c] (°C)	T_{onset} (°C)	T_{max} (°C)	ΔH (J/g)
PVCL	330,000	–	–	31.8±0.2	31.8±0.1	34.9±0.1	32±3
PVCL-g-6	71,000	6.3	0.4	33.1±0.2	33.6±0.1	37.5±0.1	27±2
PVCL-g-13	310,000	13.0	0.9	33.1±0.2	33.6±0.1	37.5±0.1	25±2
PVCL-g-16	250,000	15.8	1.2	33.4±0.2	34.0±0.1	37.8±0.1	25±2
PVCL-g-18	300,000	18.3	1.4	33.5±0.2	34.1±0.1	37.4±0.1	23±2
PVCL-g-34	260,000	34.0	3.3	33.8±0.2	34.3±0.1	38.2±0.1	21±2

[a] The molecular weights were measured with static light scattering and should be taken as apparent ones.
[b] Number of $MAC_{11}EO_{42}$ grafts per 100 repeating units.
[c] Cloud point temperature by turbidometry.

band (λ 376 nm) and the [0,3] band (λ 387 nm). The ratio decreases, from a value of ~1.60 recorded in water, to a value of ~0.60, in hydrocarbon media [29]. Changes in the ratio I_1/I_3 for Py dissolved in solutions of increasing macromonomer concentration are shown in Fig. 2. In solution of low macromonomer concentration (<0.2 mmol/L) the ratio I_1/I_3 is constant, taking a value typical for pyrene dissolved in water. As the macromonomer concentration exceeds a concentration of ~0.5 mmol/L, the I_1/I_3 ratio decreases, an indication that micellization of the macromonomers is taking place in the solution. The ratio I_1/I_3 reaches a constant, low, value in solutions of macromonomer of concentration higher than ~3 mmol/L. The concentration region (0.5–3 mM) corresponds to the progressive solubilization of pyrene within the hydrophobic domains of the macromonomer micelles. The lowest I_1/I_3 value (~1.20) recorded in solutions of $[MAC_{11}EO_{12}]>$ 3 mmol/L is similar to the value of 1.18 measured for pyrene in micellar solution of PEO surfactants, such as Brij 35 ($C_{12}EO_{23}$) [29].

We also observed that, as the macromonomer concentration increases, the total fluorescence intensity decreases, a trend opposite to the commonly observed increase in emission intensity as Py passes from hydrophilic to hydrophobic environments. The decrease in Py emission intensity may reflect the fact that pyrene solubilized in the micellar core is placed in close proximity to the methacrylate end groups of the macromonomer, known to act as quenchers of Py fluorescence [30]. A similar observation was made earlier for styrene functionalised reactive surfactants [31].

The micellization of $MAC_{11}EO_{42}$ was monitored also by isothermal titration calorimetry, adding dropwise into water a concentrated solution of the macromonomer (8.62 mM, i.e.>cmc). Each injection produced an exothermic signal which, with the number of injections, remains constant, then decreases. The dilution enthalpy of the macromonomer micelle as a function of macromonomer concentration was determined by integration of each peak. The resulting enthalpogram (Fig. 3) presents a transition point (~0.95 mmol L^{-1}) corresponding to the onset of association of the macromonomer. The heat absorbed upon injection of the macromonomer into water before reaching the cmc (~−12.5 kJ/mol) is the sum of several contributions: the heat of dilution of the micelles, the enthalpy of demicellization, and the heat of dilution of individual macromonomer molecules. When the macromonomer concentration in the cell exceeds 0.95 mM, the heat evolved decreases, signalling that an increasing fraction of the macromoner micelles injected per aliquot remains in the associating form, rather than disintegrating into isolated macromoners. The transition point noted in the enthalpogram corresponds to the value recorded by the fluorescence

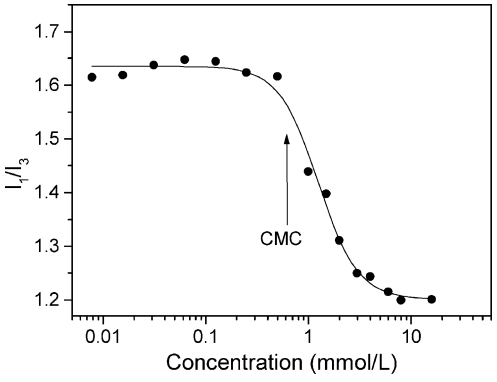


Fig. 2. Semilogarithmic plot of the changes of ratio I_1/I_3 for pyrene in aqueous solutions of $MAC_{11}EO_{42}$ as a function of macromonomer concentration.

