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THERMOREVERSIBLE HYDROGELS FROM HYDROLYTICALLY DEGRADABLE POLY(N-ISOPROPYLACRYLAMIDE)-POLY(ETHYLENE GLYCOL) TRIBLOCK COPOLYMER AND GELATIN

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Novel thermoreversible hydrogels that may find applications as biomaterials were prepared by adding gelatin to the aqueous solution of an ABA triblock copolymer with poly(ethylene glycol) middle block and poly(N-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane) as side blocks. The gelatin addition decreased the gelation temperature and improved the viscoelastic properties of the hydrogel, indicating the formation of a more resistant hydrogel, as a consequence of a semi-interpenetrating network structure formation. The increase of triblock copolymer concentration led to higher maximum values of the dynamic viscosity, storage modulus and viscous modulus. The mixed hydrogels partially degraded in phosphate buffer saline solution at 37 °C.

Keywords: poly(N-isopropylacrilamide); 5,6-benzo-2-methylene-1,3-dioxepane; gelatin; thermoresponsive polymer; degradable polymer; injectable hydrogel;

1. Introduction

Hydrogels are tridimensional polymeric networks which can swell/retain a large amount of water/biological fluids [1]. They have received a special attention within the last decades due to their potential applications especially in the biomedical field, because of their flexibility very similar to that of the human tissue imparted by the high-water content [2,3]. In order to obtain a more complex hydrogel, synthetic hydrogels with well-defined structures, which can be modified to obtain the desired degradability and functionality, have often been combined with natural polymers, thus leading to improvement of the biocompatibility, biodegradability and mechanical properties of the new materials [4].

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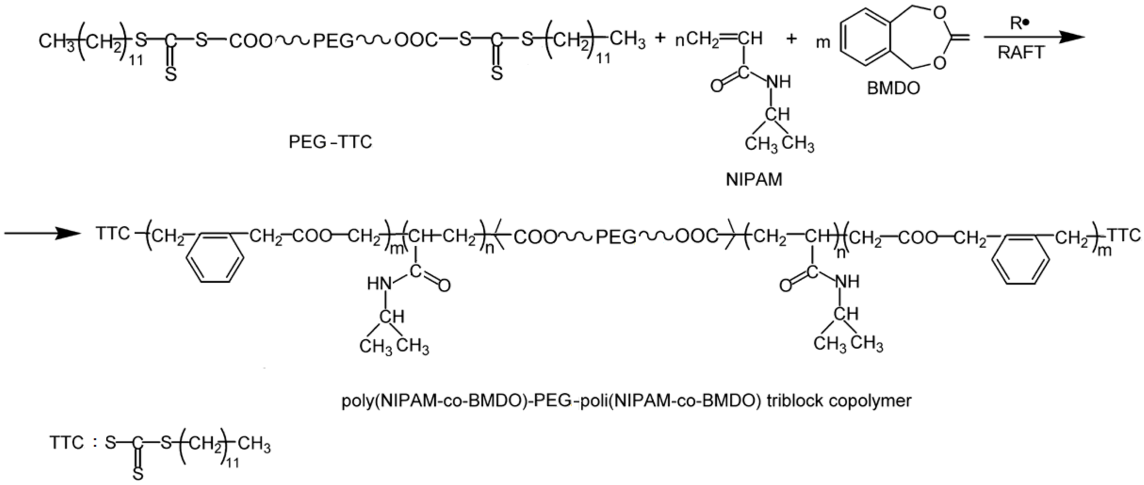
A special category of hydrogels is represented by the injectable ones, which are initially fluid aqueous solutions, but gel rapidly after injection in response to external stimuli or by means of a chemical crosslinking. Unlike to chemical crosslinked injectable hydrogels which require the presence of the crosslinking agents, enzymes or organic solvents, the physical crosslinked ones are formed via self-assembly and represent a favorable method to prepare hydrogels used for biomedical applications [5,6].

As temperature is a stimulus very easily to apply, thermoresponsive hydrogels represent smart materials used in a wide range of applications including sensors, control released of drugs, delivery of genes and in tissue engineering [7-12]. Thermosensitive injectable hydrogels are obtained at temperatures that exceed the phase transition temperature (T_{ph}), due to a hydrophilic-hydrophobic transition. This behavior makes them very attractive for biomedical application, due to the advantages they have, do not require surgical procedures; their precursor solutions can be mixed easily with drugs/cells before injection; there will be a good fit with the body cavity to be filled [5,13].

Poly(N-isopropyl acrylamide) (PNIPAM) is one of the most studied thermosensitive polymers regarding the biomedical applications, due to its lower critical solubility temperature (LCST, approx. 32 °C) close to body temperature and to its rapid transition in the hydrogel phase [14,7]. According to literature data [15-18], PNIPAM-based hydrogel undergoes a phenomenon of syneresis, affecting both their mechanical properties and the chance of being applied in biomedical systems. To overcome this inconvenience, a possible solution may be attaching the PNIPAM chain to a polymeric block which has permanent solubility in water, as it is for example poly(ethylene glycol) (PEG) [17-19]. An extra solution to avoid or at least delay the formation of the syneresis phenomenon may be mixing PNIPAM with a natural, hydrophilic polymer [20]. Another major disadvantage of PNIPAM hydrogels is their non-biodegradable character, which makes them difficult to apply in the biomedical field, such as tissue engineering. Thereby, to be completely removed from the body through the kidney, the PNIPAM hydrogel should be broken in smaller segments that are both soluble and easy to remove from the body. In our previous studies [21-23], we have demonstrated that these methods are favorable when remedying the syneresis phenomenon and the biodegradation of PNIPAM, leading to hydrogels with much improved mechanical properties. Thus, we have previously reported the synthesis of some ABA triblock copolymers with poly(ethylene glycol) (PEG) middle block and statistical copolymers of NIPAM and 5,6-benzo-2-methylene-1,3-dioxepane (BMDO) side chains, prepared through the reversible addition-fragmentation chain transfer (RAFT) free radical copolymerization of NIPAM and BMDO in the presence of a PEG chain transfer macroagent (Scheme 1) [21].



