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"Smart" polymeric nanospheres as new materials for possible biomedical applications

KRZYSZTOF SZCZUBIAŁKA*, MONIKA JANKOWSKA, MARIA NOWAKOWSKA*
Faculty of Chemistry, Jagiellonian University, 30-060 Kraków, Ingardena 3, Poland
E-mail: nowakows@chemia.uj.edu.pl; szczubia@chemia.uj.edu.pl

Novel random terpolymers of *N*-isopropylacrylamide (NIPAM), sodium 2-acrylamido-2-methyl-1-propanesulfonate (AMPS), and cinnamoyloxyethylmethacrylate (CEMA) were synthesized by free radical copolymerization using AIBN as an initiator. 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 water-soluble. Because of their amphiphilic nature they undergo self-organization in the aqueous solution with the formation of micelles capable of solubilizing sparingly water soluble organic compounds, such as drugs. The terpolymers are susceptible to three external stimuli, i.e. temperature, ionic strength and UV light. Due to the presence of NIPAM in the terpolymers they display the lower critical solution temperature (LCST), the presence of AMPS makes them sensitive to the ionic strength of the solution, while the light-responsiveness of the terpolymers is due to the presence of cinnamoyl chromophores, which undergo photodimerization when irradiated with UV light at about 280 nm. Application of any of these stimuli alone or in combination with other stimuli allows changing the copolymer properties in a controlled way.

Introduction

"Smart" or intelligent polymers are those able to respond to external stimuli such as, for example, pH, temperature, ionic strength, light, or magnetic field. These polymers draw growing interest, both in academia and in industry, due to their very broad range of possible practical applications. The examples of these applications include drug delivery [1], wound healing [2], chemical memory units [3], intelligent switches and control valves [4, 5], multifunctional separation membranes [6] to mention only a few.

The responses of the polymeric materials to the applied stimuli may be very different. The responsiveness to ionic strength is a typical property of the polymers containing ionizable groups, amphiphilic polyelectrolytes in particular [7]. The changes in the ionic strength of the solution may change, e.g. the size of the polymeric micelles, polymer solubility [8] and the fluorescence quenching kinetics of the chromophores bound to a polyelectrolyte [9, 10].

pH-responsive polymers, which may be considered as a class of ionic strength responsive polymers, constitute a very intensively studied group of intelligent polymers. These polymers usually contain monomer(s) bearing weakly acidic or weakly basic group, whose degree of dissociation, and consequently the charge of the polymeric chain, is pH-dependent. Changes in pH may affect solubility [8] or changes in the micellization behavior of both random [11, 12] and block polyelectrolytes [13, 14].

Most of the currently studied temperature-responsive polymers are based on NIPAM [15–19] thus it is their solubility that responds to the temperature changes. The homopolymer of NIPAM (PNIPAM) shows a thermoreversible phase separation in the aqueous solution when the temperature exceeds the LCST (ca. 31–35 °C) [20]. The solubility of the 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 [21]. 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.

The response of the polymers to the absorbed light may result from the photochromic isomerization, as in the case of the isomerization of azobenzene chromophores [22], or from the photoreaction, in which polymeric chromophores take part, e.g. hydrogel formation by photocrosslinking of acrylated dextran [23] or photocrosslinking of polyoxazoline substituted

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*Authors to whom all correspondence should be addressed.

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with coumarin chromophores [24]. The possible responses of the polymers to the absorbed light involve reversible changes in solubility [25], shape of polymer gel [26] or sol–gel transitions [27].

Most of the studies on the stimuli-responsive materials concern polymers responding to one stimulus. Less frequent are the publications on polymers responding to two stimuli [28]. This paper, continuing our work on stimuli-responsive polyelectrolytes containing AMPS and cinnamoyl-containing monomer [29] and NIPAM [30] is, to our best knowledge, the first study on the polymers found to be sensitive to three external stimuli, i.e. ionic strength, temperature and UV light. The polymers respond to each of these stimuli independently. Moreover, the response to a stimulus may be changed by application of another stimulus, e.g. both ionic strength and photocrosslinking change the LCST value, although their influence is opposite, i.e. increasing ionic strength decreases LCST, while photocrosslinking increases it.

