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GELATION PROPERTIES OF THE AQUEOUS SOLUTIONS OF HYDROLYTICALLY DEGRADABLE THERMOSENSITIVE POLY(N-ISOPROPYLACRYLAMIDE-CO-5,6-BENZO-2-METHYLENE-1,3-DIOXEPANE) COPOLYMERS

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Hydrolytically degradable poly(N-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane) copolymers with different 5,6-benzo-2-methylene-1,3-dioxepane (BMDO) units content were prepared by RAFT radical polymerization and the thermogelation properties of their 15 wt.% phosphate buffer saline (PBS) solution were investigated by temperature-sweep rheological measurements. The analyses showed that the phase transition temperature, as well as the maximum values attained by the dynamic viscosity, storage modulus and viscous modulus, depended on the BMDO content. The influence of sodium alginate and dextran on the gelation properties of the copolymer solutions was also studied. Hydrolysis tests, carried out at 37°C in PBS solution, revealed a reduced degradability of the copolymers under these conditions.

Keywords: poly(N-isopropylacrylamide), 5,6-benzo-2-methylene-1,3-dioxepane, dextran, sodium alginate, thermosensitive hydrogels, degradation

1. Introduction

Poly(N-isopropylacrylamide) (PNIPAM) is a low critical solution temperature (LCST) – displaying thermosensitive polymer [1,2], which was intensely researched for biomedical applications, such as injectable hydrogels for controlled release of therapeutics and tissue engineering [3-5], due to its LCST (around 32°C) slightly below the human body temperature and quick thermal response [1,6]. At temperatures below LCST the polymer is water-soluble because of hydrogen bonding with water molecules and displays a coil conformation, while above LCST the polymer is dehydrated and precipitates out of solution,

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simultaneously with a coil-to-globule transition of its chains [7]. After precipitation, the more concentrated PNIPAM solutions are converted to a shrunken gel and an aqueous solution, rather than to a uniform gel [8].

Beside this syneresis phenomenon, another important drawback of PNIPAM for such applications is represented by its non-biodegradable character, which impedes its elimination from the body. More recently, progress was made in solving this problem by the synthesis of hydrolytically degradable PNIPAM with ester groups in the main chain by: i) conventional [9-11] or controlled [12] free radical copolymerization of NIPAM with cyclic ketene acetals, and ii) simultaneous chain- and step-growth Cu^I-catalyzed radical copolymerization of NIPAM and 3-butenyl-2-chloropropionate [13]. However, to the best of our knowledge, the gelation properties of these P(NIPAM-co-ester) degradable polymers in aqueous solution have not been reported yet.

The present paper aims at investigating the gel-forming properties of some hydrolytically degradable copolymers of NIPAM and 5,6-benzo-2-methylene-1,3-dioxepane (BMDO) as the in-chain ester group forming monomer, in aqueous solution, as well as those of their mixtures with some biopolymers, like dextran and sodium alginate. We will show within the paper that both the BMDO units content of the copolymer and the presence of the biopolymer strongly affect the thermogelation process and the viscoelastic properties of the resulting gels.

2. Experimental

2.1. Materials

N-Isopropylacrylamide (NIPAM, Aldrich, 97%) was recrystallized from hexane and stored in a freezer. 1,2-Dichlorobenzene (DCB, Sigma-Aldrich, 99%) was dried over 3 Å molecular sieves. 5,6-Benzo-2-methylene-1,3-dioxepane (BMDO) [14] and S-1-dodecil-S'-(α,α'-dimethyl- α''-acetic acid) trithiocarbonate (TTC) [15] were synthesized according to literature data. 1,1'-Azobis(cyclohexanecarbonitrile) (ACHCN, Aldrich, 98%) was recrystallized from methanol and stored in the refrigerator. Sodium alginate from brown algae (ALG, Sigma, 4–12 cP viscosity of the 1% aqueous solution at 25°C), dextran from Leuconostoc spp (DXT, Sigma, $M_r \approx 6000$) and all the other reagents and solvents employed were used as received. The phosphate buffer saline solution (PBS, pH = 7.4) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 0.24 g KH_2PO_4, 3.6 g $Na_2HPO_4 \cdot 12H_2O$ and 0.02 g $NaNO_3$ (preservative) in distilled water up to 1 L solution and stored in a refrigerator until use.

