Colloid and Polymer Science (2018) 296:557–565
https://doi.org/10.1007/s00396-018-4269-z

ORIGINAL CONTRIBUTION

Thermo- and pH-responsive phase separation of *N*-isopropylacrylamide with 4-vinylpyridine random copolymer in aqueous solutions

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Received: 19 October 2017 / Revised: 12 January 2018 / Accepted: 16 January 2018 / Published online: 7 February 2018

Abstract

Linear statistic *N*-isopropylacrylamide copolymer containing 11.6 mol% of 4-vinylpyridine was synthesized by free radical polymerization. The copolymer was investigated in 0.015 g cm^{-3} aqueous solutions using methods of static and dynamic light scattering and turbidimetry in the temperature region from 10 to 50 °C. Phase separation was registered in solutions in pH interval from 1.9 to 11.5 while heating. Temperatures of the onset and the termination of the phase separation decreased with pH increase in acid and neutral conditions. Intensive aggregation of macromolecules was registered starting from the temperatures of the onset of the phase separation. The aggregate size and their fraction in solution increased rapidly within the phase separation temperature interval; the composition of the dissolved species was strongly dependent on temperature as well as on pH.

Keywords Poly(*N*-isopropylacrylamide-co-4-vinylpyridine) · Double thermo- and pH-responsiveness · Phase separation · Dynamic light scattering · Turbidimetry · Phase separation temperature

Introduction

Among a number of stimuli-responsive polymers, poly-*N*-isopropylacrylamide (poly(NIPAAm)) takes undoubtedly prominent place as a subject for multiple investigations. Despite broad and intensive studies, it still attracts much attention of researchers as it undergoes a sharp phase transition at a temperature close to physiological temperatures [1–5]. Being copolymerized with some charged monomers, it results in double, namely thermo- and pH-sensitive polymers possessing random or block structures [6]. The property of thermo- and pH-sensitivity is of great importance in biological and chemical systems. Thermo- and pH-sensitive macromolecule can take coiled or uncoiled conformation in response to small changes in temperature and pH, which are some of the most important medium parameters for human body life. In this respect, management of the polymer behavior by varying pH and temperature has high potential for medical and biotechnological applications [6–10]. In particular, such double-responsive polymers can be used for controlled and self-regulated drug delivery systems [6, 7, 11]. The poly(NIPAAm) lower critical solution temperature (LCST) is around 32 °C, which implies phase separation of its aqueous solutions at heating due to hydrophilic–hydrophobic balance change. The introduction of a hydrophilic ionic comonomer into poly(NIPAAm) chain endows the copolymer pH-sensitivity and besides, as a rule, changes its hydrophilicity, thus shifting phase transition temperature closer to physiological temperatures [6, 11]. The choice of the weak anionic or cationic unit as an ionizable comonomer allows tuning the character of copolymer response to the increase or decrease in pH [6].

A number of studies were undertaken on NIPAAm copolymerized with acidic comonomers such as maleic (MA), acrylic (AA), or methacrylic (MAA) acid or other comonomers, which provide pH-sensitivity [12–15]. Carboxylic groups of anionic comonomers release protons in basic medium and accept them with the decrease in pH becoming poorly soluble. The phase separation temperatures, as well as the size and morphology of scattering particles, can be directly changed by the choice of copolymer composition [16, 17], architectural features of

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macromolecules [18], comonomer regularity [19], solution concentration [20, 21], and other parameters, such as complexing with drugs [17]). Phase separation temperatures are also dependent on the acidity of the surrounding medium [22], presence of ions [12], and other conditions.

The peculiarities of the chemical structure of charged comonomers noticeably affect the thermoresponsiveness of NIPAAm copolymers. For example, a comparative study was undertaken of the behavior in aqueous solutions of NIPAAm linear copolymers with weak polyacids bearing pending carboxylic groups in the main chain. It was shown that poly(NIPAAm) with close content of statistically embedded units of MAA (poly(NIPAAm-co-MAA)) and MA (poly(NIPAAm-co-MA)) revealed different behavior in aqueous media in a wide range of temperatures *T* and pH, in spite of the similar structure of charged comonomers and their relatively small amount along the copolymer chain. It was found that the phase separation temperatures for poly(NIPAAm-co-MAA) [21–23] are lower than those for poly(NIPAAm-co-MA) [24] in acid conditions, whereas they grow more rapidly with pH, thus demonstrating higher pH-sensitivity of poly(NIPAAm-co-MAA) in a wide pH region. The peculiarities in double responsiveness are conditioned by the presence of methyl group in poly(NIPAAm-co-MAA) and two neighboring carboxylic groups in poly(NIPAAm-co-MA) chemical structures, respectively.

