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Response of micelles formed by smart terpolymers to stimuli studied by dynamic light scattering

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Abstract

A series of novel terpolymers of sodium 2-acrylamido-2-methyl-1-propanesulfonate (AMPS), *N*-isopropylacrylamide (NIPAM) and cinnamoyloxyethylmethacrylate (CEMA) have been synthesized in a radical polymerization process using AIBN as an initiator in DMF at 60 °C. Five terpolymers were obtained by copolymerization of the monomer mixtures containing a fixed amount of 10 mol% of AMPS while the content of CEMA ranged from 5 to 25 mol% and was changed in 5 mol% increments. The terpolymers obtained are soluble in water. Due to their amphiphilic nature the terpolymers undergo self-organization in the aqueous solution with the formation of micelles. The terpolymers are sensitive to three stimuli, i.e. temperature, UV light and ionic strength. Terpolymers with small and medium content of CEMA formed both small ($R_h = 8-11$ nm) and large ($R_h = 22-51$ nm) micelles, while the terpolymer with the highest content of CEMA formed only large and relatively monodisperse micelles ($R_h = 28$ nm), as found in the DLS measurements. The size of the micelles increases in response to both temperature and irradiation with UV light at $\lambda = 280$ nm. The sensitivity to temperature is due to the presence of NIPAM which imposes the occurrence of lower critical solution temperature (LCST) while the sensitivity to UV light is due to the content of cinnamoyl chromophores which undergo photodimerization resulting in the photocrosslinking of the micelles. Irradiation of the micelles in aqueous solution of relatively high ionic strength results in the loss of their temperature-sensitivity.

Keywords: Micelles; LCST; DLS

1. Introduction

'Intelligent' or 'smart' polymers are polymers which are able to undergo spectacular changes in their physicochemical properties in response to applied stimuli, both external ones, e.g. temperature, UV or visible light and magnetic field, or internal ones such as pH or the ionic strength. There has been increasing interest in these polymers because of growing range of their possible practical applications. The examples of these applications include drug delivery [1,2], reduction of the cholesterol level in blood [3], wound healing [4], attenuators for ultrasonic waves [5], intelligent switches and control valves [6,7] just to mention only a few of them.

The above mentioned stimuli may induce very different effects in the smart polymers. The sensitivity to the ionic strength is a typical property of the polymers containing ionizable groups, amphiphilic polyelectrolytes in particular [8]. Changes in the ionic strength of the solutions of these polymers lead to the changes in the size of the polymeric micelles, polymer solubility [9] and the fluorescence quenching kinetics of the chromophores bound to a polyelectrolyte [10,11].

Most of the currently studied temperature-responsive polymers are based on *N*-isopropylacrylamide (NIPAM) [12–17], although other thermosensitive polymers are recently also intensively studied, e.g. those containing bile acid residues [18]. It is the solubility of these polymers that responds to the temperature changes. The polymers based on NIPAM show a thermoreversible phase separation in the aqueous solution when the temperature exceeds their LCST. In the case of the NIPAM homopolymer (PNIPAM) it is about 31–35 °C [19]. The copolymers of NIPAM with hydrophobic or hydrophilic monomers may show the LCST that is lower or higher, respectively, than that of PNIPAM. Also, the addition of surfactants may shift the LCST to higher or lower values. The solubility of the

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NIPAM-containing polymers below the LCST is due to the formation of hydrogen bonds between water and N-isopropyl groups on the polymer chain. The hydrogen bonds are broken when the temperature is raised above the LCST. The phase separation of NIPAM homopolymer is known to take place in two steps [20]. In the first step the collapse of individual polymer chains from a hydrated coil into a globule takes place. In the second step, the aggregation of globules occurs resulting in the observed phase separation.

Irradiation of smart polymers containing deliberately introduced polymeric chromophores with the UV or visible light may result in the photoreactions in which these chromophores take part e.g. photochromic isomerization [21] or photocrosslinking observed for acrylated dextran [22] and for coumarin-containing polyoxazoline [23]. The possible responses of the polymers to the absorbed light involve reversible changes in solubility [24], shape of polymer gel [25], or sol–gel transitions [26].