2.2. Synthesis of the PNIPAM and P(NIPAM-co-BMDO) polymers

In a typical experiment (PNB2 sample, Table 1), a dry 10-mL Schlenk flask was charged with NIPAM (0.8 g, 7.0 mmol), BMDO (0.2 g, 1.24 mmol) and TTC (11.4×10^{-3} g, 3.2×10^{-5} mol) and the mixture was dried at 0.1 torr at room temperature for 30 min. Then, 3 mL dry DCB and ACHCN (3.9×10^{-4} g, 1.6×10^{-6} mol) as a 2.6×10^{-2} g/mL solution in dry DCB were added, the flask was sealed by a rubber septum and the mixture was stirred until complete dissolution of the monomers and the agent for RAFT (Reversible Addition–Fragmentation chain Transfer) polymerization. The reaction mixture was then degassed by cycling the flask between vacuum (3-5 min) and nitrogen for six times, and finally left under nitrogen. Next, the Schlenk flask was placed in an oil bath at 85 °C for 24 h. At the end of the reaction time, the mixture was cooled down, diluted with CH_2Cl_2 and added dropwise to cold diethyl ether. The precipitated polymer was recovered by filtration and dried under vacuum to constant weight. Table 1 shows the experimental conditions and the molecular characteristics of the polymers synthesized.

Table 1

Experimental conditions for the syntheses and molecular characteristics of the polymers synthesized[a]

Sample code	NIPAM/ BMDO mol/mol	monomer/TTC /initiator mol/mol/mol	reaction time/reaction temperature h/°C	Conv[b] %	M_n/PDI[c]	$(BMDO)_{copol}$[d] mol%
PN	100/0	176/1/0.1	7/90	88	21500/1.12	-
PNB1	90/10	264/1/0.05	24/85	69	19400/1.30	2.3
PNB2	85/15	264/1/0.05	24/85	60	18300/1.20	4.0
PNB3[e]	90/10	176/1/0.3	48/120	90	18700/1.38	6.1

[a] monomer concentration - $2.76\cdot10^{-3}$ mol/mL solvent; solvent - DCB; initiator - ACHCN;
[b] overall monomer conversion, determined gravimetrically;
[c] determined by gel permeation chromatography (GPC);
[d] BMDO units molar content in copolymer, as determined by ^{1}H NMR [16];
[e] initiator – di-t-butyl peroxide

2.3. Removal of the dodecyltrithiocarbonate end group

A similar procedure to that previously described [17] was employed.

2.4. Preparation of the (co)polymer aqueous solutions

The appropriate amount of biopolymer (2 wt.% ALG or 6 wt.% DXT based on the entire solution) was charged into a 7-mL screw cap glass vial, followed by the addition of PBS, and the mixture was stirred at room temperature until homogeneity is reached. Then, the PNBx copolymer (15 wt.% based on the whole solution) was added, and the mixture was stored into a refrigerator at 4 °C overnight. Alternatively, in the absence of the biopolymer, PN or PNBx was weighed first into the glass vial followed by PBS addition. The resulted solution was stirred for about one hour in an ice-water bath the next day, and kept for one more day in the refrigerator, before measuring it. When the biopolymer was present, the resulting solution contained two immiscible liquid layers at rest. Before any measurement, the solution was stirred for a few minutes in an ice-water bath to homogenize it, and became turbid after that if the biopolymer was present. However, the reformation of the two liquid layers was relatively slow, leaving unaffected the reproducibility of the measurements. The sample code indicates both the NIPAM (co)polymer and the biopolymer employed to prepare the solution, their concentrations being the ones mentioned above.