The choice of the ionic fragment in the thermosensitive copolymer will define the mode of the pH-dependence of the T-response. Copolymers with positively ionizable comonomers show the dependence of the phase separation temperatures on pH, which is opposite to that of anionic copolymers. Due to ionization of basic functional groups, they become soluble in neutral and acid media, while being unprotonated in basic environment, they are insoluble [7]. For example, polymers containing amino groups, such as poly(allylamine) or poly(vinylamine), are polybasic [11]. Other examples of positively charged pH-responsive polymers are polylysine, polyethylenimine, chitosan, and poly(2- or 4-vinylpyridine) [6, 7].

2- or 4-vinylpyridines go through phase transition because of deprotonation of pyridine groups [7]. Being incorporated into poly(NIPAAm) or other polymer chains, they can impact a copolymer the properties of double (thermo- and pH-) responsiveness with basic character. Synthesis of monodisperse cationic microgel particles formed from a crosslinked random 2-vinylpyridine and styrene was reported [25]. The effect of styrene content, crosslinking density, and microgel diameter on the pH-dependent swelling was studied. Synthesis and thermoresponsive swelling/deswelling in aqueous solutions for a gel of NIPAAm copolymerized with 4-vinylpyridine and *N*,*N*′-methylenebisacryl amide was described, and electrochemical behavior of this copolymer coordinated to ruthenium was investigated. The gel was found to be shrunken above 40 °C [26]. Cationic poly(NIPAAm-co-4VP) microgel particles with varying percentage of 4VP were synthesized, and the dependence of their swelling/deswelling on temperature, pH, and ionic strength was investigated [27, 28]. The studies were carried out within the 20–60 °C temperature range and over the pH range from 3 to 8 using different approaches based on the measurements of micro-particle diameter by means of dynamic light scattering (DLS) or their electrophoretic mobility. The most significant result of these studies was probably that the degree of ionization of the polyelectrolyte system as well as the 4VP content in the copolymer has a notable effect on the microgel particle swelling. Reversible thermoresponsive core–shell micellization was reported for a block-copolymer of poly(NIPAAm) with poly(4-vinylpyridine) (poly(4-VP)), where the core is formed by poly(NIPAAm) or by poly(4-VP), depending on the pH [29]. However, the works on the study of NIPAAm copolymers with 4VP are not numerous. We only know three articles on random poly(NIPAAm-co-4VP) thermo- or/and pH-sensitivity investigation [26–28]. Meanwhile, 4VP cationic units can serve for DNA or protein transport that makes their double-responsive copolymers with amphiphilic comonomers rather prospective.

The present paper is aimed at synthesizing a linear poly(NIPAAm-co-4VP) sample with 4VP comonomers statistically distributed along the polymer chain and performing its detailed study. The investigation of linear poly(NIPAAm-co-4VP) is of scientific significance for tuning its behavioral features in water; besides, the linear chain can be regarded, for example, as a structural unit of poly(NIPAAm-co-4VP) microgels or star arms. In the current work, the behavior of the poly(NIPAAm-co-4VP) sample in aqueous solutions was investigated over a wide range of temperatures (10–50 °C) and pH (1.9–11.5) using the methods of turbidimetry and static light scattering, as well as UV–Vis spectroscopy in order to directly obtain the phase separation temperatures during solution heating. The content of 4VP was comparable to that of the NIPAAm copolymers with anionic comonomers studied by the authors before. Systematic study of the polymer response to the change of the surrounding medium is an important task in developing “smart” polymer systems intended for different purposes including drug delivery.

Materials and methods

Materials

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

Synthesis of poly(NIPAAm-co-4VP)

Poly(NIPAAm-co-4VP) was synthesized by free radical copolymerization of NIPAAm and 4VP in 1,4-dioxane with AIBN as an initiator.

In a typical example, 2.26 g of NIPAAm (2× 10 mmol), 162 μL of VP (2× 1.5 mmol), and 0.025 g AIBN (0.15 mmol) were solubilized in 12 mL of 1,4-dioxane. Dried nitrogen was purged through the solution for 60 min before starting the polymerization. The reaction mixture was allowed to react at 80 °C for 20 h. The polymer was then precipitated into diethyl ether and dried under vacuum. Finally, the copolymer was dissolved in distilled water, dialyzed for 7 days at 20 °C (molecular weight cut off of dialysis tube 10,000–12,000 Da; from MediCell International, England), and recovered by freeze–drying. The copolymer was obtained in 78% yield.

Copolymer composition

The copolymer structure and composition were determined by ^{1}H–NMR analysis. ^{1}H–NMR spectra of poly(NIPAAm-co-4VP) were recorded in D_2O on a Varian Mercury Plus 400/ Varian VXR 200 spectrometer operating at 400 MHz frequency. The molar fraction of comonomers was calculated according to Eqs. (1) and (2):

$$9x + 3y = \mathrm{A}_1 \tag{1}$$

$$2y = \mathrm{A}_2 \tag{2}$$

where x and y are the relative molar fractions of NIPAAm and 4VP, respectively. The first equation describes the total peak area ($\mathrm{A}_1 = 35.88$) between 0.5 and 2.5 ppm corresponding to the main backbone protons (*3 NIPAAm + 3 (4VP)*) plus the methyl protons (*6 NIPAAm*). The second equation represents the area of aromatic protons (4 and 4′) at 8.20 and 8.33 ppm of 4VP ($\mathrm{A}_2 = 1$).

