Thermoresponsive Properties of *N*-Isopropylacrylamide with Methacrylic Acid Copolymer in Media of Different Acidity

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Abstract: Linear statistic *N*-isopropylacrylamide copolymer containing 16.8 mol % of methacrylic acid was studied using methods of static and dynamic light scattering and turbidimetry. The experiments were performed in aqueous solutions at the concentration $c = 0.015$ g cm^{-3} in a wide temperature region from 10 to 60 °C within pH interval from 1.7 to 11.2. The rapid increase of scattered light intensity and decrease of the transmitted light intensity at heating of the solution was observed, which implied phase separation in the solution. The phase separation was confirmed by intensive aggregation of macromolecules, which was evidenced by dramatic changes in the size of the dissolved species and their fraction in the solution. Thermoresponsiveness of the copolymer was strongly dependent on pH. The specific behavior of the *N*-isopropylacrylamide with methacrylic acid copolymer as compared to that with maleic acid was shown, which is caused by the presence of hydrophobic methyl group in the chemical structure of the methacrylic acid monomer.
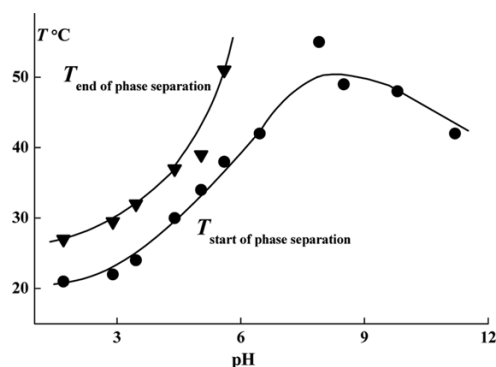
1. Introduction

Stimuli-responsive polymer systems have attracted much attention during the recent years because of their high application potential in different fields. Small changes in the surrounding medium can trigger dramatic changes in the characteristics of their solutions. Double stimuli-responsive polymers, which are sensitive both to temperature and pH, are especially interesting for biomedical applications.[1-6] Some of them become soluble or insoluble in response to varying of the corresponding aqueous media parameters close to physiological, which can be promising for drug delivery.

The simplest way to endow polymer double responsiveness is copolymerization of monomers consisting of the units of appropriate stimuli-responsive properties (pH- or thermosensitive). For example, N-isopropylacrylamide (NIPAAm), which is well-known as thermo-responsive component,[7-10] can be copolymerized with hydrophylic ionic monomers such as maleic (MA), acrylic (AA) or methacrylic (MAA) acid or other comonomers, which provide pH-sensitivity.[11-14] Poly(NIPAAm) becomes

insoluble in water on heating due to the change in hydrophilic-hydrophobic balance. The lower critical solution temperature (LCST) of poly(NIPAAm) aqueous solutions is about 32 °C. Combination of NIPAAm with hydrophilic ionic comonomers not only allows macromolecules to respond to pH, but can shift the phase transition temperature closer to human body temperature.[15] Carboxylic groups of acidic comonomers accept protons in acid media and release them in basic conditions impacting the copolymer properties of polyelectrolytes.

The phase transition temperature, *i.e.*, phase separation temperature, and the character of phase separation in polymer solutions can be varied in a wide range by varying copolymer composition,[16-18] solvent acidity,[19] solution concentration,[20] presence of ions,[14] as well as synthesis conditions,[18] macromolecular architecture,[21,22] and other parameters. For a linear statistic poly(NIPAAm-*co*-MA) containing 6.1 mol% of MA it was shown[19,20,32] that in dilute solutions the starting and terminating temperatures of the phase separation and the width of the temperature interval where the phase separation takes place, grow with the increase of pH and the decrease in the copolymer concentration. The observed phase separation temperatures proved to be shifted relative to the cloud point of poly(NIPAAm) to the region of higher temperatures at all pH and concentrations. Further introduction of hydrophobic or hydrophilic elements into the thermo- and pH-sensitive systems (for example, their complexing with drugs) also results in the change of hydrophilic-

hydrophobic and electrostatic balance in the solutions and as a result, leads to the variation of phase separation characteristics.[17]

The effect of different parameters on thermo- and pH-sensitivity of polymers is not only of practical but primarily of a fundamental importance for directional control of the properties of "smart" polymer systems targeted for drug delivery. That is why it is important to study the interrelations between the copolymer parameters and its thermo- and pH-induced response. Special attention seems to be paid to the influence of the chemical structure of the ionic comonomers on these properties.

The reported work intends to study the effect of pH on thermosensitivity of a NIPAAm with MAA copolymer. Poly(NIPAAm-*co*-MAA) is promising as a polymer carrier for pulse drug delivery. Being crosslinked to a hydrogel, the copolymer can swell and absorb a drug or shrink and, consequently, release it in response to changes of surrounding medium (*T* or pH). The swelling behavior of a macromolecular network is to a great extent a function of the structure and properties of its linear segments. Hence, the behavior of linear units of the network in different media conditions should be studied. Though poly(NIPAAm-*co*-MAA) is commercially available and has been studied by different authors in various aspects,[23-29] it is undoubtedly worth to systematically analyze the behavior of aqueous solutions of linear poly(NIPAAm-*co*-MAA) in a wide range of *T* and pH. A charged MAA comonomer possesses a specific structure containing, besides the pending hydrophilic carboxyl group, a hydrophobic methyl group.

It was shown that conformational properties of poly(MAA) in aqueous solutions are influenced by the presence of this -CH_3 group, resulting in aggregate formation and conformational transitions from coiled to uncoiled state in different media.[30, 31] It can be suggested that such a structural peculiarity of the comonomer can also affect the copolymer properties, in particular, its responsive behavior. In the reported work we compare the thermosensitivity of the poly(NIPAAm-*co*-MAA) with that of poly(NIPAAm-*co*-MA) previously studied by the authors.[19,20,32]

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAAm), purchased from Aldrich Chemical Corp. (Milwaukee, WI, USA), was re-crystallized from hexane. Methacrylic acid (MAA), *N,N′*-azobisisobutyronitrile (AIBN), and 1,4-dioxane were purchased from Fluka AG (Buchs, Switzerland). MAA was purified by distillation under reduced pressure, AIBN was solubilized in warm methanol and re-crystallized, and 1,4-dioxane was purified by refluxing. All chemicals were of analytical or reagent grade and were used without purification unless stated.