2.5. Hydrogel degradation

The 7-mL screw cap vial containing 1 g of copolymer solution in PBS was introduced at 37±0.5 °C in the aluminum block of a chilling/heating dry plate (Torrey Pines Scientific, Inc, USA) and the formation of the hydrogel was visually observed. After certain time intervals, the vial was removed from the plate and placed in the refrigerator overnight to dissolve the hydrogel, the resulting solution being then homogenized and analyzed by rheometry. After the whole analysis was done, the remaining solution was placed back at 37 °C to form the hydrogel.

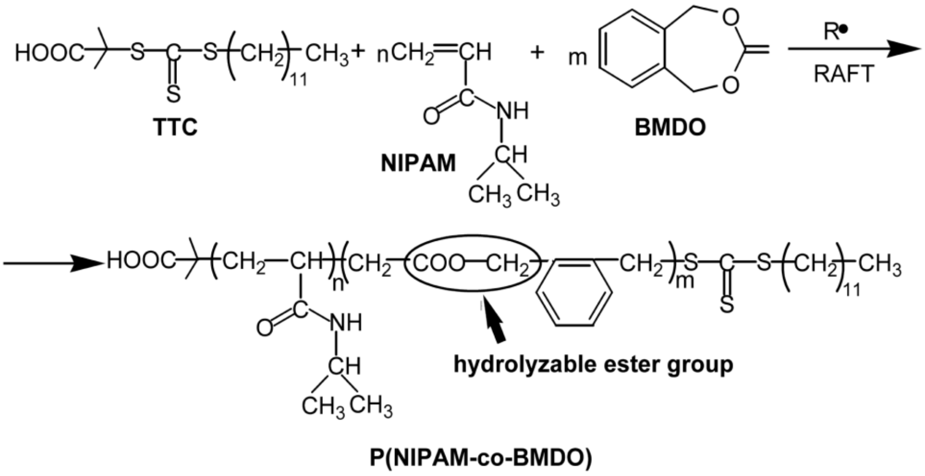
2.6. Characterization

The ^{1}H NMR spectra were recorded on a 300 MHz Varian Gemini 300 BB spectrometer by using deuterated chloroform as the solvent. The molecular weights and molecular weight distributions of the polymers synthesized were determined by gel permeation chromatography (GPC), in DMF with 0.05 M LiBr added, using a Viscotek RImax system (Malvern Instr. UK), made up of a GPCmax autosampler, Viscotek Dguard, T6000M and T3000 columns in series and a VE 3580 refractive index (RI) detector. The temperatures of the column oven and RI detector were 50 °C and 40 °C, respectively. The flow rate was 0.8 mL/min, while the calibration was made with narrow poly(methyl methacrylate) standards. The rheological measurements were carried out on a Kinexus Pro (Malvern Instr. UK, software 1.60) rheometer equipped with a Peltier element for temperature control, by employing a 20 mm parallel plate geometry with 0.5 mm gap, in oscillating mode. A constant 0.2% strain within the linear viscoelastic region was applied in all rheological measurements. The temperature-sweep experiments were carried out from 10 °C to 60 °C at a frequency of 1 rad/s and a heating rate of 0.5 °C/min. To prevent water evaporation a solvent trap was used.

3. Results and discussion

The P(NIPAM-co-BMDO) statistical copolymers were prepared by the reversible-addition-fragmentation chain transfer (RAFT) radical copolymerization of the two monomers in the presence of TTC as the RAFT agent (Scheme 1). The

RAFT living/controlled polymerization technique was used in order to obtain well defined copolymers with low polydispersities (Table 1) and made up of macromolecules with the same composition [16]. As BMDO acts as a retarder for NIPAM polymerization [10], different experimental conditions were employed in order to synthesize polymers with the desired molecular characteristics, i.e. similar molecular weights and polydispersities but with different monomer compositions. The presence of the hydrolysable ester groups within the copolymer chain was proved by ^{1}H NMR analyses (spectra not shown here).