Static and dynamic light scattering and turbidimetry

Static (LS) and dynamic (DLS) light scattering as well as turbidimetry were used to investigate the behavior of aqueous solutions of poly(NIPAAm-co-4VP) upon heating. Experiments were carried out using the Photocor Complex instrument (Photocor Instruments Inc., Russia) equipped with the Photocor-DL diode laser (wavelength $\lambda = 658.7$ nm, power 30 mV), Photocor-PC2 correlator with 288 channels, and Photocor-PD detection device for measurements. Millipore filters with hydrophobic PTFE membrane and pore size of 0.45 μm (Merck) were used to filter the solutions into dust-free vials. The solutions were studied at concentration $c = 0.015$ g cm^{-3} in de-ionized water. To investigate the limits of poly(NIPAAm-co-4VP) thermoresponsiveness, the pH of the initial solution (pH = 4.7) was changed within the interval from 1.9 to 11.5 by adding 1 N HCl or 1 N NaOH.

The solutions were heated from 10.0 to 50.0 °C, temperature T being changed discretely with the steps ranging from 1.0 K (near cloud point) to 2–3 K (at low T). The precision of the temperature regulation was 0.1 K. The heating rate was about 1.5 K min^{-1}. The equilibrium state when the solution characteristics were constant in time after temperature change was always achieved before the experimental values were measured upon heating. For this purpose after reaching the given temperature, the light scattering intensity I and optical transmittance I^* were measured upon time until their constant values were achieved. Hydrodynamic radius R_h of the dissolved particles was measured when I became independent of time. The contribution of different types of particles to the summary light scattering intensity was estimated using the areas S under the corresponding peaks of the R_h (I) distribution. The temperature dependences of the characteristics in question were plotted. The measurements were conducted at the scattering angle of 90°. In equilibrium conditions, within the interval 45–135°, the angle dependences of light scattering intensity I and the hydrodynamic radii R_h of the scattering objects were analyzed in order to justify the diffusion process. Time t_{eq} required for equilibrium state achievement in aqueous solutions of poly(NIPAAm-co-4VP) after changing temperature was measured. Figure 1 demonstrates the progress of relative light scattering intensity I/I_0 and optical transmittance I^*/I^*_0 in time after the temperature jump, I_0 and I^*_0 being respectively the light scattering intensity and optical transmittance at $T = 10$ °C, and shows the way in which the t_{eq} value was determined.

Weight average molar mass of the sample M_w was obtained by means of static light scattering in dimethylformamide (DMF) dilute solutions. The concentration c ranged from 0.0307 to 0.0121 g cm^{-3}. The Debye method was used for calculations [30]. Calibration was carried out in relation to toluene. Refractive index increment dn/dc necessary for molar mass calculation was measured on a refractometer RA-620 (Kyoto electronics MFG. CO, Japan) supplied with the light source LED Na-D Line (589.3 нм).

Results and discussion

Synthesis and characterization of poly(NIPAAm-co-4VP)

The copolymerization of NIPAAm with charged hydrophilic monomers containing functional groups such as 4VP was

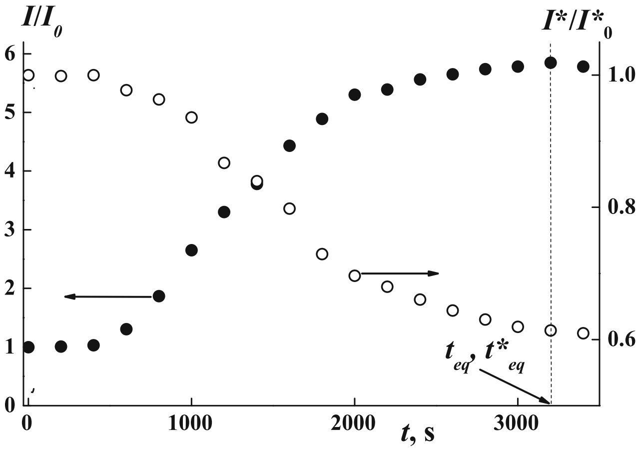


Fig. 1 Dependence of relative intensities of I/I_0 and I^*/I^*_0 on time for poly(NIPAAm-co-4VP) solution at $T = 30$ °C and pH = 5.1

aimed at producing copolymers sensitive to both temperature and pH. The presence of the amino groups in 4VP confers to copolymer high sensitivity to the variation of pH. Copolymers of NIPAAm with 4VP were prepared by free radical copolymerization in dioxane using AIBN as initiator. Then, the copolymer was solubilized in distilled water and dialyzed against water to remove the un-reacted monomers, low-molecular-weight copolymer, and traces of solvent. As reported in Table 1 and proven by ^{1}H–NMR spectrum (Fig. 2), a copolymer was obtained; however, the percentage of 4VP in the copolymer determined from ^{1}H–NMR was slightly lower than that observed in the feed.