Scheme 1. Synthesis of the NIPAM-BMDO statistical copolymers

The ester linkages were introduced into PNIPAM backbone through radical ring-opening copolymerization of NIPAM with BMDO, a cyclic ketene acetal [24]. So, PNIPAM can be degraded into small polymeric chains, which can be rapidly cleared from the body by renal filtration.

Gelatin is a natural biopolymer, derived from denatured collagen, of animal origin, which has been proven to have good biocompatibility in vivo [26,27]. It is commercially available at a low price, being often used in biomedical application especially due to its excellent characteristics like biocompatibility, biodegradability, as well as for the fact that it facilitates cell adhesion and enzymatic degradation [28-30].

A semi-interpenetrating polymer network (SIPN) is a material comprising one or more of polymers which entangled within another crosslinking network. By comparison with a normal polymer blend, in a SIPN the polymers are more homogenously mixed and mechanically interlocked, thus providing improved mechanical properties [31].

The SIPN hydrogels are very attractive for pharmaceutical (drug delivery) and biomedical (tissue engineering) application due to the properties resulting from the polymer blends [32]. In many cases, natural polymers characterized by their biodegradability and biocompatibility are combined with synthetic polymers with well-defined structures, to improve the characteristics necessary of some biomaterials.

The present work deals with the synthesis and characterization of novel thermosensitive mixed hydrogels based on degradable triblock copolymers with PEG middle block and P(NIPAM-co-BMDO) side blocks, containing hydrolysable ester groups within the chain, and a natural biopolymer – gelatin. After hydrogel formation, a SIPN should be obtained through the immobilization of gelatin within the P(NIPAM-co-BMDO)-PEG-P(NIPAM-co-BMDO) physical network resulting after solution gelation at temperatures above T_{ph} of the block copolymer. This should improve the gel strength and biocompatibility in comparison with the hydrogel obtained from the triblock copolymer alone. To the best of our knowledge, such kind of hydrogels have not been described in the literature yet. The gelation properties of aqueous solutions as well as the viscoelastic properties and degradability of the resulting hydrogels will be analyzed as a function of the gelatin concentration and molecular weight and also on the concentration of the triblock copolymer.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAM, Aldrich, 97 %) was recrystallized from hexane. 5,6-Benzo-2-methylene-1,3-dioxepane (BMDO) was prepared as described in literature [33] and stored at 4 °C in a container filled with nitrogen. The poly(ethylene glycol) bis(S-1-dodecyl-S'-(α,α'-dimethyl-α"-acetate) trithiocarbonate (PEG-TTC) macromolecular RAFT chain transfer agent was synthesized with a functionality of 1.9 (as determined by ^{1}H NMR) from α,ω-dihydroxy PEG with 10,000 g/mol molecular weight (PEG, Aldrich) and S-1-dodecyl-S'-(α,α'-dimethyl-α"-acetic acid) trithiocarbonate (TTC) [34] as described elsewhere [21]. The poly(NIPAM-co-BMDO)-PEG-poly(NIPAM-co-BMDO) triblock copolymer (NT) was prepared by the copolymerization reaction of NIPAM and BMDO (9/1 mol ratio) in the presence of PEG-TTC and 1,1'-azobis(cyclohexanecarbonitrile) (ACHCN) as the initiator (monomers/PEG-TTC/ACHCN = 176/0.5/0.1 mol ratio), at 90 °C for 24 h, as reported before [20]. The dodecyl trithiocarbonate end groups of the triblock copolymer were replaced by hydrogen in the presence of tributyltin hydride, using a radical-induced reduction process, as described in the literature [21]. The triblock copolymer without dodecyl terminal groups (denoted as NTH) employed in these experiments had the following characteristics: M_n = 34,500 Da, M_w = 60,000 Da, M_w/M_n = 1.73 (measured by gel permeation chromatography (GPC)) and NIPAM units/BMDO units mole ratio 97/3, as determined by ^{1}H NMR.

Type A gelatin from porcine skin with various gel strengths: 50-80 g Bloom (Fluka, GEL50), 90-110 g Bloom (SIGMA, GEL110) and 300 g Bloom (SIGMA, GEL300) were employed without further purification. Besides providing information about the strength of a gel resulted from a 6.67 wt.% gelatin solution, the Bloom number offers indications about the average molecular weight of gelatin. The higher the Bloom number, the larger the gelatin molecular weight is [35]. The phosphate buffer saline (PBS) solution was prepared with a pH about 7.4 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 NaN_3 as a preservative in 1 L distilled water.