Scheme 1. Synthesis of the P(NIPAM-co-BMDO) statistical copolymers

The thermogelation properties of the copolymer PBS solutions were investigated at 15 wt.% concentration by temperature-sweep dynamic rheometry experiments (Fig. 1). When the phase transition temperature (T_{ph}) is reached, the thermoresponsive P(NIPAM-co-BMDO) chains should dehydrate and become water insoluble, leading to the formation of molecular aggregates. As a consequence, a strong increase of the solution viscosity, as well as gel formation, should be noticed. The temperature at which the viscosity begins to increase, T_{vi}, can be determined from rheological measurements and it may be taken as a measure of T_{ph} [18,19] because it characterizes the initial stages of the copolymer aggregation process due to the hydrophilic-hydrophobic transition of the chains.

Indeed, the temperature-sweep rheological measurements showed that both dynamic viscosity (Fig. 1a) and viscoelastic moduli (Fig. 1b) strongly increased at temperatures higher than T_{ph}, in agreement with the thermoresponsive character of the P(NIPAM-co-BMDO) copolymers. As the BMDO units content in the copolymer became larger, T_{vi} shifted toward lower values, simultaneously with a strong increase of the maximum value attained by the dynamic viscosity (DV_{max}), storage modulus (G'_{max}) and viscous modulus (G''_{max}) (Fig. 1, Table 2).

The progressive decrease of T_{vi} with the BMDO units proportion in the copolymer may be explained through the increasingly hydrophobic character of the copolymer due to the BMDO units being more hydrophobic than the NIPAM units [10,12]. The more hydrophobic copolymer once precipitated led very likely to stronger physical interactions amongst the polymer chains having as a result higher values for DV_{max}, G'_{max} and G''_{max}.

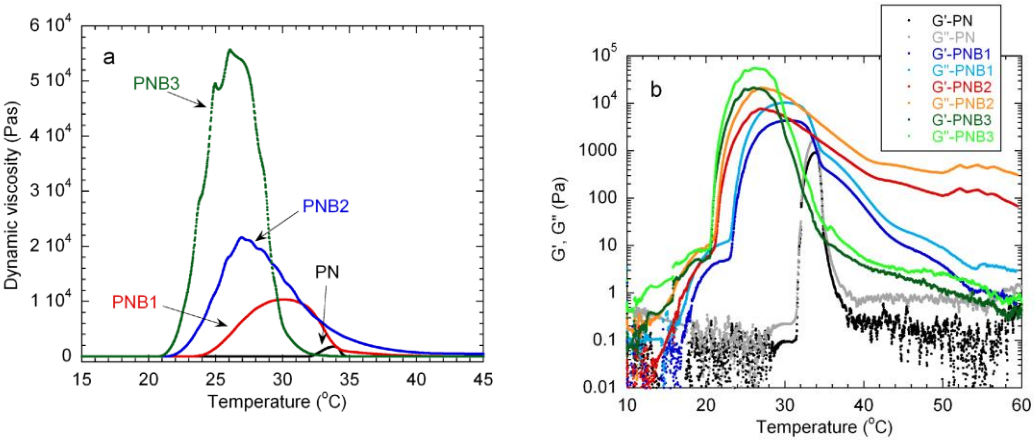


Fig. 1. Temperature-sweep rheological measurements upon the P(NIPAM-co-BMDO) solutions; a) dynamic viscosity vs. temperature; b) G', G" vs. temperature. Polymer concentration – 15 wt.%; solvent – PBS.