Experimental

Materials

AMPS (Aldrich) was used as received, NIPAM (Aldrich) was purified by dissolving in toluene followed by precipitation in a large excess of hexane. 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 until spectrally clean eluent was obtained. Methanol was then removed from CEMA by freeze-drying. α,α'-Azobisisobutyronitrile (AIBN) was recrystallized from methanol at 40 °C under nitrogen. Anhydrous Na_2CO_3 and NaCl were purchased from POCh, Gliwice, and used as received. LiCl (POCh, Gliwice) was dried in an oven at 105 °C. DMF (HPLC grade, Aldrich) and diethyl ether (Lachema, pure analytical) was used as received. Doubly distilled water was used in all experiments.

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 till Na_2CO_3 was completely dissolved. AIBN (0.1% based on total monomers), CEMA and NIPAM were then added. 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 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 found from elemental analysis and from UV (absorption at 280 nm) and ^{1}H-NMR spectra.

Instrumentation

Irradiation of the terpolymer solutions were carried out in a Rayonet photochemical reactor, model RPR-100, equipped with 16 lamps (RPR-3000), each having power of approximately 21 W. Irradiations at higher temperatures were carried out using a cell with jacket through which thermostated water was circulated. UV spectra of the terpolymers were measured on a Hewlett-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. LCST of the terpolymer solutions was measured using a Hewlett-Packard 8452A diode-array spectrophotometer equipped with a Hewlett-Packard 89090A Peltier temperature-control accessory. The accessory allowed digital control of the temperature ($\pm$ 0.1 °C) by programmed thermoelectrical heating in the range from 15 °C up to about 70 °C. Temperature was measured with a Hewlett-Packard 89102A temperature sensor immersed in a 1-cm optical path cuvette with the polymer solution at 1.0 g/dm^3. The polymer solution was continuously stirred with a magnetic stirrer at a rate of 5 s^{-1}. Cloud points were determined from changes in the transmittance at $\lambda = 400$ nm of the solutions heated at about 0.5 °C/min. The ionic strength of the solutions was adjusted by the addition of LiCl.

Results and discussion

We have synthesized five terpolymers of AMPS, NIPAM and CEMA. The structure of the terpolymers is given in Fig. 1, while the monomer compositions of the reaction mixtures and polymers obtained are given in Table I. All the reaction mixtures contained a fixed amount of AMPS (10 mol %), the content of CEMA ranged from 5 to 25 mol % in 5 mol % increments, and the content of NIPAM was changed from 65 to 85 mol %. The terpolymers were assigned symbols A*x*C*y*, where *x* and *y* mean AMPS and CEMA content (in mol %) in the reaction feed, respectively. Such a monomer composition gives the polymers the ability to react to three different external stimuli, i.e. ionic strength, temperature and UV light. The content of AMPS gives the polymers the polyelectrolyte character and therefore the ability to respond to changes in the ionic strength of the solutions. The reaction of the polymers to ionic strength was most clearly seen in the LCST shift to lower temperatures when the ionic strength was increased (see below). Our previous studies on the AMPS–NIPAM copolymers have shown [30] that quite small content of AMPS results in considerable shift in the LCST values of the polymers toward lower temperatures, therefore the polymerization

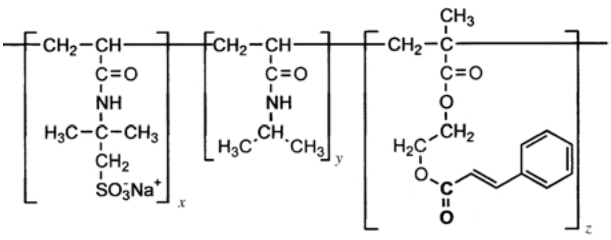

Figure 1 Structure of the terpolymers.