This paper describes the synthesis and properties of novel polymers containing three components: 2-acrylamido-2-methyl-1-propanesulfonate (AMPS), NIPAM and cinnamoyloxyethylmethacrylate (CEMA). The terpolymers, described in this paper are, to the best of our knowledge, the first polymers sensitive to three external stimuli, i.e. ionic strength, temperature and UV light. The synthesis and studies on these polymers were preceded by the studies on the UV-light sensitive terpolymers containing AMPS and CEMA [27] and temperature-sensitive copolymers containing AMPS and NIPAM [28]. Previously, we have also described the LCST response of terpolymers described in this paper, to the various stimuli [29]. In this paper we report the studies on the effect of temperature, ionic strength and UV light on the formation of micelles in aqueous solutions of the terpolymers using dynamic light scattering (DLS) measurements.

2. Experimental part

2.1. Materials

CEMA (Polysciences, Inc.) was purified from the inhibitor (MEHQ) chromatographically by passing it through a column filled with De-hibit 200 resin (Polysciences, Inc.) and using methanol as an eluent. The resin was previously washed with methanol till spectrally clean eluent was obtained. Methanol was then removed from CEMA by freeze-drying. AMPS (Aldrich) were used as received. NIPAM (Aldrich) was purified by dissolving in toluene followed by precipitation in a large excess of hexane. α,α'-Azobisisobutyronitrile (AIBN, Aldrich) was recrystallized from methanol at 40 °C under nitrogen. Pyrene (Aldrich) was recrystallized from methanol at 40 °C under nitrogen. Anhydrous Na_2CO_3 (analytical grade) and NaCl (analytical grade) were purchased from POCh, Gliwice and used as received. DMF (HPLC grade, Aldrich) and diethyl ether (Lachema, analytical grade) was used as received. Doubly-distilled water was used in all experiments.

2.2. Polymer synthesis

The terpolymers of AMPS, NIPAM and CEMA were synthesized by free-radical polymerization using AIBN as an initiator. AMPS was dissolved in 12 ml of DMF. To this solution an equinormal amount of Na_2CO_3 was added and the resulting solution was stirred until Na_2CO_3 was completely dissolved. AIBN (0.1% based on total monomers), CEMA and NIPAM were then added. The total weight of the monomers in each synthesis was 2 g and the compositions of the reaction mixtures are given in Table 1. The reaction mixtures were transferred into ampoules, degassed by bubbling with nitrogen for at least 30 min, sealed and placed in a thermostated bath at 60 °C for 60 h. The polymers were then precipitated by pouring the reaction mixture into about 400 ml of diethyl ether, dried in vacuum, and dissolved in water. The solutions were then dialyzed using dialysis tubes with a cutoff molecular weight of 14,000 for four days against deionized water and then freeze-dried. The composition of the terpolymers obtained was determined based on results from the elemental analysis and from UV (absorption at 280 nm) and ^{1}H NMR spectra.

2.3. Instrumentation and procedures

Irradiation of the terpolymer solutions was carried out at room temperature using a 100 W Ushio lamp and a 270 nm cutoff filter. The extent of photodimerization was followed by measurement of decrease of absorption band characteristic of cinnamoyl chromophores (maximum at 280 nm). After 4 h of irradiation the maximum photocrosslinking of 70–80% was achieved. Extension of the irradiation time did not increase substantially the extent of dimerization. UV spectra of the terpolymers were measured on a Hewlett–

Table 1
The monomer content of the reaction mixtures and the terpolymers studied

Polymer	AMPS content (mol%)		NIPAM content (mol%)		CEMA content (mol%)	
	Feed	Polymer	Feed	Polymer	Feed	Polymer
A10C5	10.0	12.8	85.0	76.4	5.0	10.8
A10C10	10.0	8.7	80.0	71.3	10.0	20.0
A10C15	10.0	8.3	75.0	69.0	15.0	22.7
A10C20	10.0	8.6	70.0	60.8	20.0	30.6
A10C25	10.0	7.8	65.0	55.8	25.0	36.4

The studies on the smart polyelectrolytes containing 2-acrylamido-2-methyl-1-propanesulfonate (AMPS), *N*-isopropylacrylamide (NIPAM) units and monomer with pendant photocrosslinkable cinnamoyl chromophores respond to three stimuli, i.e. temperature, UV light and ionic strength. The terpolymers undergo self-association in the aqueous solution as demonstrated by DLS measurement.

Packard 8452A diode-array spectrophotometer using 1-cm optical path quartz cuvettes. ^{1}H NMR spectra were recorded in D_2O at ambient temperature on a Bruker 500 MHz spectrometer.