Because the polymerization reactions were carried out in the presence of TTC as the RAFT agent, the resulted copolymers had a dodecyl end group (evidenced by ^1H NMR analyses; spectra not shown here), which may additionally increase the hydrophobic character of the copolymers and induce supplementary interactions among the polymer chains. To assess their influence upon the thermogelation process of the polymer solution, the dodecyl end groups were removed in the case of the PNB1 sample and replaced by a hydrogen atom by a radical-induced reduction process carried out in the presence of tributyltin hydride and azobis(isobutironitrile) as previously described [17]. The resulted polymer, denoted as PNB1H, was dissolved in PBS (15 wt.% solution) and the thermogelation process was studied by temperature-sweep measurements (Fig. 2).

The results showed that, after the dodecyl group removal, T_{vi} shifted to higher values (from 23.5 °C in the case of PNB1 to 26 °C for PNB1H), while DV_{max}, G'_{max}, and G''_{max} decreased, thus confirming the reduction of the hydrophobic character in comparison with the PNB1 copolymer.

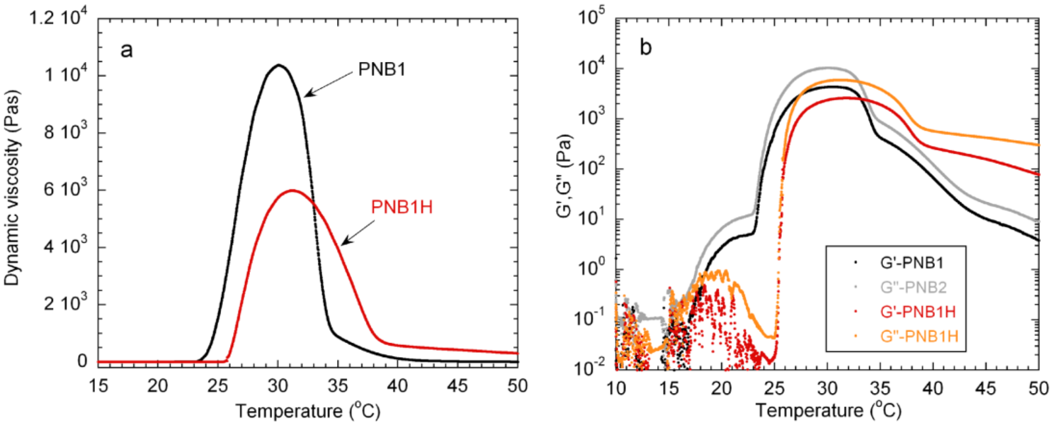


Fig. 2. Influence of the dodecyl end group on the thermogelation process of the PNB1 copolymer solution. a) dynamic viscosity vs. temperature; b) G’, G” vs. temperature. Polymer concentration – 15 wt.%; solvent – PBS

Table 2

Characteristics of the thermogelation process of the thermosensitive (co)polymer solutions[a]

Polymer	T_{vi}[b] °C	DV_{max}/T_{DVmax}[c] Pa·s/°C	$G'_{max}/T_{G'max}$[c] Pa/°C	$G''_{max}/T_{G''max}$[c] Pa/°C
PN	31.5	1800/34	920/34	1800/34
PNB1	23.5	10400/30	4380/30.5	10400/30
PNB2	21.5	21600/27	7770/27	21600/27
PNB3	20.5	55700/26	21550/26	55700/26

[a] polymer concentration – 15 wt.%; solvent – PBS;

[b] temperature at which the dynamic viscosity started increasing, determined from the intersection of the extrapolated linear parts of the horizontal and ascending arms, respectively, of the temperature-sweep rheological curves.

[c] the maximum values of the dynamic viscosity (DV_{max}), elastic modulus (G'_{max}) and viscous modulus (G''_{max}) and the corresponding temperatures on the temperature-sweep rheological curves.