2.2. Poly(NIPAAm-*co*-MAA) synthesis

Poly(NIPAAm-*co*-MAA) copolymer was synthesized by free radical polymerization of the corresponding *co*-monomers in 1,4-dioxane. 1.13 g of NIPAAm (10 mmol), 169 μL of MAA (2 mmol), and 0.016 g of AIBN (0.1 mmol) were dissolved in 6 mL of 1,4-dioxane. The solution was purged with nitrogen for 30 min, then, the temperature was increased up to 75 °C. Polymerization was achieved within 12 h. The polymer solution was precipitated in diethyl ether, recovered by filtration, and dried under vacuum at 50 °C. Subsequently, the polymer was re-dissolved in distilled water, dialyzed against water for 7 days (molecular weight cut off 10,000-12,000 Da; from Medicell International, London, United Kingdom), and recovered by lyophilization.

2.3. ^1H NMR analysis

The copolymer structure and composition were determined by ^1H NMR analysis. ^1H NMR spectra of poly(NIPAAm-*co*-MAA) were recorded in deuterated water on a Varian Mercury Plus 400/Varian VXR 200 spectrometer operating at 400 MHz frequency. The molar fraction of co-monomers was determined by comparing the peak area of the methynic proton at 3.86 ppm of NIPAAm (the integral was set to equal 1 proton) with the total peak area between 0.5 and 2.5 ppm corresponding to the main backbone protons (3 NIPAAm + 2 MAA) plus the methyl protons (6 NIPAAm + 3 MAA) according to the following Eq. (1):

$$9 \text{ NIPAAm} + 5 \text{ MAA} = 10.06 \tag{1}$$

2.4. Conductometric analysis

The content of MAA in copolymer was also determined by conductometric titration at 22 °C using the CDM 210 conductivity meter (Radiometer, Copenhagen, Denmark) equipped with a CDC 865 cell. Briefly, an excess of 0.1 N HCl was added to polymer solution, then the solution was back titrated with aqueous 0.1 N NaOH solution in portions of 200 mL each time. The volume of the NaOH solution used for the ionization of carboxyl groups was determined from the conductometric titration curve and the content of carboxyl groups in the copolymer (*C*) was calculated according to Eq. (2):

$$C(\text{meq/g}) = \frac{(V_2 - V_1)_{NaOH} \times f \times c}{m}, \tag{2}$$

where V_{NaOH} is the used volume of aqueous NaOH solutions in mL, f is the factor of NaOH solution, c is the concentration (mol/L) and m is the weight of dried sample, in grams.

2.5. Potentiometric titration

A 0.005 N aqueous solution of poly(NIPAAm-*co*-MAA) was passed through an ion exchange column filled with a sulfonic Dowex 50WX8 resin in order to obtain a protonated form of the copolymer. The solution was then titrated with 0.1 N NaOH using the all-purpose t 716 DMS Titrino titrator, Metrohm (Switzerland) equipped with a combined glass electrode. The obtained potentiometric titration data pH=*f* (base volume) were then used to calculate the apparent dissociation constant pK_{app} values according to the Henderson-Hasselbach Eq. (3):

$$pK_{app} = pH + \log\frac{1-\alpha}{\alpha} \tag{3}$$

where α is the total fraction of ionized carboxylic groups. The latter is calculated as $\alpha=\alpha_N+\alpha_H$, where α_N is the degree of neutralization, and α_H is the degree of auto-dissociation (α_N=[NaOH]/C_p, α_H=([H$^+$]-[OH$^-$])/C_p, where C_p is the equivalent concentration of the copolymer).

2.6. Static and dynamic light scattering and turbidimetry

The behavior of aqueous solutions of poly(NIPAAm-*co*-MAA) on heating was analyzed by the static (LS) and dynamic (DLS) light scattering and turbidimetry methods. Experiments were carried out using the Photocor Complex instrument (Photocor Instruments Inc.) equipped with the Photocor-DL diode laser (wavelength λ=658.7 nm), Photocor-PC2 correlator with 288 channels, and Photocor-PD detection device for measuring the transmitted light intensity. The solutions were filtered into dust-free vials using Millipore filters with hydrophobic PTFE membrane and pore size of 0.45 μm (Merck). The solutions were studied at concentration c=0.015 g cm^{-3} in bi-distilled water. The pH was changed within the interval from 1.7 to 11.2 by adding 1 N HCl or 1 N NaOH to the initial solution (pH=3.5).

The solution temperature T was changed discretely within the interval from 10 to 60 °C, with the steps ranging from 1.0 °C (near cloud point) to 5 °C (at low T). The temperature was regulated with the precision of 0.1 °C. The heating rate was about 1.5 °C min^{-1}. The procedure was as follows: after reaching the given temperature, the analysis of light scattering intensity I and optical transmittance I^* started. The hydrodynamic radius R_h of dissolved particles was measured when I became independent from time. The contribution of different types of particles to the summary light scattering intensity was estimated using the value S of squares under the curved line of the corresponding peaks of the I distribution on R_h. The scattering angle was 90°. In equilibrium conditions, when the characteristics of solution did not change with time, the angle dependences of light scattering intensity I and the hydrodynamic radii R_h of the scattering objects were also analyzed in order to justify the diffusion process. The temperature dependences of the characteristics in question were plotted with experimental values measured after the equilibrium state achievement.