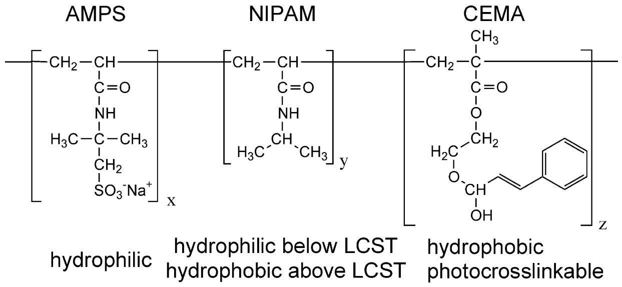
2.4. DLS measurements

The apparent hydrodynamic radii and their distribution were measured with an Otsuka Electronics Photal DLS-7000 light scattering spectrometer equipped with a 75 mW Ar laser operating at $\lambda = 488$ nm. Data were collected using an ALV wide-band multi-τ digital autocorrelator allowing characterization of relaxation time distributions over eight decades. The measurements were performed in a thermostated cell in the temperature range 25–70 °C and in the angle range from 30 to 150°. After each increase of temperature of the solution the measurements were performed when the scattering intensity stabilized, usually after about 30 min. The polymer solutions were filtered before measurements using a 0.20 or 0.45 μm disposable membrane filter. The ionic strength of the polymeric solutions was adjusted using NaCl. Mathematical treatment of the data obtained has been described elsewhere [30].

3. Results and discussion

We have synthesized five terpolymers of AMPS, NIPAM, and CEMA (Scheme 1) in a free radical polymerization reaction.

All five reaction mixtures contained 10 mol% of AMPS, while the content of CEMA ranged from 5 to 25 mol% and was changed in 5 mol% increments. The terpolymers were assigned symbols AxCy, where x and y denote AMPS and CEMA content in mol%. The content of the reaction mixtures and the composition of corresponding polymers (found from elemental analysis, UV and NMR spectra) are given in Table 1. It can be found that the monomer content in the reaction mixtures and in the polymers is quite different. In particular CEMA content in the polymers is much higher than that in the reaction mixtures. In our previous studies we have determined the reactivity coefficients for a pair of the monomers AMPS and NIPAM [28] and have found that both of them are larger than one. This may indicate that the copolymers are rather blocky in nature.



Scheme 1. The structure of the terpolymers.

The qualitative and quantitative composition of the polymers was designed to achieve two goals. On one hand, the monomers introduced to the terpolymers are to give them the sensitivity to three external stimuli, i.e. temperature, ionic strength and UV light. Namely, NIPAM is well-known as a monomer used to obtain polymers which show LCST. AMPS is an ionic monomer therefore polymers containing it have a polyelectrolyte character and, as a consequence, their conformation is sensitive to the ionic strength. In our previous work on AMPS-NIPAM copolymers it was found that even very low content of AMPS (2–10 mol%) significantly increases the LCST value of AMPS-NIPAM copolymers compared to NIPAM homopolymer (PNIPAM) [28]. Therefore, we have chosen the content of AMPS (10 mol% in the reaction feed) which should result in a terpolymer showing measurable LCST taking into account the fact that the content of hydrophobic CEMA should decrease the LCST value. CEMA is a monomer, which contains a cinnamoyl chromophore. The chromophore, like other derivatives of unsaturated carboxylic acids, undergoes photodimerization when irradiated with UV light at $\lambda = 280$ nm. We have previously found that the photodimerization of the cinnamoyl chromophore present in the terpolymers of AMPS, CEMA and *N*-dodecylmethacrylamide (DodMAm) is efficient and may be used to control the physicochemical properties of the polymer solutions [27].

On the other hand, the monomers used differ very much in their affinity to water, i.e. AMPS is very well soluble ionic monomer, CEMA is hydrophobic, while NIPAM is hydrophilic at temperatures below LCST and hydrophobic above LCST. Therefore the synthesized terpolymers are amphiphilic and are expected to undergo micellization in an aqueous solution with the formation of micelles in the nanometer range and their micellization behavior should be temperature-dependent. The formation of micelles was confirmed by DLS measurements. The interior of the micelles in the aqueous solutions is expected to be hydrophobic and composed mostly of CEMA units while the micellar shells are expected to be composed of AMPS and NIPAM.

3.1. DLS studies of the non-irradiated terpolymers at room temperature

We have measured the DLS relaxation time distributions for all five studied terpolymers before irradiation at room temperature in 0.1 M NaCl (Fig. 1).