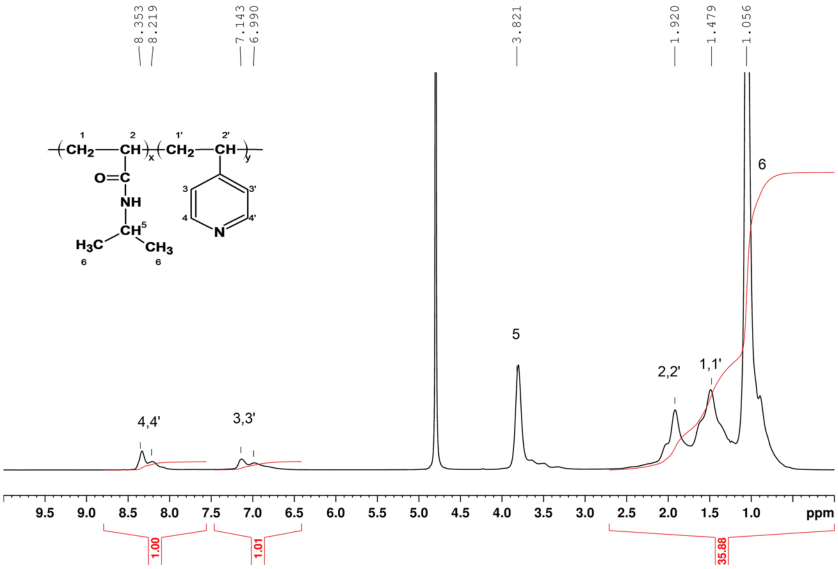
Table 1 Composition of the comonomers in the feed and in poly(NIPAAm-co-4VP) copolymer

Comonomer composition			
In the feed × 10^{-3} M (% mol ratio)		In copolymer (% mol ratio)	
NIPAAm	4VP	NIPAAm	4VP
10 (86.9)	1.5 (13.1)	(88.4)	(11.6)

The distribution of the comonomers in copolymer was estimated from copolymerization parameters Q and e proposed by Alfrey and Price [31]. Therefore, the values $Q_1 = 0.40$ and $e_1 = 0.47$ were taken for NIPAAm [32] while $Q_2 = 1$ and $e_2 = 0.40$ were taken for 4VP [33]. The Q value represents the reactivity or resonance stability of the macroradical as a result of the presence of withdrawing or donating electron substituents. Therefore, the reactivity of 4VP seems to be higher than that of NIPAAm, suggesting that the electronic stabilization effect exerted by the pyridine ring is larger than that exerted by the carbonyl group of the amide moiety and isopropyl group from NIPAAm. On the other hand, the e values of NIPAAm and 4VP, representing the polarity of the monomers, are almost equals. Based on these values, we calculated the reactivity ratios r_1 and r_2 and subsequently the $r_1 \times r_2$ product equal to 0.995, suggesting that the distribution of monomers in copolymer is statistic.

The molar mass value was obtained from the dependence cH/R on c (Fig. 3), where R is the excessive (in respect to the solvent) intensity of light scattering and H is the optical constant. The parameters obtained from LS measurements in DMF are exposed in Table 2, A_2 being the second virial coefficient. One mode was observed in the solutions within the interval of c investigated. Hydrodynamic radius of macromolecules was independent of concentration, thus the average value R_h^0 was calculated, and diffusion coefficient D_0 was defined in accordance with the Stokes–Einstein formula (Table 2).

Fig. 2 ^{1}H–NMR spectrum of poly(NIPAAm-co-4VP) in D_2O



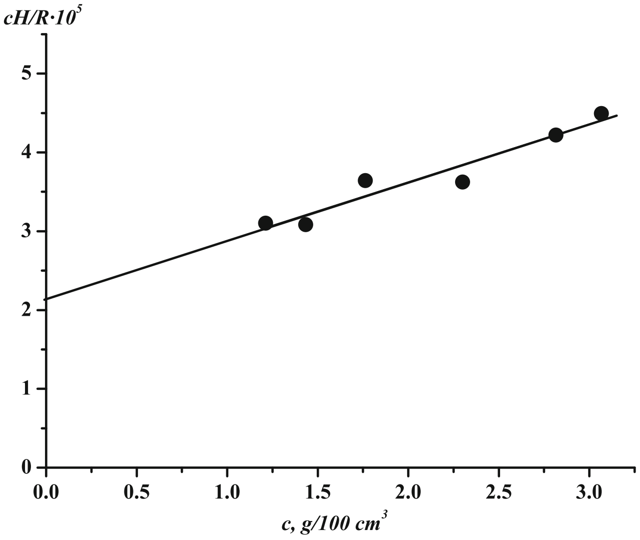


Fig. 3 Dependences of reciprocal excessive intensity of scattered light on concentration for poly(NIPAAm-co-4VP) solution in DMF

Temperature dependence of solution parameters for poly(NIPAAm-co-4VP) at different pH.