TABLE I Monomer composition of the reaction mixtures and the polymers

Polymer symbol	AMPS				NIPAM				CEMA				Yield (%)
	Reaction mixture			Polymer	Reaction mixture			Polymer	Reaction mixture			Polymer	
	m (g)	n (mmol)	mol %	mol %	m (g)	n (mmol)	mol %	mol %	m (g)	n (mmol)	mol %	mol %	
A10C5	0.3207	1.55	10	12.75	1.4890	13.16	85	76.42	0.1906	0.77	5	10.83	20.00
A10C10	0.3050	1.47	10	8.71	1.3320	11.77	80	71.29	0.3620	1.47	10	20.00	11.25
A10C15	0.2908	1.40	10	8.26	1.1908	10.52	75	69.03	0.5184	20.10	15	22.71	22.50
A10C20	0.2778	1.34	10	8.60	1.0618	9.38	70	60.85	0.6603	2.68	20	30.55	16.50
A10C25	0.2659	1.29	10	7.82	0.9439	8.34	65	55.75	0.7902	3.21	25	36.43	12.50

mixtures contained only 10 mol % of AMPS. The responsiveness of the terpolymers to temperature was due to the content of NIPAM, as discussed in the Introduction section, while the responsiveness to the light was due to the content of CEMA, a monomer containing the cinnamoyl chromophore. The derivatives of cinnamic acid and other unsaturated carboxylic acids, e.g. 2-indenecarboxylic acid, are known to undergo photodimerization when irradiated with light around 280 nm [31]. Moreover, such a composition of the terpolymers also determines their amphiphilic character; AMPS and CEMA are ionic and hydrophobic monomers, respectively, while NIPAM is hydrophilic below LCST and hydrophobic above LCST. Therefore, the terpolymers tend to undergo self-organization in the aqueous solution with the formation of micelles of the size in the nanometer range. The results of the studies on the micelle size and its response to the external stimuli are the subject of the paper under preparation.

Photocrosslinking of the micelles

Due to the content of a photodimerizable chromophore the polymeric micelles could be photocrosslinked. Photocrosslinking of the polymers was achieved by irradiation of the polymer solutions with 300 nm lamps with the emission band wide enough to be absorbed by the polymeric cinnamoyl chromophores which absorb at $\lambda_{max} = 280$ nm (see Fig. 2). An example of the changes in the UV absorption spectra upon irradiation is also shown in Fig. 2. The cyclobutane derivative which is formed during photodimerization of the cinnamoyl chromophores does not absorb at 280 nm, therefore photocrosslinking can be followed by monitoring the decrease in time of the solution absorbance at this wavelength. As an example, Fig. 3 shows a plot of the conversion of the cinnamoyl chromophores in the A10C5 solutions irradiated at room temperature without added electrolyte and plots for the polymer irradiated at elevated temperature and/or ionic strength. It can be seen that the photodimerization reaction is very fast during the first minute of irradiation and then remarkably slows down. High conversion of the cinnamoyl chromophores after a very short period of irradiation may result from the fact that a substantial part of the cinnamoyl chromophores are close to each other thereby forming a hydrophobic domain. Formation of the hydrophobic domains could be also concluded from the fluorescence probe measurements (data not shown). The sudden decrease of the photoreaction rate after such a short time may be caused by the mobility of the polymeric chains decreasing very fast during initial stages of irradiation. Due to increasing microviscosity inside the micelles cinnamoyl chromophores require more time to come into close contact necessary for the photodimerization to occur. It was found that increasing the temperature of irradiated solution up to 60 °C noticeably decreases the conversion, especially at higher ionic strengths. On the other hand, increasing ionic strength of the polymer solution decreases the conversion at the initial stages of the photoreaction, but after longer irradiation time the conversion is higher than that for the solution irradiated without added salt. The decrease of the conversion at the initial irradiation stage may be a result of the quenching of the cinnamoyl chromophores