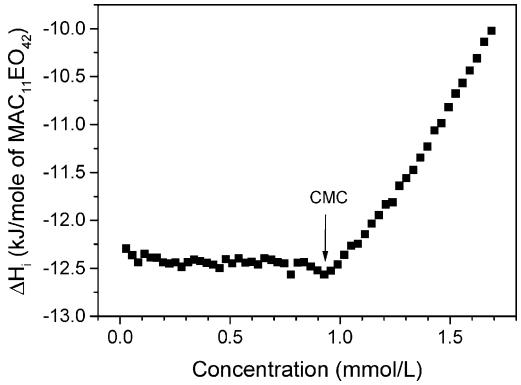


Fig. 3. Reaction enthalpy (ΔH_i) observed upon dilution of 8.62 mmol L^{-1} $MAC_{11}EO_{42}$ into water at 20 °C.

probe experiment for the onset of micellization. The fluorescence measurement also led us to conclude that the micellization process takes place over a large macromonomer concentration range (Fig. 2). It was not possible to record an ITC enthalpogram for $[MAC_{11}EO_{42}] > 1.5$ mmol/L and to reach a $[MAC_{11}EO_{42}]$ in the cell for which the micellization process is complete, which in the case of a normal surfactant is indicated by a constant enthalpy of dilution [32].

3.3. Amphiphilically modified PVCL in water at temperatures below the LCST

The hydrodynamic radius of the various copolymers in dilute aqueous solutions at 20 °C, well below the cloud point, was determined by dynamic light scattering for solutions ranging in concentration from 1 to 10 g/L. Similar measurements were conducted as well for solutions of a non-modified PVCL of similar molecular weight (Fig. 4(a)). The latter polymer has a hydrodynamic radius of the same order of magnitude (5–30 nm), whether it is dissolved in water or in THF, a good solvent of PVCL (data not shown). Thus, the polymer is dissolved as single chains in water at 20 °C, and this is the situation over the entire concentration range covered.

Two different patterns emerged from the DLS studies of the copolymers, depending on their grafting density. The hydrodynamic radius distribution recorded with solutions of the copolymers of high-grafting density, such as PVCL-g-34, is monomodal ($c = 1$–10 g/L), with no indication of the presence of larger objects (Fig. 4(d)). Thus, in aqueous solutions, PVCL-g-34 forms mainly intrapolymeric associates: the amount of amphiphilic grafts is high enough to force the polymer chain to adopt a stable intrapolymeric structure. A similar observation has been previously reported in a study of PNIPAM grafted with amphiphilic perfluorooctyl groups [10], another type of amphiphilically modified polymers which tend to form unimers rather than interpolymeric associates.

In contrast, the hydrodynamic radius distribution recorded for aqueous solutions of copolymers with a low grafting density, such as PVCL-g-13 and PVCL-g-18, is bimodal, with a contribution of small size entities of R_h 5–30 nm, assigned to single polymer chains, and a contribution of larger particles of R_h 80–150 nm (Fig. 4(b) and (c)). The relative importance of the two size populations depends on the copolymer concentration: the relative amount of the larger particles increases with increasing copolymer concentration.