Note that time t_{eq} required for equilibrium state achievement in aqueous solutions of poly(NIPAAm-*co*-MAA) after changing temperature was not so large: t_{eq} was close to 500 s at low temperatures and about 3000 s near and within the phase separation region. Such values are typical for linear thermo- and pH-sensitive polymers[33-36] and much lower than t_{eq} for star-shaped poly(2-isopropyl-2-oxazolines)[37–40] and graft copolymers of polyimide backbone and poly-*N,N'*-dimethylaminoethyl methacrylate side chains.[41]

3. Results and discussion

3.1. Preparation and characterization of poly(NIPAAm-*co*-MAA)

As largely recognized, poly(NIPAAm) is the most common thermosensitive polymer for different applications since it possesses a sharp phase transition at the temperature close to that
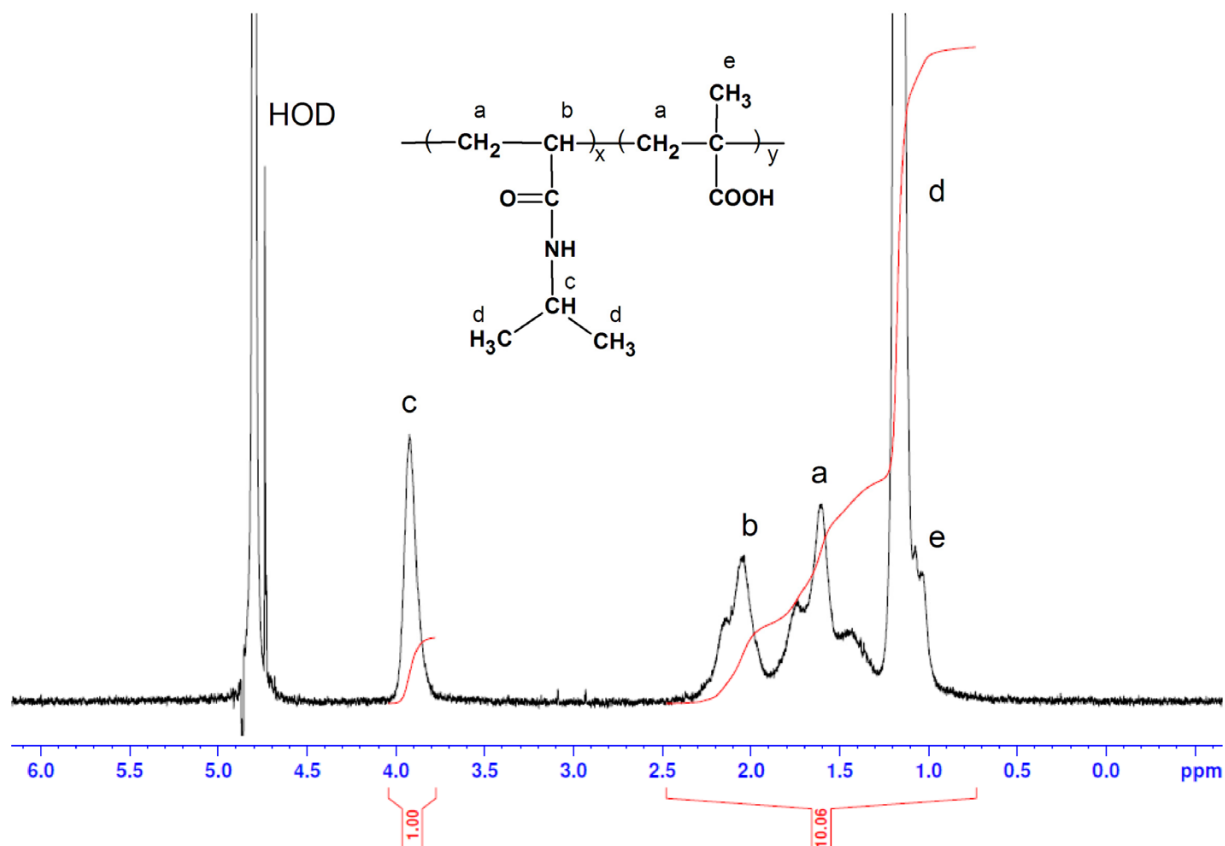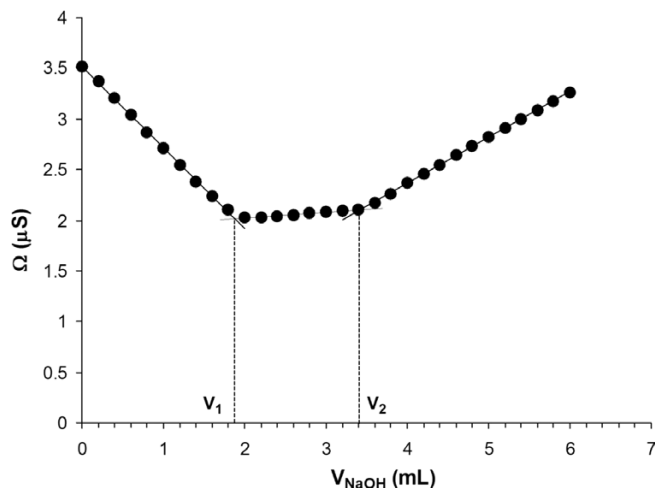

Figure 1. ^{1}H NMR spectrum of poly(NIPAAm-co-MAA) in D$_2$O.