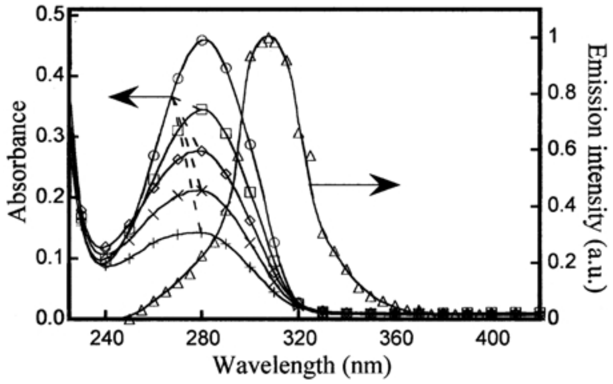

Figure 2 UV absorption spectra of A10C5 solutions at 0.1 g/dm^3 after 0 s (○), 10 s (□), 30 s (◇), 10 min (×) and 1 h (+) of irradiation and an emission spectrum of RPR-3000 lamps used for irradiations (△).

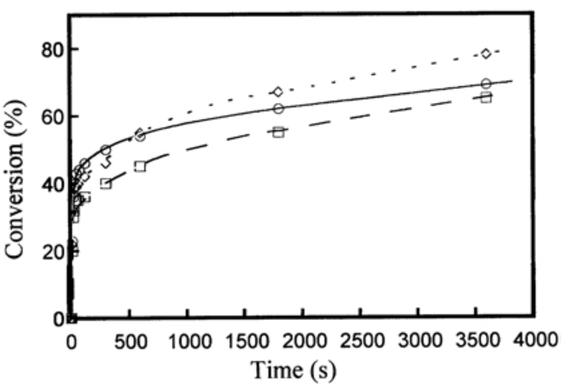

Figure 3 Conversion of the cinnamoyl chromophores in A10C5 solution during irradiation with 300 nm lamps at 25 °C and ionic strength $I = 0$ (○), at 25 °C and $I = 0.3$ (□) and at $T = 25$ °C and $I = 0.45$ (◇).

by added salt, while increased conversion for longer irradiation times at higher ionic strength of the solutions may be a result of more compact conformation of the polymeric chains and smaller distances between reacting chromophores.

Dependence of LCST on the stimuli

None of the terpolymers showed LCST below 70 °C (i.e. maximum temperature achieved in the Peltier device) in the aqueous solution. However, all the terpolymers, except for A10C25, showed LCST when the ionic strength of the solution was increased by the addition of LiCl. LCST of the terpolymers was found to be strongly dependent on the ionic strength of the solutions. Fig. 4 shows the results of the measurements of turbidity (expressed as $1-T$ where T is the transmittance of the solution) as a function of the temperature for different ionic strengths. It can be seen that the increase of the temperature above some value is accompanied by sudden increase in the solution turbidity which is a consequence of the formation of larger aggregates. The temperature at which the turbidity starts growing is considered to be LCST of the polymer. The increase of the ionic strength results in a significant decrease in the LCST value. Also, the increase in the ionic strength is accompanied by a decrease of the transmittance of the solutions even before the LCST is attained which may indicate that the micelles increase their size below LCST. For the polymers with decreasing NIPAM content the rise in turbidity is less and less abrupt. Finally, for A10C25 no turbidity increase could be found. The dependence of the LCST values on the ionic strength for different polymers is shown in Fig. 5. It can be seen that the lower NIPAM content in a polymer is, the lower ionic strength is necessary for LCST to appear. For the polymer with the highest NIPAM content, i.e. A10C5, LCST may be controlled in the widest temperature range, i.e. from about 22 to 63 °C.

It was found that the LCST of the terpolymers depends not only on the ionic strength, but it is also changed by the photocrosslinking of the terpolymers with UV light. Fig. 6 shows the results of the measurements of LCST for nonirradiated A10C5 solutions and for the solutions irradiated at room temperature and at 60 °C. It can be seen that due to photocrosslinking of the micelles LCST appears at higher ionic strength (at 0.35 and 0.4 for solutions irradiated at 25 °C and 60 °C, respectively) compared to nonirradiated solutions (at 0.3). Moreover, for a given ionic strength the irradiated solutions show higher LCST than nonirradiated solutions. It can be also found that irradiation of the polymer solution at higher temperature results in a larger increase in the LCST compared to the polymer irradiated at lower temperature. The LCST values of the polymer photocrosslinked at room temperature at lower ionic strengths are close to the LCST values for nonirradiated polymer while at higher ionic strength are close to the LCST values of the polymer irradiated at 60 °C.