Fluorescence probe measurements were conducted using copolymer solutions of increasing concentration, following experimental conditions identical to those used to monitor the macromonomer micellisation and described in the preceding section. The variations of the ratio I_1/I_3 with increasing polymer concentration expressed either in terms of polymer concentration (in g/L) or in terms of amphiphile concentration (mmol/L) are represented in Fig. 5(a) and (b), respectively. We note that for all copolymer solutions the ratio I_1/I_3 decreases with increasing polymer concentration from its value in water (~ 1.65) and levels off eventually for solutions of copolymer concentration in excess of 0.3–1.0 g/L, depending on the grafting level. The onset of the decrease in I_1/I_3, which is often taken as an indication of the onset of micellization, depends on the grafting level of the copolymer: the higher the grafting level, the lower the onset of micellization (Fig. 5(a)). However, when the data are plotted in terms of amphiphile concentration (Fig. 5(b)), it appears that micellization begins when $[C_{11}EO_{42}] > 4 \times 10^{-3}$ mmol/L, for all copolymers. Thus, micellization of the copolymers is controlled to a large extent by the hydrophobic assembly of the substituents, as noted earlier

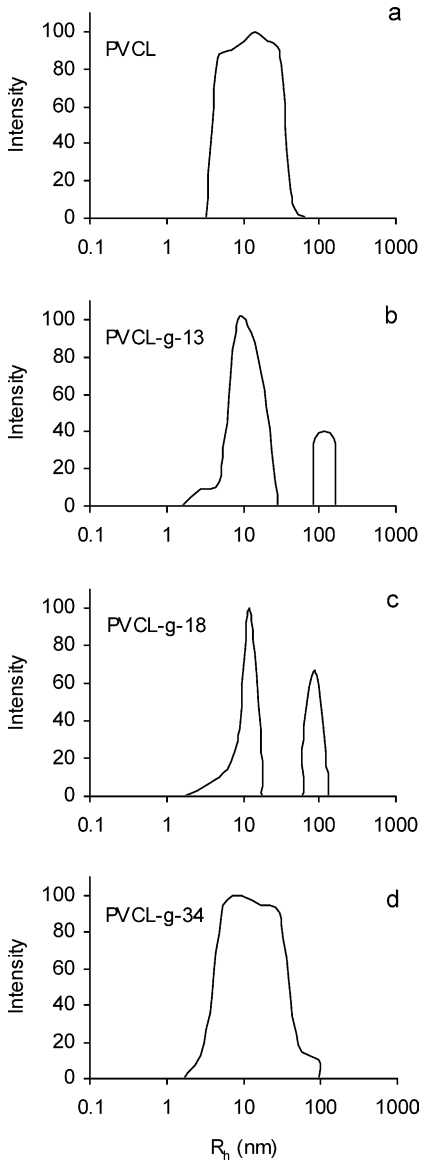


Fig. 4. Size distributions of PVCL and the graft copolymers in water at 20 °C. Homopolymer PVCL (a), grafted copolymers PVCL-g-13 (b), PVCL-g-18 (c), and PVCL-g-34 (d). Polymer concentration 10 g/L.

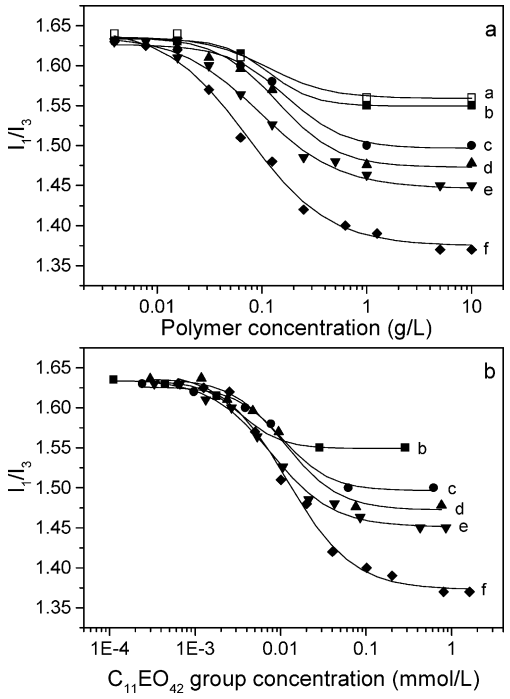


Fig. 5. Semilogarithmic plots of the changes of ratio I_1/I_3 for pyrene in aqueous solutions of homopolymer PVCL and grafted copolymers at 20 °C as a function of polymer concentration expressed in g/L (5a) and as a function of macromonomer graft concentration in mmol/L (5b). Homopolymer PVCL (a); PVCL-g-6 (b); PVCL-g-13 (c); PVCL-g-16 (d); PVCL-g-18 (e); PVCL-g-34 (f).

in the case of various hydrophobically modified polymers [33,34]. The fact that the minimum $C_{11}EO_{42}$ concentration required for copolymer micellization is lower by more than 2 orders of magnitude than the cmc of the macromonomer also is in agreement with previous observations.