The behavior of poly(NIPAAm-co-4VP) aqueous solutions upon heating is in general similar to that of thermoresponsive polymers, in particular, to the behavior of the previously studied poly(NIPAAm-co-MA) and poly(NIPAAm-co-MAA) [21–24]. Three temperature intervals can be distinguished on the $I(T)$ and $I^*(T)$ plots (Fig. 4): a region of a very slight increase of I (see also insert in Fig. 4) which extends from low temperatures up to the temperature T_1 (interval I), a region of rapid increase of scattering intensity ($T_1 < T < T_2$, interval II), and a region where I decreases ($T > T_2$, interval III). These regions can be separated by characteristic temperatures T_1 and T_2 which are the temperatures of the onset and termination of phase separation, respectively. Interval II can be regarded as the phase separation interval. Phase separation was controlled by the decrease of the transmitted light intensity I^*, which starts at temperature T^*_1 (Fig. 4), being accompanied with the loss of solution transparency. The transmission I^* achieves zero values at temperature T^*_2, determining the termination of the phase separation. The temperatures of the phase separation onset T_1 and T^*_1, obtained separately using $I(T)$ and $I^*(T)$ plots for each pH, practically coincided (Fig. 4). However, $T_2 > T^*_2$ for all pH except pH = 4.7 and 5.1. The values of T^*_2 were determined as phase separation termination temperatures. In the III interval, the I decreased and the I^* remained about 0, since compaction of scattering particles and probably their precipitation occurs.

The phase separation temperatures in aqueous solutions can be tuned by pH variation due to ionizable amino groups of the poly(NIPAAm-co-VP) pyridine ring. As expected, the phase separation temperatures were strongly influenced by the solution acidity (Fig. 5). The decrease in pH led to an increase in both T_1 and T_2, as well as T^*_1 and T^*_2 provided the surrounding medium is acidic (pH < 6.7). In acidic conditions, amino groups of 4VP being protonated were positively charged, more hydrophilic, and therefore, higher energy is necessary to produce dehydration and then hydrophobic association. At pH ≥ 6.7, the values of T^*_1 and T^*_2 demonstrated independence of pH. The macromolecules of poly(NIPAAm-co-4VP) in polyelectrolyte form became highly soluble—the higher the charge, the better the solubility. On the contrary, at pH ≥ 6.4, the amino groups were deprotonated. This resulted in high hydrophobicity of 4VP, and the solubility of the copolymer was supported by only NIPAAm comonomer. The width of the phase separation interval $dT^* = T^*_2 - T^*_1$ did not change significantly with pH, being $dT = (4–5)$ K at pH > 4.7 and in highly acidic conditions, the value of dT increased from 4.5 K at pH = 4.7 to 7 K at pH = 1.9 due to improvement of the total hydrophilicity of the copolymer.

The time t_{eq} required for achievement of the I and I^* equilibrium values after the temperature change proved to be a function of the temperature. Figure 6 demonstrates that at temperatures below T_1, the time t_{eq} is close to 1000–1500 s. The values of such magnitudes at low temperatures are typical for linear thermo- and pH-sensitive polymers [21, 34–36]. Time t_{eq} increases in the vicinity of T_1 and becomes maximal near the temperature of phase separation onset. These maximal values t_{eq}^{max} can reach 9000 s at some pH; however, no systematic change with pH was observed. Such big values are not typical for linear polymers; they rather remind the t_{eq} values obtained for star-shaped and grafted polyoxazolines [37, 38] and grafted poly-*N*,*N*′-dimethylaminoethyl methacrylates [39]. Note that for the polymers having complex architecture, the dependences of t_{eq} on T were discussed in details previously [40].

Composition and hydrodynamic size of the scattering species in poly(NIPAAm-co-4VP) solutions

In aqueous solutions of poly(NIPAAm-co-4VP), two or three modes were always registered, the hydrodynamic size and the composition being dependent on temperature and pH. Let us first consider the solutions at low temperatures ($T < T^*_1$). Figure 7 demonstrates the dependences of hydrodynamic radii of scattering species in poly(NIPAAm-co-4VP) solutions at

Table 2 Molecular and hydrodynamic characteristics of poly(NIPAAm-co-4VP) in DMF

$M_w \times 10^{-4}$ (g/mol)	$A_2 \times 10^4$ (m^3·mol·g^{-2})	dn/dc (cm^3·g^{-1})	R_h^0 (nm)	$D_0 \times 10^7$ (cm^2 s^{-1})
4.7 ± 0.5	3.7 ± 0.5	0.077 ± 0.003	4.9 ± 0.3	4.7 ± 0.3