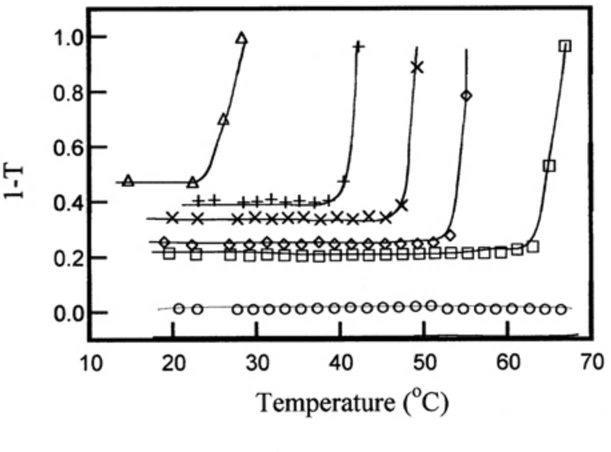

Figure 4 Turbidity of 1.0 g/dm^3 solutions of A10C5 at $I=0$ (○), 0.3 (□), 0.35 (◇) 0.4 (×), 0.45 (+), and 0.7 (△).

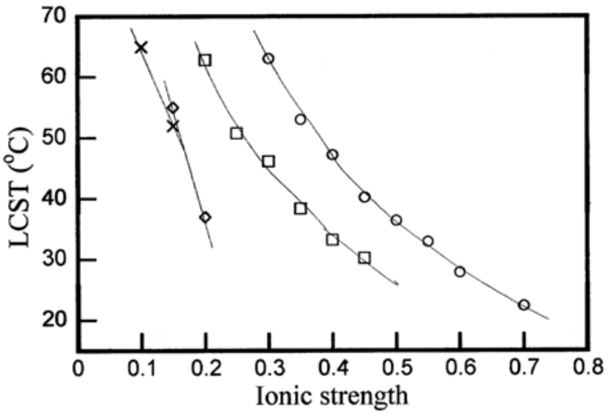

Figure 5 Dependence of LCST on ionic strength for A10C5 (○), A10C10 (□), A10C15 (◇), and A10C20 (×) at 1.0 g/dm^3.

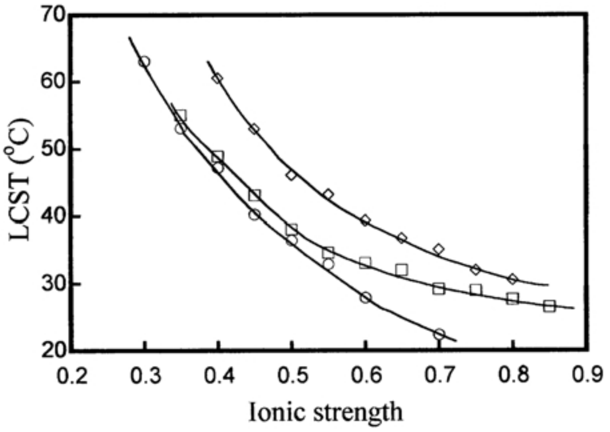

Figure 6 LCST of A10C5 at 1.0 g/dm^3 nonirradiated (○) and irradiated at 25 °C (□) and at (◇) 60 °C.

Conclusions

The physicochemical properties of the terpolymers of AMPS, NIPAM and CEMA can be modified by the application of three external stimuli: ionic strength, temperature and UV radiation at $\lambda = 280$ nm. The terpolymers are amphiphilic polyelectrolytes undergoing self-organization in the aqueous solution. The polymeric solutions show LCST at increased ionic strengths. The LCST of the polymers decreases with increasing ionic strength and increases after irradiation with UV light. Irradiation at higher temperature causes greater increase of LCST than irradiation at room temperature. Such water-soluble and self-organizing systems may find applications as new materials for biomedical applications.

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