The plots of I_1/I_3 vs copolymer concentration reveal yet another characteristic of the copolymer solutions, namely the micropolarity of the hydrophobic domains created upon association of the copolymers in water. A qualitative assessment of this property is given by the I_1/I_3 value determined in the copolymer solutions of highest concentration when the plateau value is attained (Fig. 5). We note that this value depends significantly on the grafting level: the solution of the most densely grafted copolymer yielding the lowest I_1/I_3 value (1.40) and the pure homopolymer the highest. Moreover and in all cases, this value is higher than the value (1.20) recorded for micellar solutions of the macromonomer (Fig. 2). From grafting density dependant trend exhibited by the minimal I_1/I_3 value, which is a measure of the polarity averaged over all pyrene solubilisation sites, we can conclude that the assemblies formed with lightly modified PVCL are more polar than the more heavily grafted samples, a possible indication of differences in the hydration of the micellar assemblies.

The fluorescence probe experiments yield information on the minimum copolymer concentration needed for association and on the micropolarity of the assemblies, but they do not allow us to assess whether the hydrophobic assemblies are formed intra- or intermolecularly. Dynamic light scattering measurements performed on copolymer solutions in the same concentration region indicate that in some cases either intra- or inter-molecular association can take place, or even a combination of both. Thus, in the studied polymer concentration range, the pyrene molecules are solubilised either by hydrophobic domains formed by single polymer chains or by the interpolymeric associates or by both of these. If the concentration is increased well above the overlap concentration, very strong interpolymeric association is likely to form. Association behavior and rheological properties of a more concentrated aqueous solution of PVCL-co18 have been studied in detail in a separate paper [35].

3.4. Thermosensitivity of aqueous solutions of the copolymers

3.4.1. Thermodynamics of the phase transition

When an aqueous solution of PVCL is heated above its LCST, phase separation takes place—a phenomenon accompanied by the loss of the hydration layer surrounding the polymer chains in cold solution. The dehydration is an endothermic process. It is readily detected by microcalorimetry. The endotherms recorded for solutions of PVCL and the grafted copolymers are broad, and markedly asymmetric, with a sharp increase in heat capacity on the low temperature side (onset of the transition, T_{onset}) and a gradual decrease of the heat capacity for temperatures higher than a maximum temperature T_{max} (Fig. 6). Similar asymmetric endotherms have been reported in a study of PVCL homopolymers with varying molecular weights [19] as well as for PVCL grafted with PEO [36]. Introduction of amphiphilic grafts onto PVCL increases the onset temperature of the endothermic transition compared to the non-modified PVCL. T_{onset} is closely related to the cloud point temperature of the same solution, see Table 2. Surprisingly, the degree of grafting does not affect very much the T_{onset},

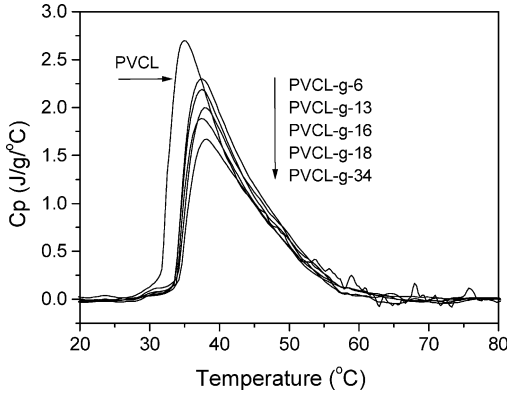


Fig. 6. Microcalorimetric endotherms for an aqueous solution of PVCL and the graft copolymers. Polymer concentration 1 g L^{-1} and heating rate 60 °C h^{-1}.

which is the same for all copolymers. The heat of the transition, ΔH (Table 2) in contrast, depends on the grafts content of the copolymers, corresponding closely to the concentration of VCL units.