Table 1. Co-monomer composition in the feed and in poly(NIPAAm-*co*-MAA) copolymer

Co-monomer feed composition × 10⁻³ M (% mol ratio)		Co-monomers in copolymer (% mol ratio) determined by			
		¹H NMR		Conductometry	
NIPAAm	MAA	NIPAAm	MAA	NIPAAm	MAA
10 (83.33)	2 (16.67)	82.51	17.49	83.84	16.16

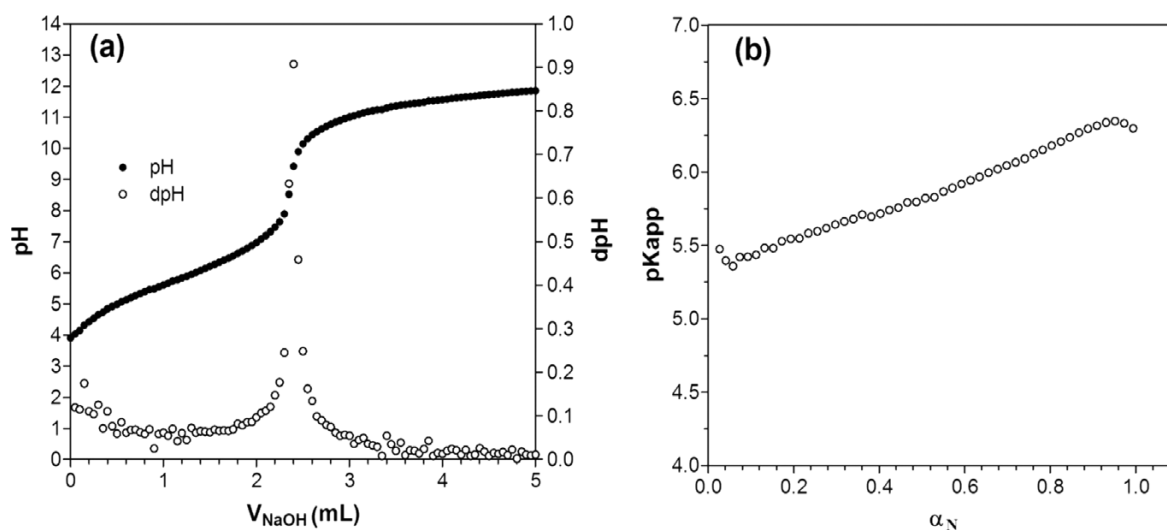**Figure 2.** Conductometric titration curve of poly(NIPAAm-*co*-MAA).

of the human body.

However, the behavior of polymers that would respond simultaneously to two kinds of stimuli including temperature and pH seems to be very attractive both from theoretical and practical point of view. As follows, NIPAAm was copolymerized with the pH-sensitive MAA by free radical copolymerization in 1,4-dioxane. As confirmed by ¹H NMR spectra (Figure 1), the co-polymer is generated. The molar ratio of the MAA in the copolymer (17.49%) determined from ¹H NMR is slightly higher than that in the feed (16.67%) (Table 1). On the contrary, the value of MAA molar ratio in copolymer determined by conductometry (16.16%) (Figure 2) is smaller than that in the feed (see Table 2). However, these differences between the compositions in the feed and those determined by both methods are very small and are included in the error limits of the methods.

The potentiometric titration curve of poly(NIPAAm-*co*-MAA) together with the first derivative are presented in Figure 3(a). The carboxylic groups of MAA in the copolymer were added in protonated state, then were titrated with 0.1 N NaOH. Poly(MAA) is known to possess anomalous pK_{app} behavior at low dissociation degrees, which was interpreted as a conformational transition from compact coil to extended coil or unfolding of the microdomains formed by hydrophobic forces or hydrogen bonds[42,43] (Figure 3).

In the case of poly(NIPAAm-*co*-MAA) with relatively low amount of MAA units, this anomalous behavior was not observed due to the lack of MAA-MAA sequences and to the hydrophilicity of the co-monomer (NIPAAm). The acid dissociation constant, pK_a, could be obtained for this copolymer by extrapolation of pK_{app} at α=0 (Figure 3(b)), and the obtained value was pK_a=5.3, showing low acid strength.

3.2. Molar mass determination

Molar mass M_w of the copolymer was determined using light scattering in water solutions, with the addition of 0.5 M or 0.1 M NaCl to suppress polyelectrolyte effect. However, two modes were observed in the solutions implying the presence of a small amount of aggregates. The values obtained in two solvents proved to be close to each other, the average value being M_w=170,000 Da. This value seems to be high for the investigated copolymer. The aggregates are likely to have contribute noticeably to the obtained M_w value. Note that pH was about 3.5. The effect of pH on the composition of the scattering objects in poly(NIPAAm-*co*-MAA) aqueous solutions will be discussed below. Unfortunately, we failed to obtain a molecularly homogeneous copolymer solution in organic solvents as well.

Figure 3. Potentiometric titration curve of poly(NIPAAm-*co*-MAA) with 0.1 N NaOH (a) and pK_{app} *vs.* α_N (b).

3.3. Dependence of phase separation temperature on solution acidity

The behavior of the poly(NIPAAm-*co*-MAA) solutions in general is similar to that of thermoresponsive polymers, in particular, to the behavior of the previously studied poly(NIPAAm-*co*-MA).[19,20,32] For all the investigated solutions, three temperature intervals can be seen on the $I(T)$ plot (Figure 4). First, there is a region of constant or very slight increase of I, which extends from low (room) temperatures up to the definite temperature T_1 (interval I). In the second interval (II), a dramatic increase of scattering intensity is observed starting from T_1 up to the temperature T_2, where scattering intensity is maximal. The third (III) interval ($T>T_2$) is the region where I decreases.

Region II can be considered as a phase separation interval, T_1 and T_2 being the temperatures of the start and the termination of phase separation, respectively. Phase separation is accompanied with the loss of solution transparency, which is controlled by the decrease of the transmitted light intensity I^* (Figure 4). The start of I^* decrease, which takes place at temperature T^*_1, implies the beginning of the phase separation. The transmission I^* achieves zero values at temperature T^*_2, which determines the termination of the phase separation. The phase transition temperatures, obtained separately using $I(T)$ and $I^*(T)$ plots for each pH, practically coincided. That is why further discussion will concern only T_1 and T_2.

Temperature dependencies of I and I^* were measured in a wide pH interval from 1.7 to 11.2, and temperatures T_1 and T_2 were determined for different pH values. Since poly(NIPAAm-*co*-MAA) contains ionic carboxyl groups -COOH, the properties of its macromolecules in solution should depend on the medium acidity.