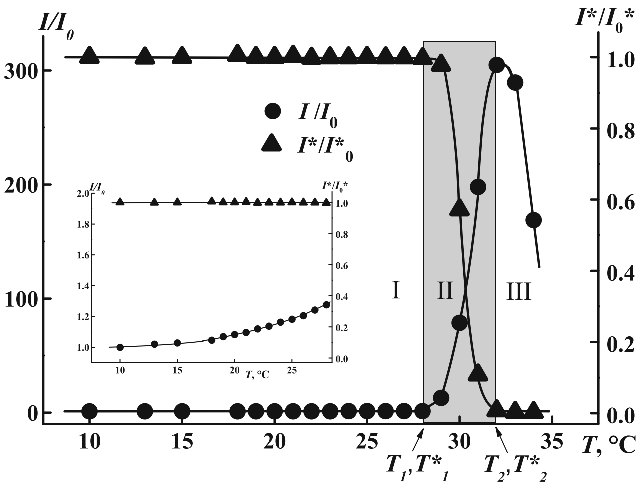


Fig. 4 Dependences of relative intensity of scattered I/I_0 and transmitted I^*/I^*_0 light on T for aqueous solution of poly(NIPAAm-co-4VP at pH = 5.1; insertion depicts these dependences below temperatures of phase deparation $T_1 = T^*_1$

temperature $T = 15$ °C on pH. Three modes are observed if pH ≤ 4.7. These modes with hydrodynamic radii R_h^f, R_h^m, and R_h^s could be attributed to macromolecules (macromolecular unimers), micelle-like structures, and loose aggregates, respectively, taking into account the literature data [6, 41–44] and our previous results [22]. Note that for solutions at pH < 4.7, a tendency to hydrodynamic radii decrease with acidity decrease is observed for all types of scattering particles. This can be explained by the polyelectrolyte deswelling of macromolecules due to worsening of the thermodynamic quality of the solvent. Note that at minimal pH = 1.9, the hydrodynamic radius of macromolecules $R_h^f = 4.6$ nm corresponds to the value $R_h = (4.9 \pm 0.3)$ nm obtained for the copolymer in DMF, which is a thermodynamically good solvent.

It can be seen from Fig. 7 that only two modes were registered provided that pH > 4.7. Their hydrodynamic radii were independent of pH. The slow moieties are undoubtedly aggregates. Their average hydrodynamic size at pH > 4.7 is $R_h^s = (120 \pm 15)$ nm. However, there is a question regarding the nature of the smaller species at pH > 4.7. Their mean radius, $R_h^f = (6.4 \pm 0.3)$ nm, is bigger than the value of R_h^f obtained both for the unimers in highly acid media and $R_h = 4.9$ nm obtained for the copolymer molecules in DMF solutions. It can be suggested that in neutral and basic conditions, there can be strong intra- and inter-molecular interactions, due to hydrophobicity of deprotonated 4VP groups, resulting in formation of small supramolecular structures (let us name them as *x*-mode). Besides the big loose aggregates observed over the whole pH range, macromolecules could form small aggregates or micelle-like structures stabilized by hydrophobic interactions of pyridine cycles. Similar solution behavior characterized by emergence of the *x*-mode instead of unimers was detected by the authors for poly(NIPAAm-co-MAA) in acid media, where ionic (acidic) units of the copolymer are insoluble [24]. Discussing the nature of the *x*-mode, the authors of [24] supposed that the second possible reason for its appearance is resolution of the DLS instrument, which is insufficient for separation of particles with very close size (unimers and micelle-like structures).

At temperatures above T^*_1, the composition of the scattering species and their size change during heating in accordance with the observed increase in I values. At pH ≤ 4.7, three modes are present at all temperatures $T < T^*_1$, the size of the scattering particles being independent of T (see Fig. 8a as an example). The values R_h^s together with R_h^m start to increase as T increases above T^*_1 due to further aggregation of the species present in solution. The R_h^f values do not change with temperature. The contributions of each mode (fast S_f, middle S_m, and slow S_s) to the total scattered light intensity are shown in Fig. 8b. For all the pHs, the values of S_f, S_m, and S_s are practically independent of temperature below T^*_I. This allows us