2.2. Preparation of the hydrogels

Stock solutions of gelatin in PBS were initially prepared. The appropriate amounts of gelatin and PBS were loaded into 22 mL screw cap glass vials and the mixture was stirred at 50 °C in the aluminum block of a chilling/heating dry plate (Torrey Pines Scientific, Inc., USA) for 2 h until homogeneous, and then stored at 4 °C in a refrigerator. The solution concentrations were chosen so that to yield hydrogel solution precursors of predetermined both gelatin (0.5, 1 and 1.5 wt.%) and triblock copolymer (10 and 15 wt.%) concentration after mixing with the appropriate amount of NTH. After a few days at cold, the gelatin solutions converted to either a viscous solution (0.5 wt.%) or to a gel (1 and 1.5 wt.%). In order to prepare the NTH – gelatin solution in PBS, the corresponding gelatin solution vial was taken from the refrigerator and placed at 30 °C for 2 hours with stirring to resolubilize the biopolymer. The temperature was then decreased at 25 °C and after 1 more hour the calculated amount of solution was weighed in a 7 mL screw cap glass vial, followed by the addition of appropriate amount of NTH. The vial was placed first at 25 °C for 2 hours and then at 20 °C with stirring overnight. The mixture was additionally agitated in an ice-water bath for 3 hours the next day and stored in the refrigerator until use. An inhomogeneous solution resulted, displaying two liquid layers at rest, which suggested the incompatibility of the NTH-gelatin pair at cold. Before measuring them, the solutions were stirred for about 1 hour in an ice-water bath, resulting in a turbid solution, whose separation rate was relatively low. It is important to mention that by raising the temperature to 15-20 °C, the mixture became homogeneous and transparent, indicating the compatibility between the two polymers at this temperature.

The hydrogel code indicates the weight concentration of both NTH and gelatin. For example, NTH10%-GEL50 0.5% denotes the solution/hydrogel sample containing 10 wt.% NTH, 0.5 wt.% GEL50, the remaining up to 100% being PBS.

2.3. Hydrogel degradation in PBS

A PBS solution (0.8 g) containing 15 wt.% triblock copolymer and 0.5 wt.% GEL50 was introduced at 37 ± 0.5 °C to form the hydrogel, and the gel condition was visually assessed from time to time. After certain time intervals, the vial was removed from the plate and placed in the refrigerator for a few hours to dissolve the hydrogel. The resulting solution was then stirred in an ice-water bath for about 45 min and immediately analyzed by rheometry. After all analyses were done, the remaining solution was frozen in a freezer and freeze dried. The resulted white powder samples were extracted for 24 h at room temperature with 5 mL CH_2Cl_2. The next day, were centrifuged at 8000 rpm for 10 min, the liquid phase was decanted and then filtered through a PTFE 0.45 µm membrane filter. The solvent was removed under vacuum and a white powder of the degraded triblock copolymer was obtained. The resulting material was analyzed by gel permeation chromatography (GPC) measurements.

2.4. Characterization

The ^{1}H NMR spectra were recorded on a 300 MHz Varian Gemini 300 BB spectrometer. Deuterated chloroform was the solvent employed.

The molecular weights analysis of the polymers synthesized was carried out by gel permeation chromatography (GPC) in DMF with 0.05 M LiBr at 0.8 mL/min flow rate. A Viscotek RImax system (Malvern Instruments, UK) made up of a GPCmax autosampler and a Viscotek Dguard, T6000M and T3000 columns in series, and a VE 3580 refractive index (RI) detector was employed, while the temperatures of the column oven and RI detector were 50 and 40 °C, respectively. The system calibration was made with narrow poly(methyl methacrylate) standards.

The rheological measurements were performed in oscillating mode on a Kinexus Pro rheometer (Malvern Instruments, UK, software 1.60) equipped with a Peltier element for temperature control. A 20-mm parallel plate geometry with 0.5 mm gap was used, and a 0.2 % strain within the linear viscoelastic region was applied in all rheological measurements. The temperature-sweep experiments were carried out by progressively increasing temperature with 0.5 °C/min heating rate from 10 to 60 °C at a frequency of 1 rad/s. The frequency-sweep measurements were carried out at 37 °C on the 0.01 – 10 Hz frequency interval. A solvent trap was always used in order to avoid water evaporation.

3. Results and discussion

3.1. Hydrogel formation

Hydrogels aqueous solution precursors of various compositions and concentrations were prepared by dissolving appropriate amounts of triblock copolymer and gelatin in PBS. Depending on temperature, the polymeric solution displayed a different appearance. At 10 °C or below the solution consisted in two layers at rest and became cloudy after stirring with a uniform aspect, meaning that the two phases were still present. This indicated that the two polymers were incompatible at cold. On the other hand, at 15-20 °C the solution was clear, with a homogeneous aspect, suggesting that the two polymers are compatible within this temperature range. The compatibility of the triblock copolymer-gelatin pair at higher temperatures was confirmed by the aspect of the hydrogels obtained at 37 °C, which were transparent and only slightly cloudy, unlike the hydrogels previously described based on alginate or dextran and the P(NIPAM-co-BMDO)-PEG-P(NIPAM-co-BMDO) triblock copolymer, where the incompatibility between the biopolymer and the block copolymer led to an opaque hydrogel at 37 °C [23].

The influence of several factors, like molecular weight and concentration of gelatin and concentration of triblock copolymers, upon the thermogelation and viscoelastic properties of the resulting SIPN hydrogels were investigated by rheometry.

The thermogelation properties of the different hydrogel compositions were compared by means of a) the temperature at which the viscosity of the initial solution starts increasing because of the association of the thermosensitive side blocks of the copolymer (T_{vi}), T_{vi} being a measure for the phase transition temperature, and b) the gelation temperature (T_{gel}), which was considered as the temperature corresponding to the storage modulus G' being equal to the viscous modulus G". The viscoelastic properties of the resulted hydrogels were analyzed by both temperature and frequency-sweep rheological measurements. Also, the hydrolytic degradability of the SIPN hydrogels at 37°C in PBS was studied.

3.2. Influence of the gelatin concentration

By adding the gelatin to the thermosensitive triblock copolymer hydrogel, T_{vi}, T_{gel} and the maximum values of the dynamic viscosities (DV_{max}), G' (G'_{max}) and G" (G''_{max}) have been modified as it can be seen from the temperature sweep rheological measurements (Fig. 1a, 1b; Table 1).