Figure 5 clearly demonstrates a strong effect of solution acidity on phase separation temperatures. The increase in pH leads to an increase in both T_1 and T_2, provided the surrounding medium is acidic. At the same time the width of the phase separation interval $dT=T_2-T_1$ does not change noticeably with pH, being $dT=(6-7)°C$. However, at pH>5.4 the value of T_2 starts growing rapidly, and at pH ≥ 6.5, T_2 was not observed below the top mea-

Figure 5. Dependence of T_1 (circles) and T_2 (triangles) on pH for aqueous solutions of poly(NIPAAm-*co*-MAA) (hard symbols) and poly(NIPAAm-*co*-MA) (open symbols).[18]

Figure 6. Temperature dependence of relative intensity of scattered light I/I_0, aqueous solution of poly(NIPAAm-*co*-MAA) aqueous solution at pH=5.6 (a) and 8.5 (b).

surement temperature of 60 °C.

The value of pH influences not only transition temperatures but also the course of the $I(T)$ dependencies. At pH < 5.6 the I value monotonically increases with temperature until it reaches T_2. However, at the values of pH ≥ 5.6, a small deflection from monotonicity is observed (Figure 6). After reaching the maxi-
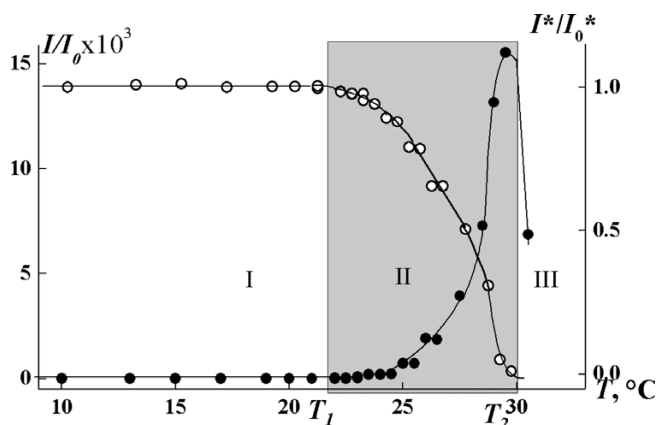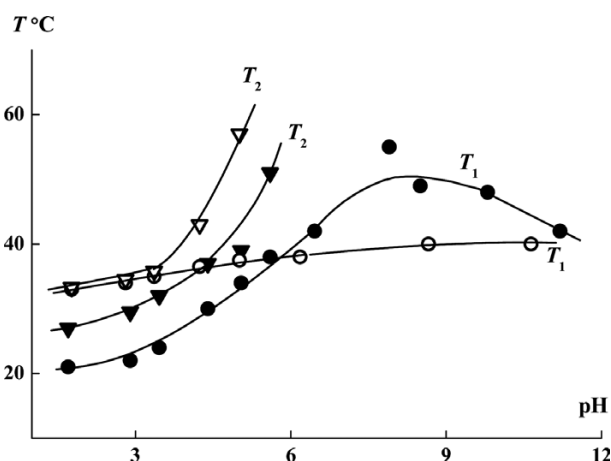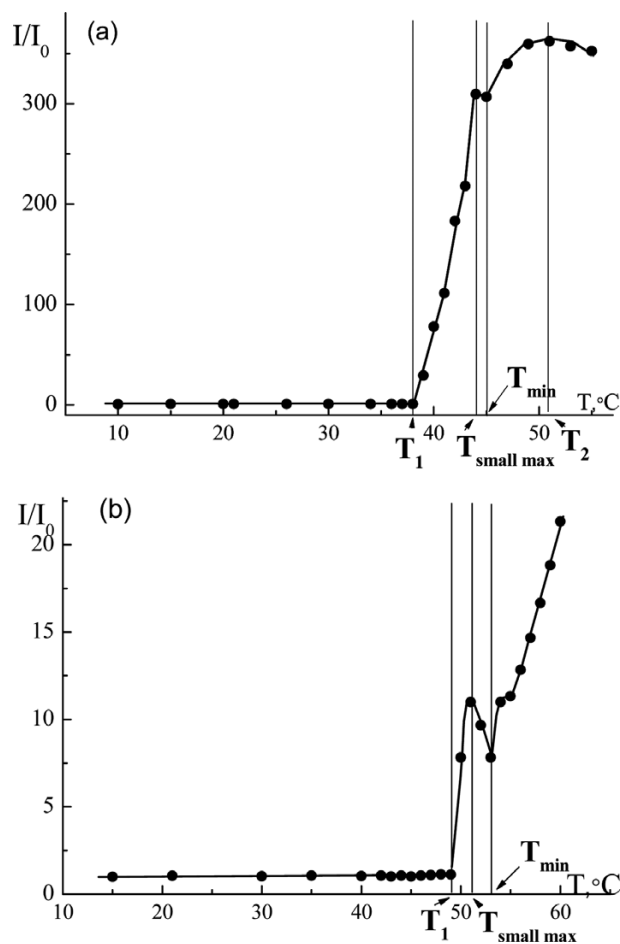
Figure 4. Dependences of relative intensity of scattered I/I_0 and transmitted I^*/I^*_0 light on T for aqueous solution of poly(NIPAAm-*co*-MAA) at pH=2.9. I_0 and I^*_0 are the intensities of scattered and transmitted light at 21 °C, respectively.
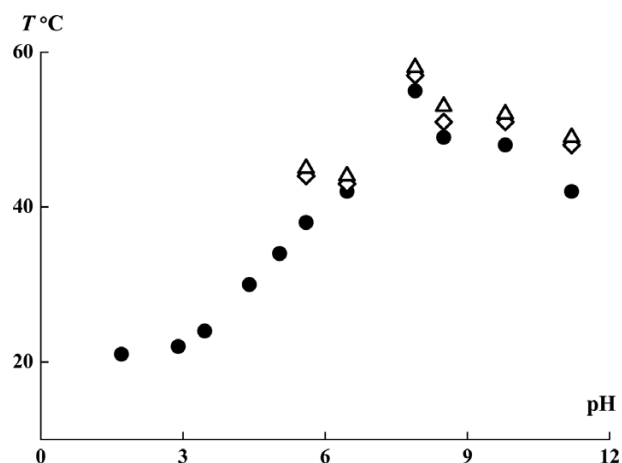
Figure 7. Dependence of T_1, (circles) $T_{small\,max}$ (rhombs) and T_{min} (triangles) on pH for aqueous solution of poly(NIPAAm-*co*-MAA).
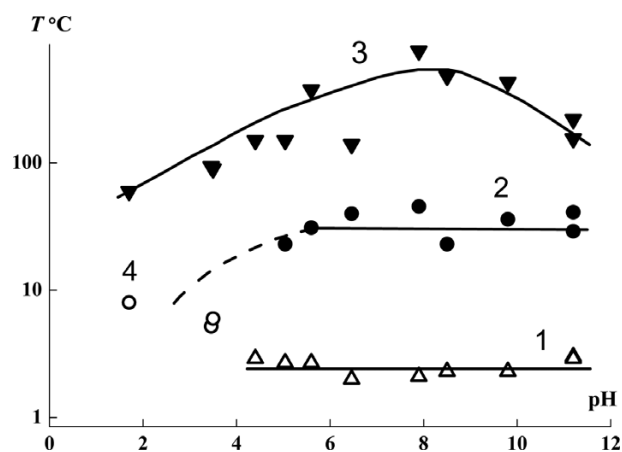