The gel-forming ability of the PNBx copolymers in 15 wt.% PBS solution was tested by placing glass vials containing 1 g of polymer solution into a chilling/heating dry plate (Torrey Pines Scientific, Inc., USA) at 37 °C and visually assessing the gel condition. The results showed that in all cases, similarly to the PNIPAM solution case [8,20], the initial copolymer solution converted directly and very fast (less than 15 min) to a shrunken gel and an aqueous phase.

To improve the water retention of the PNIPAM injectable hydrogels, we recently proposed a method consisting in the addition of some hydrophilic naturally occurring biopolymers, like sodium alginate [20] or dextran [21], to the PNIPAM aqueous solution. With the same aim in view, 2 wt.% ALG or 6 wt.% DXT were added to 15 wt.% PBS solutions of PNBx and their thermogelation properties were investigated by temperature-sweep rheological measurements. The addition of the biopolymers resulted in the formation of two immiscible layers within the solutions at rest, due to the incompatibility between PNBx and DXT or ALG, respectively. One should mention that, similarly to the PNIPAM case, the PNBx-DXT incompatibility was preserved in distilled water as well [21], while for the PNBx-ALG mixture by changing the solvent from PBS to distilled water the polymer solution became homogeneous [20]. This different behavior may be explained through the presence of an interaction occurring in distilled water among the PNBx and ALG chains by means of the Na^+ cations, similarly to the case of PNIPAM [20], making the two polymers compatible and leading to a homogeneous solution. When PBS was the solvent, the PNBx – ALG interactions were very likely cancelled due to the Na and K cations existing in the solvent that saturated the PNBx chains. Before any measurement, the mixed polymers solutions were very well stirred to homogenize them. The reformation of the two liquid layers within the resulted turbid solutions was very slow, and therefore, the reproducibility of the measurements was not affected.

The temperature-sweep rheological measurements showed that the addition of either of the two biopolymers to the PNB1 solution led to a decrease of T_{vi} (Fig. 3a), similarly to the case of the ALG – PNIPAM [20] and DXT – PNIPAM [21] aqueous solutions. This T_{vi} shift may be explained by either the dehydrating effect of the hydrophilic biopolymer upon PNB1 chains, similarly to the effect of alcohols [22] or salts [23] upon PNIPAM or a higher local concentration of the thermosensitive polymer [24] within its aqueous phase. The measurements also showed that DV_{max}, G'_{max}, and G''_{max} appreciably increased for the PNB1-ALG hydrogels, while their value did not change too much in the case of PNB1-DXT hydrogels, although the shape of the temperature-sweep curves was altered (Fig. 3). However, the addition of the biopolymer did not perceptibly improve the water retention of the P(NIPAM-co-BMDO) hydrogels at 37 °C. The gelation tests showed the same behavior of the biopolymer-containing solution as for the solution without biopolymer, i.e. very fast conversion (less than 15 min) to a shrunken gel and an aqueous phase.