It was found that all the terpolymers except for A10C25 show bimodal distributions of the relaxation times. This suggests that the solutions of these polymers contain objects of two distinct sizes. The relative intensity of the mode at shorter relaxation times, corresponding to smaller objects, decreases with increasing content of CEMA in the polymer and disappears completely for A10C25, which shows

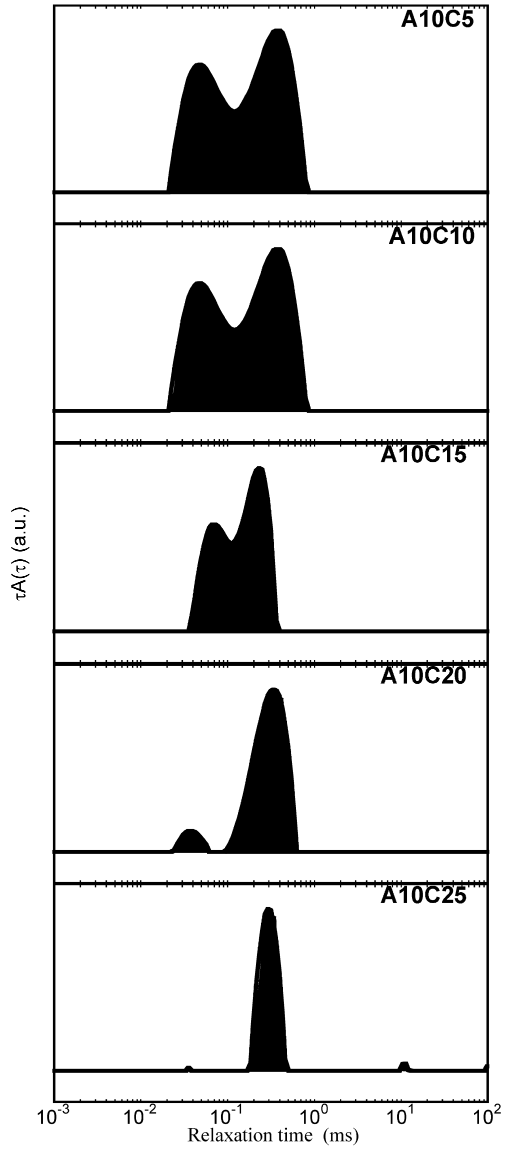


Fig. 1. DLS relaxation time distributions for the terpolymers in aqueous solutions (1 g/l at $T = 25$ °C and at the scattering angle of 90 °C).

unimodal relaxation time distribution at longer relaxation times. This means that the terpolymers A10C5, A10C10, A10C15, and A10C20 show tendency to both intra- and intermolecular association and that the intermolecular association becomes more and more prevailing with increasing content of CEMA in the polymer up to completely intermolecular association in the case of A10C25. To have an idea on the size of objects present in the polymeric solutions we have calculated the hydrodynamic radii of the objects corresponding to the relaxation times at the maxima of the relaxation time distributions. The radii of the micelles were calculated using the Einstein–Stokes relation (1):

$$R_\mathrm{h} = \frac{kT}{6\Pi\eta D} \tag{1}$$

where k is the Boltzmann constant, T the temperature, η the viscosity and D is the diffusion coefficient. Diffusion coefficient can be determined from a slope of the linear plot of relaxation rate, Γ, vs. squared scattering vector, q^2 (not shown). The averaged values of radii obtained (using a linear regression) for all the polymers at 25 °C are shown in Fig. 2.

It can be seen that for the terpolymers A10C5, A10C10, A10C15 the small micelles, which are probably unimolecular or 'oligomolecular', have similar R_h of about 7–8 nanometers. For A10C20 the R_h increases to about 11 nanometers. On the other hand, the R_h of large, or multipolymer, micelles decreases monotonically from 51 to 22 nm with increase of the content of CEMA in the polymers. Finally, A10C25 forms only multipolymer micelles with $R_\mathrm{h} = 28$ nm. As can be seen in Fig. 1 the relaxation time distribution for this polymer is quite narrow which means that the micelles of A10C25 are relatively monodisperse.