Figure 8. Hydrodynamic radii of fast (1), middle (2), slow (3) and *x*- (4) modes at different pH for aqueous solution of poly(NIPAAm-*co*-MAA).

mum, a short decrease in *I* turns into further rapid *I* increase. The pH, at which this peculiar poly(NIPAAm-*co*-MAA) behavior starts being registered, is quite close to pK_a=5.3. This observation makes it possible to suggest that the deflection from the monotonic $I/I_0(T)$ dependence is caused by the variation of the electrostatic (ionic) balance in the solution.

The position of the first (small) maximum ($T_{small\,max}$) and the minimum (T_{min}) on the T(pH) dependence (Figure 7) is symbatic to the T_1 changes, and the temperature interval ($T_{small\,max}$-T_{min}) is always about 1-2 °C. This effect seems to require a special study. However, an idea can be proposed regarding its nature. NIPAAm and MAA can establish inter- or intramolecular H-bonds between isopropyl substituted amino groups and carboxylic groups. C=O and -NH- groups of poly(NIPAAm) can interact by means of H-bonds with -COOH of MAA. With the increase of T, the desolvation of NIPAAm units starts at T_1, then at $T_{small\,max}$ the H-bonds mentioned above break down. This improves the solubility of the copolymer due to the ionized carboxylic groups of MAA, hence the decrease of *I* is observed. Then, at further heating, desolvation of NIPAAm units and aggregation of macromolecules continues to decrease the copolymer solubility, which is accompanied by the *I* increase. At pH < pK_a the copolymer is partly in neutralized state and probably cannot support the dissolution.

3.4. Composition of the scattering objects in poly(NIPAAm-*co*-MAA) solutions

To understand the change of *I* observed for the copolymer solutions under investigation, the analysis of dissolved entities responsible for light scattering in poly(NIPAAm-*co*-MAA) solutions was carried out. Two or three modes were registered, depending on the temperature and pH. Their hydrodynamic radii will be denoted as R_h^f, R_h^m and R_h^s for the fast, middle and slow modes, respectively. Taking into account the literature data[37,38,44,45] and our previous experience,[19,20,32] the slow and the middle modes can be attributed to loose aggregates and micelle-like structures, respectively.

First, let us consider the solutions at temperatures far below T_1. Figure 8 demonstrates the dependence of R_h^f, R_h^m and R_h^s in poly(NIPAAm-*co*-MAA) solutions at room temperature (T=21 °C)

on pH. Within the region of high acidity (pH≤4.4), only two modes were registered. Alternatively, at larger pH values (pH > 4.4), three types of scattering entities are present in poly(NIPAAm-*co*-MAA) solutions.

As seen in Figure 8, the value of R_h^s increases with pH growth from R_h^s=60 nm at pH=1.8 and then decreases up to 220 nm, with the maximum (R_h^s=760 nm) at pH=7.9. The increase of R_h^s values is probably conditioned by the worsening of the thermodynamic quality of the solution. Then, at high pH the aggregates can be partially destroyed because of the electrostatic repulsion of macromolecules (and ionization of NH of the NIPAAm amino groups which become weakly positive), and R_h^s decreases. Notably, the course of the R_h^s changes with the change of pH seen in Figure 8 reminds of the dependence of T_1 on pH (Figure 5). The common features of these dependences will be discussed below.

In neutral and basic surrounding medium with pH≥4.4, the hydrodynamic radii of the fast and medium modes are independent of pH. The average values are R_h^f=(2.5±0.4) nm and R_h^m=(34±4) nm. Such the R_h^f values could be attributed to the macromolecules (macromolecular unimers). As mentioned above, at pH≤4.4 only two modes were registered, namely, the slow mode and the mode whose radius R_h^x is about 7 nm (let us call it the *x*-mode). Regarding the nature of this mode, the following assumption can be made. It could involve small aggregates which are formed based on H-bonds. H-bonds in poly(MAA) play a major role in macromolecular behavior in acidic medium, because they are stabilized by hydrophobic interactions of methyl groups. In these conditions, they form cyclic intramolecular structures and aggregation of macromolecules.[43,46] Thus, at low pH there can be strong intra- and intermolecular interactions, due to H-bonds between the COOH- groups. The highly protonated poly(NIPAAm-*co*-MAA) macromolecules seem to be associated/aggregated because of the interaction characteristic for poly(MAA), and at pH<5 the polymer molecules can unite into small compact structures (small aggregates). These structures are stabilized by H-bonds, which can arise between amide fragments of NIPAAm and carboxyl groups of MAA.[21] This process can be complemented by interactions between NIPAAm and the solvent, resulting in, as shown in,[47] some chain uncoiling.