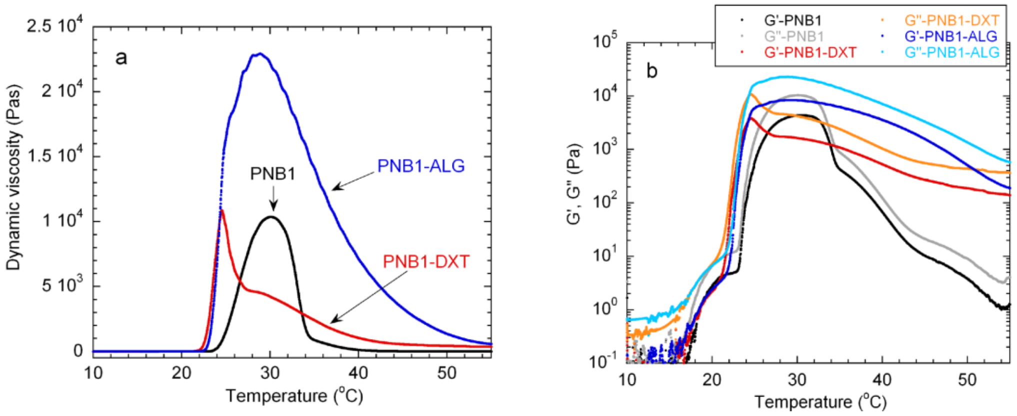


Fig. 3. Temperature-sweep rheological measurements upon the P(NIPAM-co-BMDO)-biopolymer solutions. a) dynamic viscosity vs. temperature; b) G', G'' vs. temperature. P(NIPAM-co-BMDO) – 15 wt.%, ALG – 2 wt.%, DXT – 6 wt.%; solvent – PBS.

The P(NIPAM-co-BMDO) copolymers can be hydrolytically degraded to smaller molecular weight fragments due to the presence of the in-chain ester groups. Their hydrolytic degradability was proved by employing a KOH aqueous solution as the hydrolysis agent [10,12]. However, such conditions are too harsh as compared to those an injectable hydrogel encounters inside the human body. Previously, we showed that a P(NIPAM-co-BMDO)-block-poly(ethylene glycol)-block-P(NIPAM-co-BMDO) triblock copolymer displayed hydrolytic degradation in PBS at 37 °C, which was evidenced by temperature-sweep rheological measurements. Those experiments revealed that a 10 wt.% PBS solution of this triblock copolymer lost completely its thermoresponsive character after less than 24 days [17]. Thus, hydrolysis tests were carried out under similar conditions, i.e. at 37 °C, 15 wt.% PBS solution, to check for the degradability of the PNBx copolymers. The degradation of the copolymers was followed by temperature-sweep rheological measurements, where a reduction of the molecular weight as a consequence of the degradation processes should lead to an increase of T_{vi}. The results showed that after 21 days the PNB1 solution displayed no shift of its T_{vi}, meaning that practically no degradation occurred (Fig. 4a), while a very small T_{vi} increase (about 0.5 °C) was noticed in the case of PNB1H (Fig. 4b). A little larger T_{vi} enhancement (about 1 °C) was shown by PNB2. The very small or even absent degradation of the PNBx copolymers in PBS solution at 37 °C may be explained by the formation of a shrunken gel, whose contact with PBS occurred practically only at the surface, unlike the triblock copolymers mentioned above. However, a larger ester groups content (PNB2 vs. PNB1), as well a lower hydrophobic character (PNB1H vs. PNB1) seemed to support the hydrolysis of the ester groups.