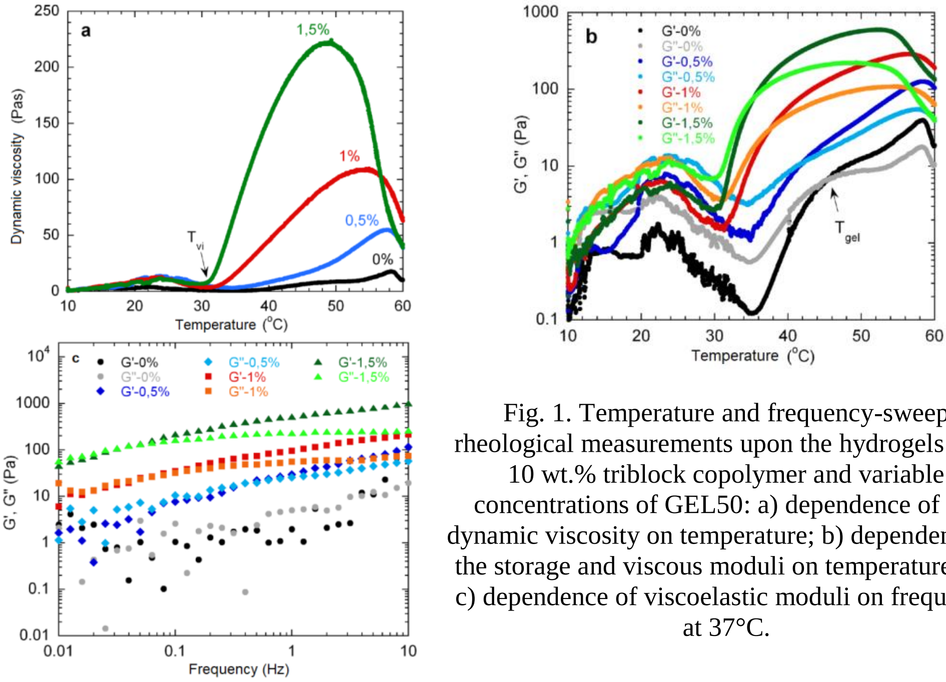


Fig. 1. Temperature and frequency-sweep rheological measurements upon the hydrogels with 10 wt.% triblock copolymer and variable concentrations of GEL50: a) dependence of the dynamic viscosity on temperature; b) dependence of the storage and viscous moduli on temperature and c) dependence of viscoelastic moduli on frequency at 37°C.

As the biopolymer concentration became higher, T_{vi} and T_{gel} decreased, probably as a consequence of the presence of the ionic groups contained within the gelatin structure, similarly to the effect of salts upon the phase transition temperature of PNIPAM [36,37]. The increase of gelatin concentration also led to higher maximum values of the dynamic viscosity, storage modulus and viscous modulus, indicating the formation of a more mechanically resistant hydrogel, in agreement with the supposition of the SIPN structure formation.

Table 1

Dependence of the gelation process characteristics on gelatin concentration

Hydrogel	T_{vi}[a] °C	DV_{max}/T_{DVmax}[b] Pa·s/°C	T_{gel}/G’=G” °C/Pa	$G'_{max}/T_{G'max}$[b] Pa/°C	$G''_{max}/T_{G''max}$[b] Pa/°C
NTH10%-GEL50 0%	35	18/58	45.5/7	40/58	18/58
NTH10%-GEL50 0.5%	34	55/58	42/11	127/58	55/58
NTH10%-GEL50 1%	32	108/54	37.5/31	290/56	108/54
NTH10%-GEL50 1.5%	31	220/47	35.5/77	600/52	220/47

[a] temperature at which the dynamic viscosity started to increase determined from the intersection of the extrapolated linear parts of the horizontal and ascending, respectively, arms of the temperature sweep rheological curves;

[b] maximum values of dynamic viscosities (DV_{max}), storage modulus (G'_{max}) and viscous modulus (G''_{max}) and the corresponding temperatures during the temperature sweep rheological measurements.

The dependence of the viscoelastic properties of the hydrogels as a function of gelatin concentration were investigated through frequency sweep rheological experiments at 37 °C (Fig. 1c). The results showed that the hydrogels had a predominantly viscous, liquid-like behavior at 37 °C, as indicated by G’ and G” crossing each other at a certain value of the frequency. However, higher concentrations of GEL50 lead to much higher values of G’ and G”.

3.3. Influence of the triblock copolymer concentration

By increasing the concentration of the triblock copolymer by 5 wt.%, i.e. from 10 wt.% to 15 wt.%, at constant GEL50 concentration (0.5 wt.%), both T_{vi} and T_{gel} decreased by about 9-10 °C, while a strong increase of the maximum values of the dynamic viscosity, G’ and G” (Fig. 2a, 2b; Table 2) was noticed, indicating the formation of a more resistant hydrogel. Also, the increase of the triblock copolymer concentration did not modify the behavior of the hydrogel at 37 °C, which preserved its predominantly viscous character, as can be seen from G’ crossing G”. However, the G’ and G” values appreciably increased by more than one order of magnitude (Fig. 2c).