The second possible reason for the *x*-mode presence can be concealed in the DLS instrument resolution. If hydrodynamic radii of scattering species differ by less than 3-4 times, their correlation functions cannot be resolved. In our case, the mode with R_h^x could be a superposition of the fast and middle modes. At the same time, the decrease of R_h^m value can be expected at acidity increase. Consequently, the size of middle-mode particles changes with pH in the same manner as the hydrodynamic radius of the slow mode.

At heating above T_1, the composition of the scattering species and their size change strictly in accordance with registered increase of *I* values. For example, Figure 9(a) demonstrates the progress of such changes observed at pH 4.4. Here, below the phase separation region ($T<30$ °C), only fast and slow modes are present in solution. As the *T* value increases above T_1, the third mode appears, demonstrating mean hydrodynamic radius R_h^m =44 nm. Alternatively, at pH≥5.04, three modes are present already at $T<T_1$ (see Figure 9(b) as an example). At all pH values, the values R_h^s start to increase rapidly as *T* increases above T_1. The increase is caused by further aggregate formation. Reduction of R_h^s value can be explained by the compaction of copolymer molecules due to the increase of hydrophobic interactions.

The contribution of each mode to the scattered light intensity can be estimated from the area under the corresponding peak on the $I(R_h)$ dependencies. They are marked as S_f, S_m, S_s, and S_x for the fast, middle, slow and *x*-modes, respectively. The mode contributions are the functions of pH. For all the investigated solutions, the values of S_f, S_m, and S_s do not depend on
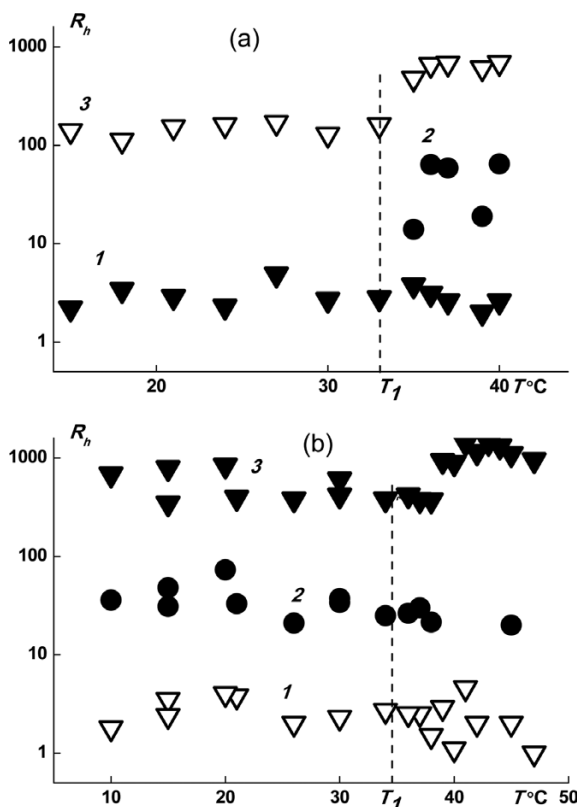

Figure 10. Contribution of fast (1), slow (2), middle (3), and *x*- (4) modes at different *T* for aqueous solution of poly(NIPAAm-co-MAA) at pH=1.7 (a) and 5.6 (b).

temperature at $T<T_1$. However, above T_1 the solution composition changes with temperature, and the character of these changes depends on the medium acidity.

Within the temperature region $T<T_1$, at pH<4.4, the light scattering from the mode with R_h^x prevails (Figure 10(a)). At further heating, the ratio between the contributions of *x*-mode particles and aggregates alternates at the phase separation point (T_1). Here the portion of light scattered from aggregates sharply increases. The increase of aggregates' contribution to light scattering is undoubtedly caused by intensive aggregation at $T>T_1$, when the size of aggregates rapidly increases (Figure 9). The amount of aggregates is also likely to increase. However, to prove this we need to know not only their contribution to the light scattering, but also the shape and density of all types of the scattering particles present in the solution, which cannot be known in advance.

In the medium close to neutral (pH≥4.4), the contribution of aggregates is dominant already at temperatures below T_1. At heating above T_1, the value of S_s increases rapidly again (Figure 10(b)), implying total aggregation over the phase separation interval, the S_f and S_m values decrease, respectively.

3.5. Discussion and comparison of double responsive properties in water solutions of MAA and MA copolymers with NIPAAm

The influence of pH on thermosensitivity of the poly(NIPAAm-*co*-MAA) is conditioned by hydrophilic-hydrophobic balance