3.2. Temperature dependence of the micelle size of the non-irradiated terpolymers

We have studied the micelle size of the terpolymers at various temperatures in 0.1 M NaCl. The R_h of the A10C5 solutions at elevated temperatures could not be calculated because the scattering intensity was too weak. It was found that the relaxation times were also bimodal at higher temperatures. The position of the mode at shorter relaxation time did not depend on the temperature. Thus, it can be concluded that the size of the unimolecular micelles of A10C10, A10C15, and A10C20 does not change with temperature (data not shown) and support the assumption that these micelles are really unimolecular. The dependence of the size of multipolymer micelles of the terpolymers on temperature found from the DLS measurements is shown in Fig. 3. It can be seen that R_h of non-irradiated multipolymer micelles of A10C10, A10C15, and A10C20 changes when the temperature exceeds the LCST growing up to 150–300 nm upon heating to 60–70 °C. On the other hand, heating of the solution of A10C25 does not result in the

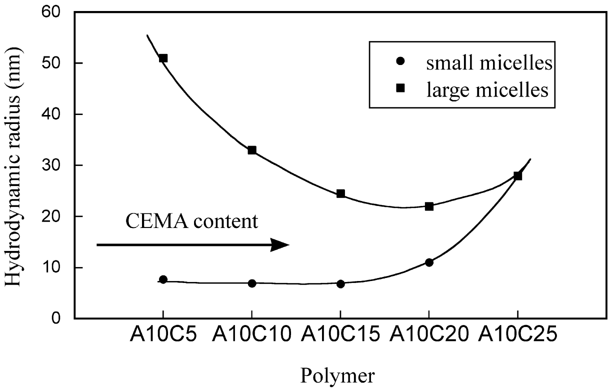


Fig. 2. Hydrodynamic radius of the micelles present in aqueous solutions of the terpolymers 1 g/l in a presence of 0.1 M NaCl at $T = 25$ °C.

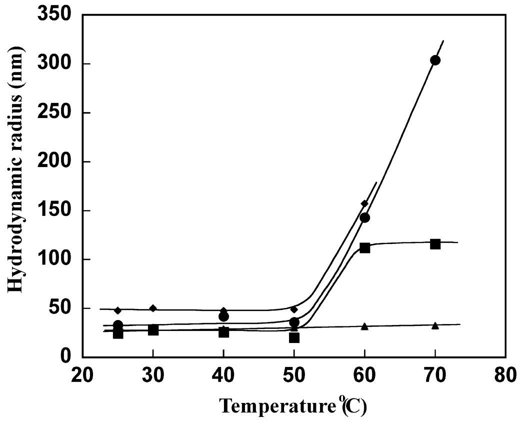


Fig. 3. The hydrodynamic radius of multipolymer micelles of A10C10 (●), A10C15 (■), A10C20 (◆), and A10C25 (▲) in aqueous solution (1 g/l) in a presence of 0.1 M NaCl before irradiation.

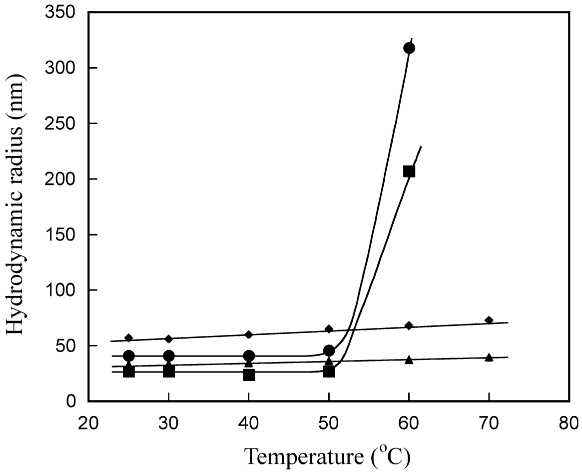


Fig. 4. The hydrodynamic radius of the irradiated multipolymer micelles of A10C10 (●), A10C15 (■), A10C20 (◆), and A10C25 (▲) in 0.1 M NaCl.

abrupt increase in the multipolymer micelle size. We have found previously that at these conditions this polymer does not show the LCST [29]. Only a slight gradual increase in R_h is observed from 28 to 32 nm when the temperature is raised from 25 to 70 °C.

3.3. Temperature-induced changes in R_h of the irradiated terpolymers

We have measured the hydrodynamic radii of the micelles after irradiation with UV light at the wavelength of $\lambda > 270$ nm, which resulted in the photodimerization of the polymeric cinnamoyl chromophores and in the photocrosslinking of the micelles. It was found that the irradiation of micelles of the polymers in 0.1 M NaCl aqueous solutions resulted in quite a small increase in the size of the micelles. This would suggest that the photocrosslinking takes place almost exclusively within the micelles. The increase of the micellar size may be a result of the crosslinks formed between multipolymer micelles and small micelles. Formation of crosslinks between multipolymer micelles is quite improbable, because it would result in a much greater increase in their R_h.