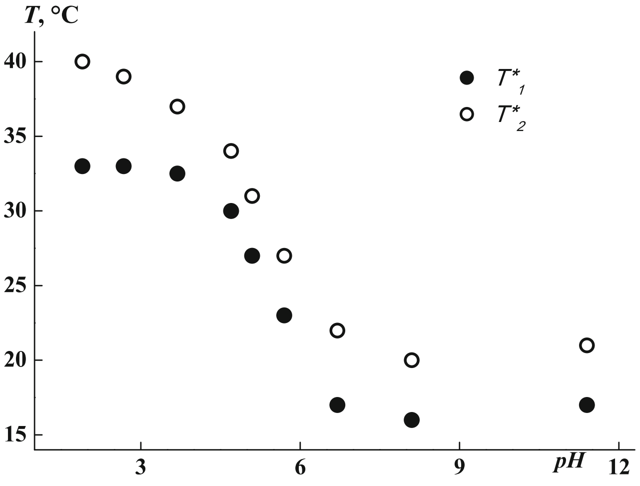


Fig. 5 Dependence of phase separation temperatures T^*_1 and T^*_2 on pH

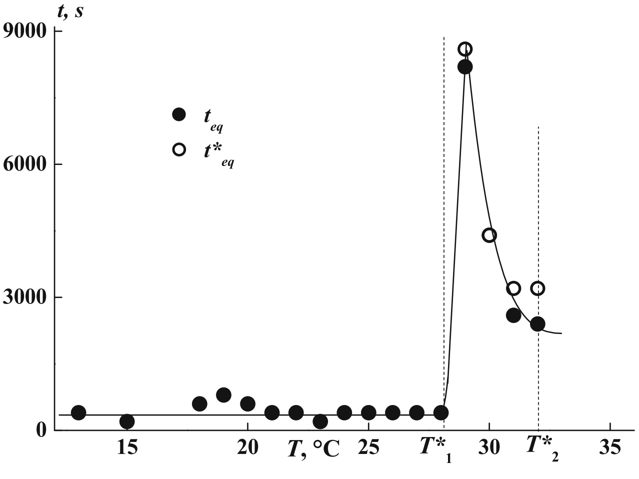


Fig. 6 Dependence of t_{eq} and t^*_{eq} on temperature for poly(NIPAAm-co-4VP) solution at pH = 5.1

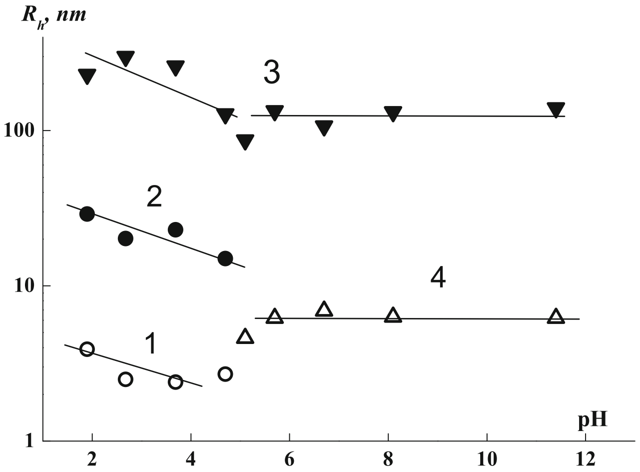


Fig. 7 Hydrodynamic radii of fast (1), middle (2), slow (3), and *x*-modes for aqueous solution of poly(NIPAAm-co-4VP) as a function of pH at $T = 15$ °C

to conclude that the composition of the scattering particles remains unchanged throughout the temperature range $T < T^*_1$.

When $T > T^*_1$, in highly acidic pH, $\mathrm{pH} \le 4.7$, unimers disappear, and the size of the aggregates and micell-like structures increases. The contribution of the slow mode sharply increases, and that of the middle mode decreases (Fig. 8b).

In weak acid, neutral and basic media (at $\mathrm{pH} > 4.7$), as mentioned above, two modes were registered below T_1, namely *x*-mode and large aggregates. Their size and contribution to the light scattering do not change with T in this temperature range. After heating above T_1, the values of R_h^s increase rapidly within the interval of the phase separation, and the portion of the light scattered from large aggregates always (at any pH) increase with temperature (Fig. 9a, b).

At $T > T^*_1$ and $\mathrm{pH} > 4.7$, three modes can be observed in Fig. 9a. The *x*-mode disappears at T around T^*_1, whereas the fast mode and the middle mode appear. In $\mathrm{pH} = 5.1$ solutions, the size of the middle mode increases with T from $R_h^m = 9$ to 74 nm within the temperature region $T^*_1 < T < T^*_2$. The hydrodynamic size R_h^f of the fast particles is close to 2.2 nm. This value is noticeably smaller than the size of macromolecules in DMF, which is the result of macromolecular contraction in thermodynamically bad conditions. The contribution of micelle-like particles to the light scattering, despite the augmentation of their size, noticeably decreases with T, simultaneously with the increase of S_s (Fig. 9b). This can be caused by the variation of the size as well as the composition of scattering particles. As for the number of scattering particles, in order to estimate their change with heating, it is necessary to know the shape and density of all types of the scattering particles in the solution [21]. This kind of information cannot be obtained using methods of molecular hydrodynamics and optics available in the study.