Figure 9. Hydrodynamic radii of fast (1), slow (2) and middle (3) modes at different *T* for aqueous solution of poly(NIPAAm-co-MAA) at pH=4.4 (a) and 5.6 (b).
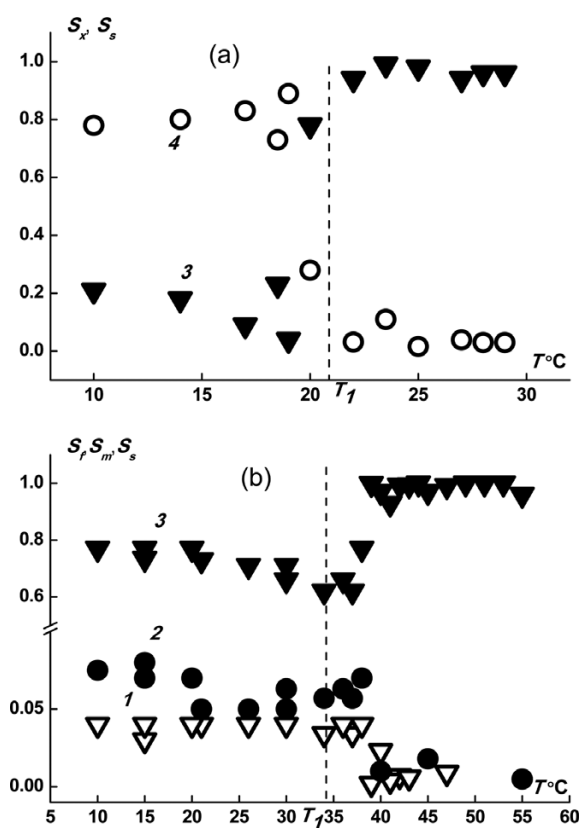
change with ionization or protonation of macromolecules. An essential role in the character of this dependence is played by MAA units of the copolymer due to the presence of carboxylic groups. In the protonated state of the MAA units, the solubility as well as thermal sensitivity of the copolymer is conditioned mainly by the NIPAAm comonomer. When the MAA carboxylic groups become ionized, the solubility of the copolymer increases, and the phase separation interval shifts to higher temperatures until the pH=7.9 is reached. At pH>7.9 the value of T_1 decreases (Figure 5). As noticed above, the similar progress of pH dependencies was observed for R_h^s (Figure 8) and the temperatures $T_{small\,max}$ and T_{min} (Figure 7).

It can be assumed that such dependencies are caused by some sensitivity of NIPAAm units to changes in pH. The effect of pH on poly(NIPAAm) network swelling was already reported.[48] The swelling ratio and the LCST of poly(NIPAAm) and poly (NIPAAm-co-acrylic acid) hydrogels proved to decrease at pH 1-7 and alternatively, increase at pH>7. This pH sensitivity is supported by the presence of isopropyl substituted amide groups. They produce H-bonds that are destroyed in strong acidic or base media. In neutral conditions, H-bonds of such type play a significant role in forming aggregates between the poly(NIPAAm-co-MAA) macromolecules.

Surprisingly, the small differences in the chemical formulas of MA and MAA result in noticeably different behavior of their copolymers with NIPAAm. Let us consider the MA and MAA structural peculiarities. First, the MA unit contains two carboxylic groups, whereas MAA, only one. Second, the MAA unit includes a hydrophobic methyl group. The latter feature is responsible for the specific behavior of poly(MAA) in aqueous solutions.[31,32,43] However, the content of the anionic comonomer seems to be not high enough in the copolymer under discussion to significantly affect the common properties.

The dependence of phase separation temperatures on pH for poly(NIPAAm-co-MA) was plotted in Figure 5 (curve 2) using the data from[19] to compare them to those for poly(NIPAAm-co-MAA) (curve 1). There is a noticeable difference in poly(NIPAAm-co-MAA) phase separation behavior as compared to that of poly(NIPAAm-co-MA). First, in the acidic region both T_1 and T_2 for poly(NIPAAm-co-MAA) are lower, and the phase separation temperature interval is wider than the corresponding interval for poly(NIPAAm-co-MA). The presence of -CH$_3$ group in MAA unit gives rise to downgraded phase separation temperatures, where both copolymers are protonated. In this region of low pH, the increased width of the phase separation interval is probably caused by better solubility of the copolymer.

Further, T_1 values increase more rapidly in the case of poly(NIPAAm-co-MAA) than that of poly(NIPAAm-co-MA), and become higher for the former in the neutral and basic regions. Apparently, the smoother T(pH) plot obtained for poly(NIPAAm-co-MA) is conditioned by two neighboring carboxylic groups ensuring two pK_a values.

Third, the dependence T_1(pH) obtained for poly(NIPAAm-co-MA) is monotonic, unlike that for poly(NIPAAm-co-MAA), where the maximum is observed in the conditions close to neutral. As discussed above, the extreme dependence of the copolymer is probably caused by the pH-sensitivity of NIPAAm unit.

The question why the same phenomenon is not demonstrated in the case of poly(NIPAAm-co-MA) is a challenge for a separate study.

4. Conclusions

The analysis of the experimental data obtained during the investigation of statistic linear copolymer poly(NIPAAm-co-MAA) aqueous solutions (c=0.015 g cm^{-3}) confirms that introduction of MAA monomers leads to the arisal of the double temperature and pH-responsiveness. At room temperature, the dimension of scattering objects and their fraction in solution depend on the pH. In particular, the radius of aggregates increases with pH increment due to the worsening of copolymer solubility. On heating, the hydrophilic-hydrophobic balance changes because of NIPAAm units desolvation and ionization/protonation of macromolecules. Consequently, phase separation in poly(NIPAAm-co-MAA) aqueous solutions takes place. The temperatures of this transition and the width of the phase separation intervals strongly depend on the pH.

Our present and previous works[19,20,32] have discovered that poly(NIPAAm) with statistically embedded acidic units can display different thermo- and pH responsiveness in spite of their similar structures and relatively small amount of anionic comonomer along the copolymer chain. It has been shown that the phase separation temperatures for poly(NIPAAm-co-MAA) are lower than those for poly(NIPAAm-co-MA) at pH<5.6. At the same time, the starting temperature of phase separation grows more rapidly for poly(NIPAAm-co-MAA) than T_1 for poly(NIPAAm-co-MA). For poly(NIPAAm-co-MAA) the dependence of T_1 on acidity reaches the maximum under the conditions close to neutral, while for poly(NIPAAm-co-MA) solution this temperature increases monotonically with the pH increase.

As a consequence, poly(NIPAAm-co-MAA) proved to be more sensitive to pH change than poly(NIPAAm-co-MA), in a wide pH region, but the thermosensitivity of poly(NIPAAm-co-MA) is manifested within the temperature range which is closer to physiological. However, it should be taken into account that the responsive behavior of the model systems will change in more complicate media containing salts, drugs, *etc*.

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