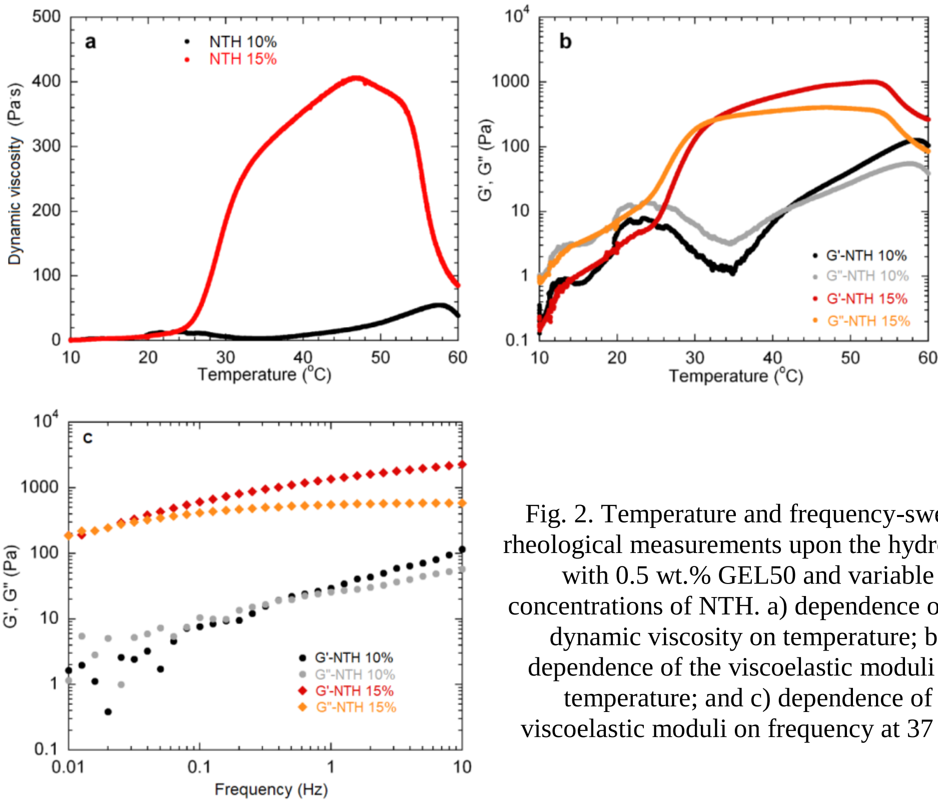


Fig. 2. Temperature and frequency-sweep rheological measurements upon the hydrogels with 0.5 wt.% GEL50 and variable concentrations of NTH. a) dependence of the dynamic viscosity on temperature; b) dependence of the viscoelastic moduli on temperature; and c) dependence of viscoelastic moduli on frequency at 37 °C.

Table 2

Dependence of the gelation process characteristics on the triblock copolymer concentration

Hydrogel	T_{vi} °C	DV_{max}/T_{DVmax} Pa·s/°C	T_{gel}/G'=G" °C/Pa	$G'_{max}/T_{G'max}$ Pa/°C	$G''_{max}/T_{G''max}$ Pa/°C
NTH10%-GEL50 0.5%	34	55/58	42 /11	127/58	55/58
NTH15%-GEL50 0.5%	25	410/47	32.5/260	1000/53	410/47

3.4. Influence of the gelatin molecular weight

Next, the influence of the gelatin molecular weight upon the hydrogel characteristics was assessed by employing gelatin samples of increasing Bloom numbers (GEL50, GEL110 and GEL300). Therefore, hydrogels prepared from 10 wt.% triblock copolymer and 0.5 wt.% gelatin were tested first through temperature sweep rheological experiments, which proved a reduced influence of the gelatin molecular weight upon the thermogelation and viscoelastic properties of the hydrogels.

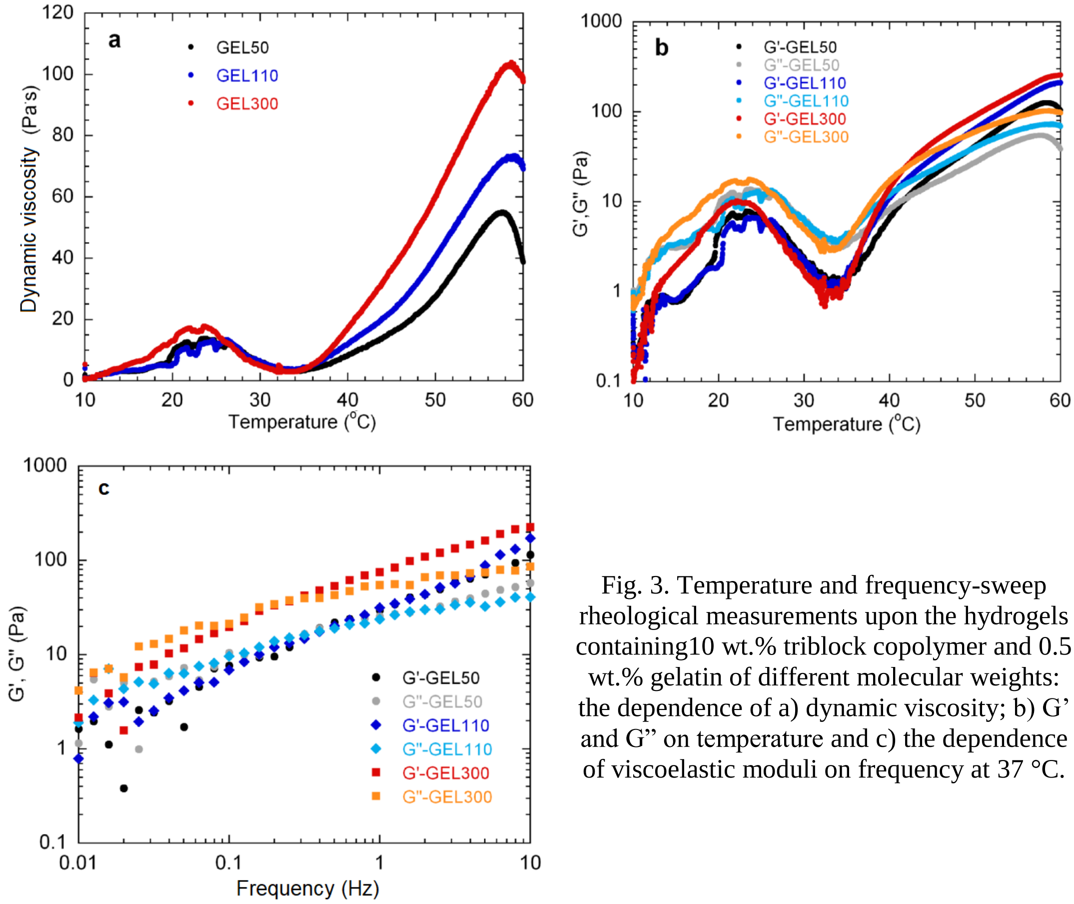


Fig. 3. Temperature and frequency-sweep rheological measurements upon the hydrogels containing10 wt.% triblock copolymer and 0.5 wt.% gelatin of different molecular weights: the dependence of a) dynamic viscosity; b) G' and G" on temperature and c) the dependence of viscoelastic moduli on frequency at 37 °C.