Conclusion

Double-responsive properties of NIPAAm copolymers with 4VP in water solutions

The behavior of linear statistic poly(NIPAAm-co-4VP) in aqueous solutions and its thermally induced phase separation is a strong function of pH.

Analysis of the composition of the dissolved scattering objects was carried out. It was shown that larges aggregates and micelle-like structures (small aggregates) are always present in solutions. In highly acid media (in better thermodynamic conditions), at low temperatures, unimers can also be observed (individual, isolated macromolecules). With solution heating above the temperature of the phase separation, intensive aggregation occurs which is manifested in the rapid increase of the aggregate size and their contribution to the total light scattering intensity.

Introduction of 4VP monomers into poly(NIPAAm) chain leads to emergence of double temperature and pH-responsiveness. Due to the cationic nature of the 4VP component, the influence of pH on thermosensitivity of the poly(NIPAAm-co-4VP) is mainly revealed in acidic conditions, where the hydrophilic–hydrophobic balance changes with protonation or deprotonation of the macromolecules. An essential role in the character of this dependence is played by pyridine cycles of 4VP units. The limits of the copolymer thermal sensitivity

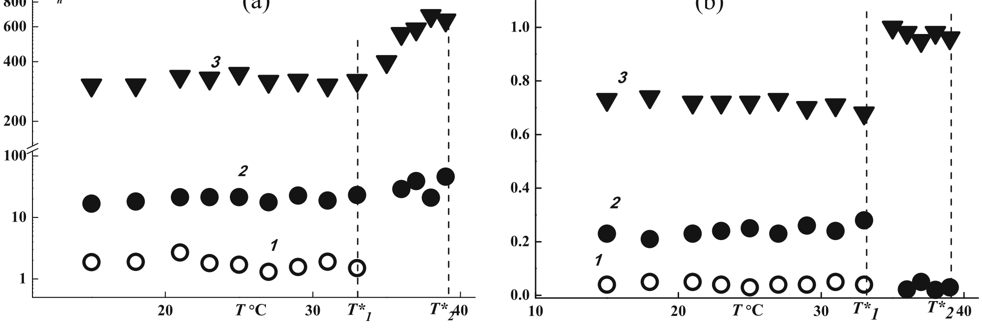


Fig. 8 Hydrodynamic radii (**a**) and contribution to light scattering (**b**) of fast (1), slow (2), and middle (3) modes at different T for aqueous solution of poly(NIPAAm-co-4VP) at $\mathrm{pH} = 2.7$

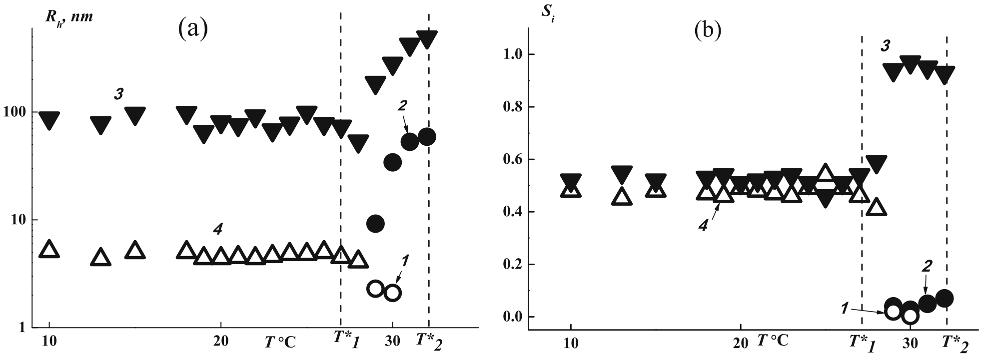


Fig. 9 Hydrodynamic radii (**a**) and contribution to light scattering (**b**) of fast (1), slow (2), middle (3) and *x*-modes (4) at different *T* for aqueous solution of poly(NIPAAm-co-4VP) at pH = 5.1

are conditioned mainly by the degree of ionization of the pyridine cycle. When the pyridine groups become deprotonated and hence insoluble in neutral and basic media, the solubility of the copolymer is supported by the NIPAAm monomer units. Because of increased hydrophobicity of the copolymer with deionization, the phase separation temperatures of its aqueous solutions decrease. Herewith, the triggering mechanism of poly(NIPAAm-co-4VP) is opposite to that observed for statistic copolymers of NIPAAm of anionic nature, such as previously studied poly(NIPAAm-co-MAA) and poly(NIPAAm-co-MA). It is important that in highly acid conditions, the thermosensitivity of poly(NIPAAm-co-4VP) is manifested within the temperature range which is closer to human body temperatures.

Funding information The research activity of coauthors from Romania was supported by a project that has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 667387 WIDESPREAD 2-2014 SupraChem Lab.

The study of the coauthors from the Institute of Macromolecular Compounds RAS was carried out under the financing of the Federal Agency for Scientific Organizations (FASO Russia), State registration No AAAA-Φ 16-116071450046-9.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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