What changes to a much greater degree after irradiation is the response of the micelle size to temperature (Fig. 4). The irradiated A10C10 and A10C15 terpolymers form greater micelles (318 and 207 nm, respectively) when their temperature exceeds the LCST than non-irradiated polymers (143 and 112 nm, respectively, see Fig. 3). On the other hand, both irradiated A10C20 and A10C25 micelles do not show an abrupt increase of the size in the temperature range studied. Instead, they show rather gradual, linear increase from 57 to 73 nm and from 33 to 40 nm, respectively, when the temperature was increased from 25 to 70 °C. In particular, the behavior of A10C20 micelles changes very much after irradiation, because the polymer does not show LCST any more. This is consistent with the irradiation-induced changes of the behavior of A10C25 at increased ionic strengths (see below).

3.4. Temperature-induced changes in R_h of the non-irradiated and irradiated A10C25 solutions at various ionic strengths

We have chosen A10C25 terpolymer to study the influence of temperature on the micelle size at various ionic strengths for both non-irradiated and irradiated solutions, because the self-association of this polymer is the least complicated (only multipolymer micelles, highly monodisperse). The results of the DLS measurements are shown in Fig. 5.

It can be seen that at lower temperatures (below 40 °C) the hydrodynamic radius of the non-irradiated micelles decreases slightly with increasing ionic strength (Fig. 5(a)). When the ionic strength of the non-irradiated solution of

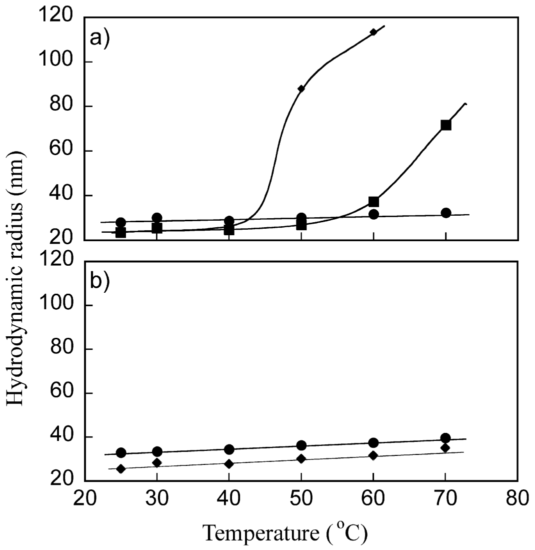


Fig. 5. The hydrodynamic radius of (a) non-irradiated and (b) irradiated micelles of A10C25 in 0.1 (●), 0.2 (■), and 0.3 M NaCl (◆).

A10C25 is increased from 0.1 to 0.2 the size of the micelles starts to increase when the temperature exceeds 50 °C so that the hydrodynamic radius increases from 24 nm at 25 °C to 72 nm at 70 °C. At the ionic strength of 0.3 the micelles start to grow at even lower temperature (around 40 °C) reaching 113 nm at 60 °C. For the irradiated micelles, however, the temperature response at elevated ionic strength is completely different. The micelles irradiated in 0.3 M NaCl solution are slightly smaller than those irradiated in 0.1 M NaCl solution and show slow and comparatively slight (from 25 to 35 nm) linear increase of the hydrodynamic radius with increasing temperature from 25 to 70 °C. Thus, irradiation of the micellar solution of A10C25 at higher ionic strengths results in the loss of the temperature-sensitivity of the micelles.

4. Conclusions

The copolymers of AMPS, NIPAM, and CEMA are sensitive to ionic strength, temperature and irradiation with UV light. They undergo micellization in the aqueous solution with the formation of both small and large micelles, except for the solutions of A10C25, which forms only larger, quite monodisperse micelles. There is a visible dependence of the type of micelles formed and their size on the content of hydrophobic CEMA monomer in terpolymer. The hydrodynamic radius of non-irradiated micelles in 0.1 M NaCl increases abruptly when the temperature exceeds the LCST, which indicates the occurrence of the aggregation process. That effect is not visible for A10C25 micelles, which show only relatively small linear increase in their size. This confirms that the hydrophobic interactions are the main driving forces for aggregation in that polymer solution. Irradiation of micelles in 0.1 M NaCl results in the larger size achieved by A10C10 micelles and A10C15 at higher temperatures while the temperature-sensitivity of the A10C20 and A10C25 micelles is almost lost. This may indicate that the irradiation carried out under conditions at which the electrostatic interactions between the ionic AMPS groups present in polymer chains are diminished (high ionic strength) can allow for the formation of well defined and stable multipolymer micelles in aqueous solutions of the polymer with relatively high content of CEMA.

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