Additional information on the solvation layer around the polymers was obtained by pressure perturbation calorimetry (PPC), a technique that allows one to evaluate the changes in the partial volume of the polymer throughout the phase transition, and to obtain information on the temperature dependant relative hydrophilicity/hydrophobicity of a polymer in solution [23]. We have reported previously an extensive PPC study of PVCL aqueous solutions [19]. Here, we assess the effect of the amphiphilic grafts on the volumetric properties of the polymers.

PPC scans recorded for solutions of PVCL and PVCL-g-34 in water (Fig. 7) present the thermal expansion coefficients of the polymers (α_{pol}) as a function of temperature. The plots can be divided into four temperature ranges. Below the transition temperature, $10<T<30$ °C, α_{pol} for PVCL remains constant, while in the case of PVCL-g-34, α_{pol} has a negative slope at low temperatures. In both cases, α_{pol} undergoes a sharp decrease, reaches a minimum for $T_{peak}\sim 33$–34 °C, then increases abruptly with increasing temperature to reach a maximum value at $T\sim 45$ °C, and it gradually decreases as the temperature further increases. The plots recorded for the other $C_{11}EO_{42}$-grafted PVCL samples present features similar to those of the copolymer with highest graft density. Values of α_{pol} recorded at 10, 30, and 80 °C for aqueous solutions of all grafted PVCL samples, and for the homopolymer PVCL are listed in Table 3. Integration of the changes of $\alpha_{pol}(T)$ with temperature yields $\Delta V/\bar{V}$, the change in the hydration volume in percent of the partial volume of the polymer, corresponding to the collapse of the polymer chain. The volume change is taken usually as the area defined by the peak of the PPC scan and a progress baseline drawn from projections of the baselines in the pre-transition and post-transition regions [23]. Applying this method to the PPC scan of PVCL in water (Fig. 7) yields a value of $\Delta V/\bar{V}=-0.10\%$ for the sharp negative transition at 32.9 °C. However, the PPC trace recorded for PVCL solutions may be viewed also as consisting of two parts, the sharp signal ($\Delta V/\bar{V}=-0.10\%$) and a broader signal ($\Delta V/\bar{V}=+0.50\%$) in the low and high temperature ranges of the transition, respectively. Correspondingly, similar values, although smaller in magnitude, are obtained for the graft copolymers, Table 3. This data analysis is supported by the asymmetry of the DSC trace (Fig. 6), which may be interpreted as the overlap of two phenomena, first the collapse of the chains, then the aggregation of the collapsed chains, each phenomenon characterized by a change in the polymer hydration volume.

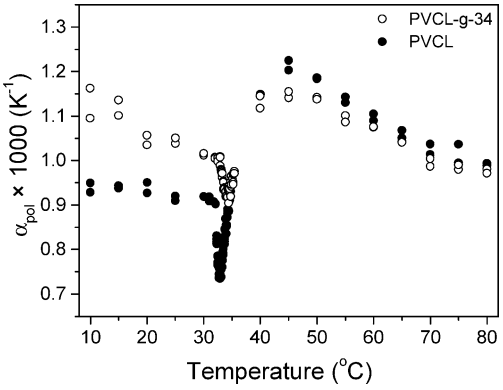


Fig. 7. Temperature dependence of the coefficient of thermal expansion (α_{pol}) of PVCL in H_2O (full circle) and in PVCL-g-34 (open circle); polymer concentration 5 g L^{-1}.

The $\alpha_{pol}(T)$ curves for the homopolymer and the graft copolymer differ in one aspect, the slope in the pre-transition region ($10<T<30$ °C). The α_{pol} value is nearly constant in this temperature domain for PVCL, whereas, in the case of the graft copolymer, it undergoes a significant decrease with increasing temperature (Table 3 and Fig. 7). Highly positive values of the thermal expansion coefficient and strong temperature dependence of α are typical characteristics of molecules that act as structure breakers in water, such as the polar hydrophilic amino acids asparagine or glutamine. In the case of $C_{11}EO_{42}$ grafted PVCL, the high PEO content seems to cause a similar effect: PEO acts as a structure breaker at temperatures between 10 and 30 °C. At elevated temperatures ($45<T<80$ °C) the slope of $\alpha_{pol}(T)$ remains negative, indicating that surfaces of the structures generated by the collapsed polymers may still be partially hydrophilic even though the polymer has expulsed the majority of the bound water molecules.