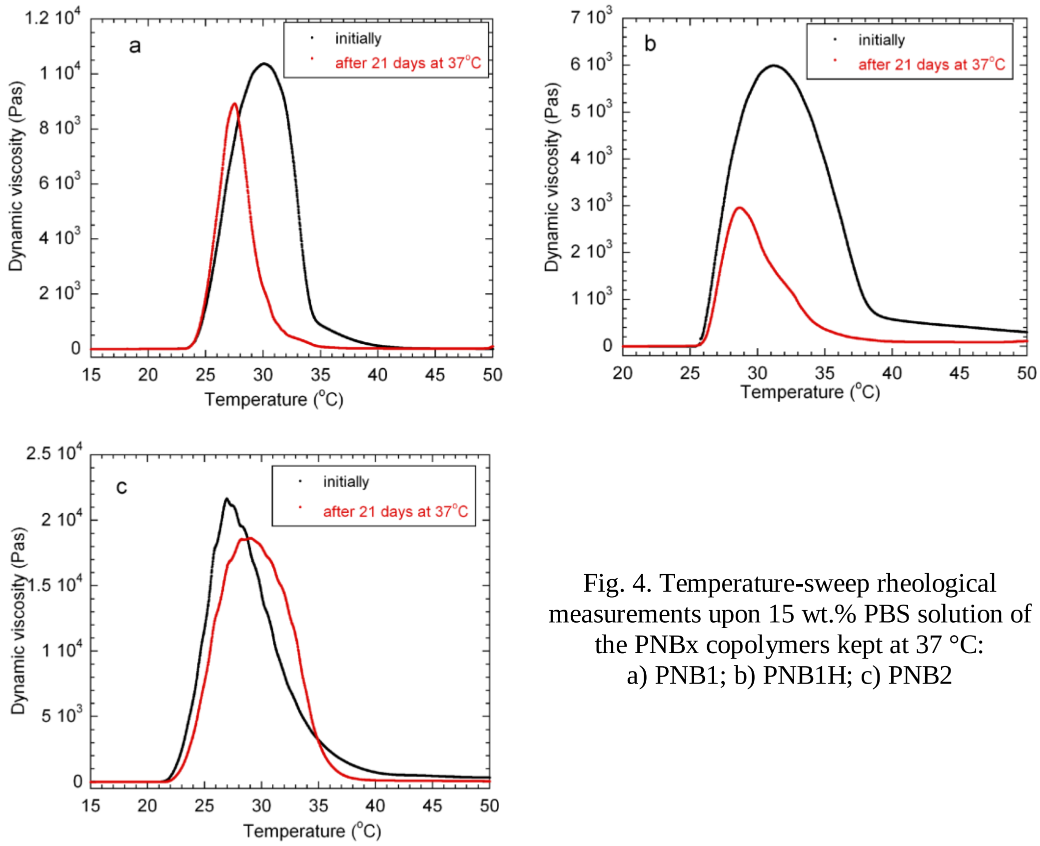


Fig. 4. Temperature-sweep rheological measurements upon 15 wt.% PBS solution of the PNBx copolymers kept at 37 °C:
a) PNB1; b) PNB1H; c) PNB2

4. Conclusions

The PBS solutions of P(NIPAM-co-BMDO) copolymers of various compositions were investigated by temperature-sweep dynamic rheometry experiments. The results showed that both dynamic viscosity DV_{max} and viscoelastic moduli (storage modulus G'_{max} and viscous modulus G''_{max}) strongly increased at temperatures higher than phase transition temperature T_{ph}, proving the thermosensitive character of the polymers synthesized. As the BMDO units content in the copolymer became larger, the temperature at which the viscosity begins to increase (T_{vi}) shifted toward lower values simultaneously with a strong increase of the maximum value attained by DV_{max}, G'_{max} and G''_{max}. This was ascribed to the copolymer hydrophobic character increasing with the BMDO units content. After the dodecyl end groups removal, T_{vi} shifted to higher values, while DV_{max}, G'_{max}, and G''_{max} decreased, confirming the reduction of the hydrophobic character. The PNBx copolymers were tested for their ability to form gel, and the results showed in all cases that the initial copolymer solution converted directly and very fast to a shrunken gel and an aqueous phase. In an attempt to improve the water retention of the injectable hydrogels, sodium alginate and dextran were added, which has as a result the formation of two immiscible layers within the solutions at rest, due to the incompatibility between PNBx and DXT or ALG, respectively. The rheological measurements showed also that the addition of sodium alginate or dextran to the PNB1 solution led to a decrease of T_{vi} and to the increase of DV_{max}, G'_{max}, and G''_{max} in the case of the PNB1-ALG hydrogels. The addition of the biopolymer did not perceptibly improve the water retention of the P(NIPAM-co-BMDO) hydrogels at 37 °C. Hydrolysis tests at 37 °C and 15 wt.% PBS solution were carried out for the PNBx copolymers and were followed up by temperature-sweep rheological measurements. The results showed that no degradation occurred for PNB1 solution, while PNB1H and PNB2 degraded in a very small proportion. The reduced or even absent degradation of the P(NIPAM-co-BMDO) copolymers under the conditions employed may be ascribed to the formation of a shrunken gel, whose contact with PBS occurred practically only at the surface.

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