Thus, while T_{vi} and T_{gel} were practically unaffected by the variation of the gelatin molecular weight, the maximum values of the dynamic viscosity, G' and G" only doubled when GEL50 was replaced by GEL300 (Table 3a, 3b; Fig. 3). The same conclusions resulted also from the frequency sweep rheological measurements: as the gelatin molecular weight raised, G' and G" increased no more than 2-3 times. Regardless the molecular weight of gelatin, the predominantly viscous character of the hydrogels at 37 °C was preserved (Fig.3c).

Table 3

Dependence of the gelation process characteristics on the gelatin molecular weight

Hydrogel	T_{vi} °C	DV_{max}/T_{DVmax} Pa·s/°C	$T_{gel}/G'=G''$ °C/Pa	$G'_{max}/T_{G'max}$ Pa/°C	$G''_{max}/T_{G''max}$ Pa/°C
NTH10%-GEL50 0.5%	34	55/58	42/11	127/58	55/58
NTH10%-GEL110 0.5%	34	72/59	41/14	210/60	72/59
NTH10%-GEL300 0.5%	34	104/59	41.5/22	257/60	104/59

3.4. Influence of the gelatin molecular weight

The hydrolytic degradability of these mixed hydrogels was studied at 37 °C for the NTH15%-GEL50 0.5% hydrogel by rheological and molecular weight (GPC) measurements. The degradation occurred mainly through the hydrolysis of the ester groups from BMDO units thus decreasing the molecular weight of the P(NIPAM-co-BMDO) thermosensitive side blocks.

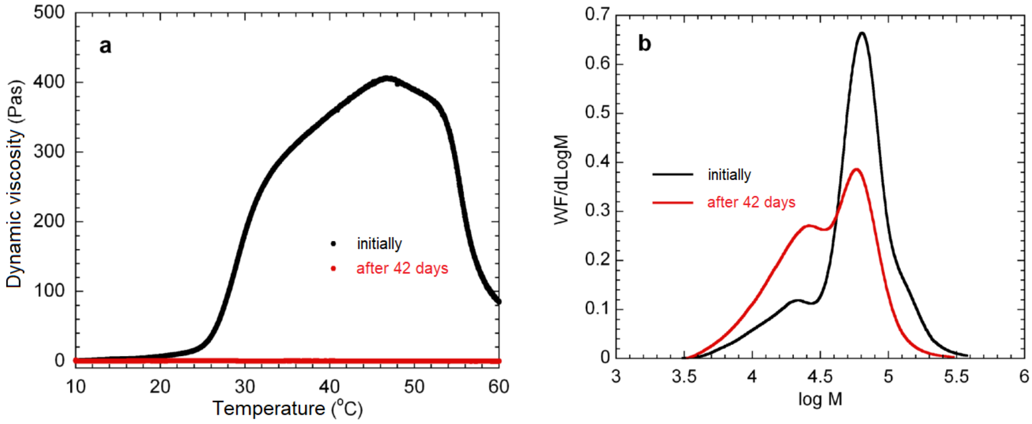


Fig. 4. Supporting evidence of the degradation of the NTH 15%-GEL50 0.5% hydrogel kept at 37 °C for 42 days. a) temperature sweep rheological measurements; b) GPC measurements.

The hydrogel was initially a slightly opaque gel at 37 °C, with no signs of syneresis, but it was converted to an opaque, white, viscous liquid after 6 weeks (42 days) at this temperature. Temperature sweep rheological measurements (Fig. 4a) showed the loss of the hydrogel thermogelation capacity at the end of this interval, while the GPC analyses (Fig. 4b) demonstrated the partial hydrolytic degradation of triblock copolymer through the decrease of the number average molecular weight (M_n) from 34,800 Da for the initial polymer to 23,100 Da in the case of the degraded polymer, the polydispersity index remaining practically unchanged (1.73 vs. 1.72). The partial degradation of the triblock copolymer was also supported by the modification of the molecular weight distribution, as proved by the GPC traces displayed in Fig. 4b. One can see that the low molecular weight peak increased, proving the formation of species with a smaller molecular weight after degradation, while the high molecular weight peak decreased and shifted toward the low molecular weights field, thus indicating the degradation of the polymer species of larger molecular weights. The lack of thermogelation ability of the degraded hydrogel (Fig. 4a) may be explained by the decrease of the molecular weight of the P(NIPAM-co-BMDO) thermosensitive side blocks, which led to both increase of T_{vi} and inability to form a gel at the triblock copolymer concentration employed.