We note also that the magnitude of the volume change for both transitions is largest for PVCL. The reduced changes in the hydration volume in the cases of graft copolymers may indicate that there are fewer molecules of 'ordered water' bound to the polymer chains possibly due to the structure breaking properties of very hydrophilic PEO chains and, hence, a smaller volume of directly interacting water. This trend is opposite to that exhibited by hydrophobically modified poly(*N*-isopropylacrylamides), which undergoes larger volume changes as a result of the introduction of structure making groups [37].

3.4.2. Heat induced mesoglobule formation.

We discovered recently [19,38], that the heat-induced dehydration of PVCL triggers the formation of colloidally stable particles in aqueous solutions kept at a temperature above their cloud point. We observed the same phenomenon here upon heating dilute aqueous amphiphilically grafted PVCL solutions above their cloud point: stable nanoparticles form, ranging in size from ~50 to 110 nm, depending on the concentration of the solution (Fig. 8). The size of the particles is only slightly dependent on the grafting density of the polymer and the size distributions of the particles are rather narrow and monomodal; μ_2/Γ^2 measured by DLS is typically between 0.020 and 0.080. The particles remained

Table 3
Thermodynamic characteristics of aqueous solutions of $C_{11}EO_{42}$ grafted PVCL

Sample	T_{peak} (°C)	$\Delta V/V_1$ (%)	$\Delta V/V_2$ (%)	$\alpha_{pol\ 10}$ (K^{-1})	$\alpha_{pol\ 30}$ (K^{-1})	$\alpha_{pol\ 80}$ (K^{-1})
PVCL	32.9±0.2	−0.10±0.01	+0.50±0.01	0.94×10^{-3}	0.92×10^{-3}	0.99×10^{-3}
PVCL-g-6	33.8±0.2	−0.11±0.01	+0.45±0.01	0.98×10^{-3}	0.91×10^{-3}	0.94×10^{-3}
PVCL-g-13	33.8±0.2	−0.08±0.01	+0.39±0.01	1.00×10^{-3}	0.91×10^{-3}	0.93×10^{-3}
PVCL-g-16	34.0±0.2	−0.08±0.01	+0.33±0.01	1.03×10^{-3}	0.96×10^{-3}	0.97×10^{-3}
PVCL-g-18	33.9±0.2	−0.07±0.01	+0.31±0.01	1.03×10^{-3}	0.95×10^{-3}	0.94×10^{-3}
PVCL-g-34	34.4±0.2	−0.05±0.01	+0.30±0.01	1.08×10^{-3}	0.98×10^{-3}	0.96×10^{-3}

Data obtained from PPC measurements.

colloidally stable, with no interparticle flocculation over periods of several days, as judged by DLS monitoring of aqueous polymer samples kept at 50 °C. The high colloidal stability may be due to steric stabilisation by the PEO chains grafted on the polymer, but since PVCL itself forms colloidally stable particle suspensions in hot water, [19, 38] intrinsic properties of the PVCL chains may be the main driving force.

We assessed next the stability of the particles against dilution with hot water. First, particles of PVCL-g-18 were prepared by heating to 50 °C filtered solutions (2 mL, 0.20 g L^{-1}) placed in the light scattering sample holder. After keeping the samples at 50 °C for one hour, a DLS measurement was performed and the first dilution step was conducted by injecting hot water (1 mL, 50 °C) into the solution kept in the cell holder. The cycle was repeated, once the intensity of the scattered light was stable. The hydrodynamic radius of the PVCL-g-18 particles at 50 °C remained remarkably constant, with no sign of particle disintegration upon dilution (Fig. 9, top). Moreover, the intensity of the scattered light from the colloidal sample was directly proportional to the concentration upon dilution (Fig. 9, bottom), providing yet another indication of the stability of the particles.

The apparent molecular weight M_w^{agg} and the radius of gyration R_g of the aggregates were estimated via static light scattering (SLS) measurements conducted for PVCL-g-18 particles of three different concentrations in hot water, obtained upon the dilution with hot water of the most concentrated solution. The Guinier method was applied, assuming that the second virial coefficient A_2 of the mesoglobules is zero in the concentration range studied (Fig. 9, inset). Accurate estimes of M_w^{agg} are somewhat difficult because of the uncertainty of the increment of refractive index, dn/dc, for the collapsed polymer at 50 °C. We calculated M_w^{agg} values using two different dn/dc values: 0.176 cm^3/g, the value measured for PVCL-g-18 in water at 20 °C and 0.232 cm^3/g, a literature value for a collapsed PVCL microgel at 40 °C [39]. Both values of M_w^{agg} are listed in Table 4, together with ρ, the density of the particles ($\rho = M_w^{agg}/(N_A(4/3)\pi R_h^3)$). The value of the particles density indicates that particles are partially hydrated, as in the case

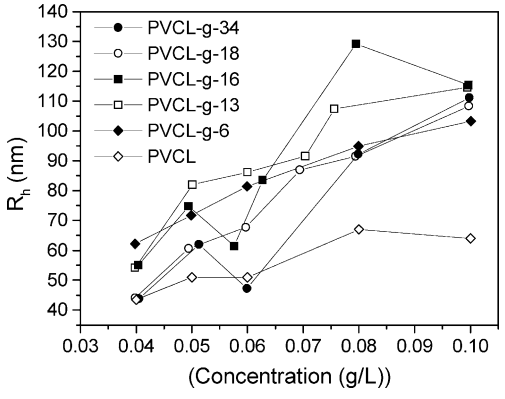


Fig. 8. Apparent hydrodynamic radius, R_h, of the mesoglobules formed by quickly heated aqueous solutions of the homopolymer PVCL or the graft copolymers with varying concentrations. Measurements were conducted at 50 °C at 90° scattering angle.

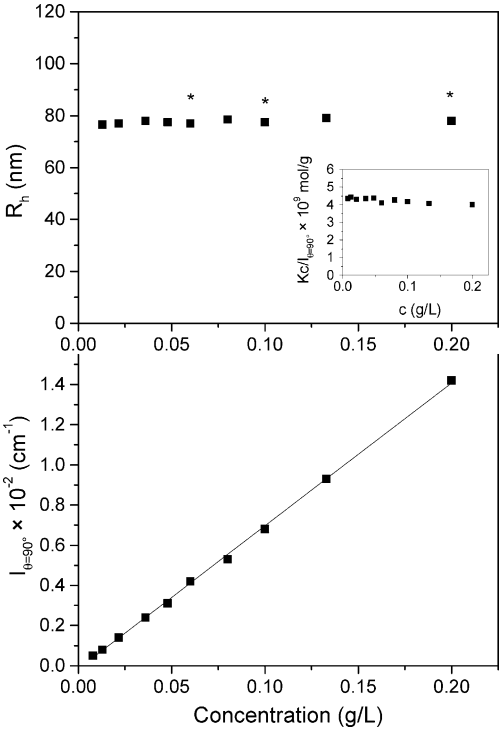


Fig. 9. (top) The effect of dilution on R_h and $K_c/I_\Theta = 90°$ of the mesoglobules (solid squares) formed upon heating of 0.2 g/L aqueous solution of PVCL-g-18. (Bottom) Scattering intensity at 90° angle from polymer solution of different concentration. Numerical results for asterisk marked data points are available in Table 4.