4. Conclusions

Novel both hydrolytically degradable and thermosensitive SIPN hydrogels with improved mechanical strength were prepared by adding gelatin to the aqueous solution of a hydrolytically degradable triblock copolymer with PEG middle block and PNIPAM containing in-chain ester groups as side blocks. The hydrogel PBS solution was heterogeneous at 10 °C or below, indicating that the two polymers were incompatible at cold, but at temperature above 15 °C the solution was homogenous and clear. The hydrogel formed at 37 °C was transparent, thus confirming the compatibility between the triblock copolymer and gelatin. The gelatin addition to the triblock copolymer hydrogel decreased both T_{vi} and T_{gel} and improved the viscoelastic properties, indicating the formation of a SIPN structure. The dependence of the viscoelastic properties as a function of gelatin showed that the resulted hydrogel had a predominantly viscous, liquid-like behavior at 37 °C. The employment of gelatin samples of various Bloom numbers for the preparation of hydrogels showed a reduced influence of the gelatin molecular weight upon the thermogelation and viscoelastic properties of the resulted hydrogels. By increasing the concentration of the triblock copolymer at a constant gelatin concentration a more resistant hydrogel was obtained. A 15 wt.% triblock copolymer concentration strongly decreased both T_{vi} and T_{gel} and increased DV_{max}, G'_{max} and G''_{max}. The predominantly viscous character of the hydrogel was preserved. The mixed hydrogels displayed a partial hydrolytic degradation in PBS at 37 °C, which occurred through the hydrolysis of the ester groups from P(NIPAM-co-BMDO) side blocks. The hydrogel turned from a slightly opaque gel at 37 °C to an opaque, white, viscous liquid after 42 days and lost thermogelation capacity at the end of the interval, as shown by the temperature sweep rheological measurements. The partial hydrolytic degradation of the triblock copolymer was also demonstrated by GPC measurements through the decrease of the molecular weight.

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REFERENCES

[1]. *D. Y. Ko, U. P. Shinde, B. Yeon, B. Jeong*, Recent progress of in situ formed gels for biomedical application, Prog. Polym. Sci., **vol. 38**, 2013, pp. 672-701;

[2]. *E. M. Ahmed*, Hydrogel: Preparation, characterization, and application: A review, J. Adv. Res., **vol. 6**, 2015, pp. 105-121;

[3]. *W. A. Laftah, S. Hashim, A. N. Ibrahim*, Polymer Hydrogels: A Review, Polym. Plast. Technol. Eng., **vol. 50**, 2011, pp. 1475-1486;

[4]. *S. J. Buwalda, K. W.M. Boere, P. J. Dijkstra, J. Feijen, T. Vermonden, W. E. Hennink*; Hydrogels in a historical perspective: From simple networks to smart materials; J. Control. Release, **vol. 190**, 2014, pp. 254-273;

[5]. *C. T. Huynh, M. K. Nguyen, D. S. Lee*, Injectable Block Copolymer Hydrogels: Achievements and Future Challenges for Biomedical Applications, Macromolecules, **vol. 44**, 2011, pp. 6629-6636;

[6]. *N. K. Singh, D. S. Lee*, In situ gelling pH- and temperature-sensitive biodegradable block copolymer hydrogels for drug delivery, J. Control Release, **vol. 193**, 2014, pp. 214-227;

[7]. *E. S. Gil, S. M. Hudson*, Stimuli-reponsive polymers and their bioconjugates, Prog. Polym. Sci., **vol. 29**, 2004, pp. 1173-1222;

[8]. *B. Jeong, S. W. Kim, Y. H. Bae*, Thermosensitive sol-gel reversible hydrogels, Adv. Drug Deliv. Rev., **vol. 54**, 2002, pp. 37-51;

[9]. *I. Dimitrov, B. Trzebicka, A. H. E. Muller, A. Dworak, C. B. Tsvetanov*, Thermosensitive water-soluble copolymers with doubly responsive reversibly interacting entities, Prog. Polym. Sci., **vol. 32**, 2007, pp. 1275–1343;

[10]. *D. Crespy, R. M. Rossi*, Temperature responsive polymers with LCST in the physiological range and their applications in textiles, Polym. Int., **vol. 56**, 2007, pp. 1461-1468;

[11]. *I. Tokarev, S. Minko*, Stimuli-responsive hydrogel thin films, Soft Matter, **vol. 5**, 2009, pp. 511-524;

[12]. *M. A. Cooperstein, H. E. Canavan*, Biological Cell Detachment from Poly(N-isopropyl acrylamide) and Its Applications, Langmuir, **vol. 26**, 2010, pp. 7695-7707;

[13]. *D. J. Overstreet, D. Dutta, S. E. Stabenfeldt, B. L. Vernon*, Injectable Hydrogels, J. Polym. Sci. B Polym. Phys., **vol. 50**, 2012, pp. 881-903;

[14]. *M. A. Ward, T. K. Georgiou*, Thermoresponsive Polymers for Biomedical Applications, Polymer, **vol. 3**, 2011, pp. 1215-1242;

[15]. *C. K. Han, Y. H. Bae*, Inverse thermally-reversible gelation of aqueous N-isopropylacrylamide copolymer solutions, Polymer, **vol. 39**, 1998, pp. 2809-2814;

[16]. *R. Motokawa, K. Morishita, S. Koizumi, T. Nakahira, M. Annaka*, Thermosensitive Diblock Copolymer of Poly(N-isopropylacrylamide) and Poly(ethylene glycol) in Water: Polymer Preparation and Solution Behavior, Macromolecules, **vol. 38**, 2005, pp. 5748-5760;

[17]. *M. Teodorescu, I. Negru, P. O. Stanescu, C. Draghici, A. Lungu, A. Sarbu*, Thermogelation properties of poly(N-isopropylacrylamide)-block-poly(ethylene glycol)-block-poly(N-isopropylacrylamide) triblock copolymer aqueous solutions, React. Func. Polym., **vol. 70**, 2010, pp. 790-797;