Table 4
Summary of the light scattering measurements on PVCL-g-18 mesoglobules at 50 °C

Concentration (g/L)	R_h (nm)	R_g (nm)	R_g/R_h	dn/dc (cm^3/g)	M_w^{agg} (g/mol)[a]	ρ (g/cm^3)
0.20	78.0	56.4	0.72	0.176[b]	8.4×10^8	0.70
				0.232[c]	4.8×10^8	0.40
0.10	77.5	56.0	0.72	0.176[b]	8.1×10^8	0.69
				0.232[c]	4.6×10^8	0.40
0.04	78.0	57.7	0.74	0.176[b]	7.9×10^8	0.66
				0.232[c]	4.5×10^8	0.38

The solutions were obtained by subsequent dilution of a 0.2 g/L solution, see Fig. 9.
[a] The molecular weights were calculated according to Guinier's method as for a hard sphere.
[b] Measured in water at 20 °C ($\lambda=514$ nm).
[c] A literature value for collapsed PVCL microgel in water at 40 °C ($\lambda=532$ nm).

of collapsed poly(*N*-isopropylacrylamide) ($\rho=0.34$ g/cm^3) [40]. In the case of PVCL-g-18, the aggregation number as estimated to be approximately 1600 and the density $\rho=0.40$ g/cm^3, using a dn/dc value of 0.232 cm^3/g. The shape of the particles was estimated from the ratio of the radius of gyration to the hydrodynamic radius, R_g/R_h which varies from 0.77 for hard spheres to 1.5 for random coils. In our experiments the ratio ranges from 0.72 to 0.75 (Table 4), indicating that the particles have a homogenous spherical geometry even after being subjected to dilution.

3.4.3. Freezing the structure of the heat induced particles

Phenols are known to complex with PVCL via hydrogen bond formation between the carbonyl groups of the lactam ring and the hydroxyl units of the phenol. These specific interactions have been used to prepare hydrogels via physical cross-linking with polyphenols, such as pyrocatechol and phloroglucinol, of linear PVCL in concentrated aqueous solutions below and above the LCST [41–43]. We have tested the same cross-linking mechanism within colloidal PVCL particles in an attempt to fix permanently the mesoglobule structure, so that the particles preserve their integrity upon cooling.

The effect of added phenols on the size of heat-induced particles was studied via DLS in the case of PVCL and PVCL-g-18 particles. Cross-linking was effected by injecting a hot aqueous 1,2-benzenediol solution (0.5 mL, 0.5 mol/L solution) into a colloidal polymer dispersion (2 mL, 0.1 g/L) placed in the light scattering system cell holder kept at 50 °C. Addition of 1,2-benzenediol at 50 °C did not affect the size of the PVCL particles, but it triggered a slight decrease of the size of the PVCL-g-18 particles, from $R_h=77$ to 64 nm. The treated solutions were allowed to cool down to 20 °C. Their hydrodynamic radius did not change, which is an indication that physical cross-linking occurred within the particles. In the absence of cross-linker, the particles disintegrate immediately as the temperature is lowered below 32 °C. Cross-linked particles were monitored at 20 °C for several weeks by DLS and no flocculation was detected.

4. Conclusions

Poly(*N*-vinylcaprolactam) grafted with amphiphilic PEO-alkyl chains forms intra- and interpolymeric associates in water at room temperature depending on the concentration and the grafting density. The associates solubilise hydrophobic substances, such as pyrene, inside their non-polar domains, which are composed of self-assembled $C_{11}EO_{42}$ grafts. Upon heating, the PVCL backbone collapses, triggering a change in the hydration of the chain and release of polymer-bound water molecules. The presence of $C_{11}EO_{42}$ grafts increases slightly the aggregation temperature, compared to that of PVCL. Thermally induced aggregation leads to the formation of colloidally stable mesoglobules. Each particle is composed of several thousands of collapsed polymer chains, although the particles still contain much water at 50 °C. The size of the mesoglobules can be altered by changing the concentration or the composition of the graft copolymer, although the effect of increasing grafting density is only minor.

The structure of the PVCL mesoglobules at 50 °C can be frozen by physically cross-linking the collapsed PVCL chains with multifunctional phenols, which leads to the formation of nano-sized hydrogel particles that are stable even at room temperature. Cross-linking thermally induced PVCL particles provides a method to produce well defined nano-sized hydrogel particles that might meet various biotechnology needs, such as enzyme stabilisation or controlled drug release, especially in view of the biocompatibility of PVCL [44]. Amphiphilic grafts in the structure stabilise the surface of the particles and may simultaneously act as solubilisation sites for a hydrophobic substance.

Acknowledgements

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