[18]. *I. Negru, M. Teodorescu, P. O. Stanescu, C. Draghici, D. M. Vuluga*, Novel crosslinked thermoresponsive hydrogels with controlled poly(ethylene glycol) – poly(propylene glycol) multiblock copolymer structure, Colloid. Polym. Sci., **vol. 292**, 2014, pp. 829-838;

[19]. *J. Virtanen, H. Tenhu*, Thermal Properties of Poly(N-isopropylacrylamide)-g-poly(ethylene oxide) in Aqueous Solutions: Influence of the Number and Distribution of the Grafts, Macromolecules, **vol. 33**, 2000, pp. 5970-5975;

[20]. *M. Teodorescu, M. Andrei, G. Turturică, P. O. Stănescu, A. Zaharia, A. Sârbu*, Novel Thermoreversible Injectable Hydrogel Formulations Based on Sodium Alginate and Poly(NIsopropylacrylamide), Int. J. Polym. Mater. PO., **vol. 64**, 2015, pp. 763-771;

[21]. *G. Turturică, M. Andrei, P. O. Stănescu, C. Drăghici, D. M. Vuluga, A. Zaharia, A. Sârbu, M. Teodorescu*, ABA triblock copolymers of poly(N-isopropylacrylamide- co-5,6- benzo-2-methylene-1,3- dioxepane) (A) and poly(ethylene glycol) (B): synthesis and thermogelation and degradation properties in aqueous solutions, Colloid Polym. Sci., **vol. 294**, 2016, pp. 743-753;

[22]. *M. Andrei, C. Drăghici, M. Teodorescu*, Gelation properties of the aqueous solutions of hydrolytically degradable thermosensitive poly(N-isopropylacrylamide-co-5,6-benzo-2-

methylene-1,3-dioxepane) copolymers, Sci. Bull. B Chem. Mater. Sci. UPB, **vol. 78**, 2016, pp. 63-74;

[23]. *M. Andrei, P. O. Stănescu, C. Drăghici, M. Teodorescu*, Degradable thermosensitive injectable hydrogels with two-phase composite structure from aqueous solutions of poly(N-isopropylacrylamide-co-5,6-benzo-2-methylene-1,3-dioxepane)-poly(ethyleneglycol) triblock copolymers and biopolymers, Colloid Polym. Sci., **vol. 295**, 2017, pp. 1805-1816;

[24]. *S. Louguet, V. Verret, L. Bédouet, E. Servais, F. Pascale, M. Wassef, D. Labarre, A. Laurent, L. Moine*, Poly(ethylene glycol) methacrylate hydrolyzable microspheres for transient vascular embolization, Acta Biomater, **vol. 10**, 2014, pp. 1194-1205;

[25]. *S. Deshayes, A. M. Kasko*, Polymeric Biomaterials with Engineered Degradation, J. Polym. Sci. A Polym. Chem., **vol. 51**, 2013, pp. 3531-3566;

[26]. *F. Ayhan, S. Demirci, H. Ayhan*, Poly (Ethylene Glycol)/Gelatin Composite Hydrogels for Drug Delivery, J. compos. biodegrad. polym., **vol. 2**, 2014, pp.36-45;

[27]. *X. Geng, X. Mo, L. Fan, A. Yinb*, J. Fang, Hierarchically designed injectable hydrogel from oxidized dextran, amino gelatin and 4-arm poly(ethylene glycol)-acrylate for tissue engineering application, J. Mater. Chem., **vol. 22**, 2012, pp. 25130-25139;

[28]. *S. T. Koshy, T. C. Ferrante, S. A. Lewin, D. J. Mooney*, Injectable, porous, and cell-responsive gelatin cryogels, Biomaterials, **vol. 35**, 2014, pp. 2477-2487;

[29]. *A. Chetouani, M. Elkolli, M. Bounekhel, D. Benachour*, Synthesis and properties of novel hydrogels from oxidized pectin crosslinked gelatin for biomedical applications, Polym. Bull., **vol. 71**, 2014, pp. 2303-2316;

[30]. *M. A. Daniele, A. A. Adams, J. Naciri, S. H. North, F. S. Ligler*, Interpenetrating networks based on gelatin methacrylamide and PEG formed using concurrent thiol click chemistries for hydrogel tissue engineering scaffolds, Biomaterials, **vol. 35**, 2014, pp. 1845-1856;

[31]. *D. S. Desphande, R. Bajpai, A. K. Bajpai*, Water Sorption and Biocompatibility Evaluation of Poly(Vinyl Alcohol – Acrylonitrile) Based Hydrogels, Soft Mater, **vol. 11**, 2013, pp. 221-230;

[32]. *F. Alhaique, T. Coviello, P. Matricardi*, Polysaccharide Hydrogels, Characterization and Biomedical Applications, CRC Press, Boca Raton, 2016 ;

[33]. *H. Wickel, S. Agarwal*, Synthesis and characterization of copolymers of 5,6-benzo-2-methylene-1,3-dioxepane and styrene, Macromolecules, **vol. 36**, 2003, pp. 6152-6159;

[34]. *Y. Z. You, D. Oupicky*, Synthesis of temperature-responsive heterobifunctional block copolymers of poly(ethylene glycol) and poly(N-isopropylacrylamide), Biomacromolecules **vol. 8**, 2007, pp. 98-105;

[35]. www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/2/g9382pis.pdf;

[36]. *Y. Zhang, S. Furyk, D.E. Bergbreiter, P.S. Cremer*, Specific ion effects on the water solubility of macromolecules: PNIPAM and the Hofmeister series, J. Am. Chem. Soc., **vol. 127**, 2005, pp. 14505-14510;

[37]. *T. Patel, G. Ghosh, S. Yusa, P. Bahadur*, Solution behavior of poly(N-isopropylacrylamide) in water: effect of additives, J. Disp. Sci. Technol., **vol. 32**, 2011, pp. 1111-1118.