ABA triblock copolymer based hydrogels with thermo-sensitivity for biomedical applications

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Abstract

Oligo(ethylene glycol)-oligo(propylene glycol)-oligo(ethylene glycol) (OEG-OPG-OEG) triblock copolymers are hydrogel forming and extensively investigated in the field of drug release due to their biocompatibility and thermo-sensitivity. Here the synthesis and characterization of OEG-OPG-OEG based polymer networks from methacrylated oligomers by photo-irradiation are reported. Two precursors were selected to have comparable hydrophilicity (80 wt% OEG content) but different molecular weights of M_n = 8400 g·mol^{-1} and 14600 g·mol^{-1}. The precursor solutions were prepared in concentration 10 to 30 wt%. The resulting polymer networks prepared from high M_n precursors exhibited higher swellability at equilibrium (up to 3400%) and mechanical properties in the range of G' ~ 0.1 to 1 kPa at 5 °C compared to networks based on low M_n precursors. A more significant thermo-sensitive behavior in terms of swellability, volumetric contraction and mechanical transition, starting at 30 °C could also be observed for the networks based on high M_n precursors, thus promoting future application in the field of drug release.

Introduction

Hydrogels are an emergent class of polymer materials especially because their properties such as swellability and mechanical properties, and function such as stimuli-sensitivity can be adjusted to the requirements of specific applications to mimic e.g. the extracellular matrix [1]. Hydrogels based on synthetic compounds such as poly(vinyl alcohol) or poly(ethylene glycol) or natural polymers such as gelatin have been extensively explored [2, 3] due to their biocompatibility. However, other polymers such as poly(*N*-isopropylacrylamide) (PNIPAm) or block copolymers such as ABA triblock copolymer based on oligo(ethylene glycol) segments A and an oligo(propylene glycol) segment B (OEG-OPG-OEG) exhibit thermal transition when in aqueous solution, creating a physical gel at higher temperatures. They have attracted strong interest for pharmaceutical technologies, because of their biocompatibility and thermo-sensitivity [4]. In particular, the micellization of a OEG-OPG-OEG monomer solution upon its critical micellization temperature (cmt) can induce a self-gelation [5]. In this context, a drug can be incorporated in a solution and injected at room temperature and form a physical gel at body temperature. However, such a gel based on physical crosslinks can quickly dissolve when surrounded by body fluids, which could cause a burst release of the active compounds. On the other hand, a covalently crosslinked hydrogel with thermo-sensitivity used as template for drug loading might induce a more gradual release of the drug because its diffusion might be slowed down by the

polymer network. In addition, the covalent crosslinking enables the variation of composition parameters and therefore tailorable swellability and elastic properties can be achieved, which might interfere with the micellization process. In this context, we explore the crosslinking efficiency, swellability, and mechanical strength of hydrogels prepared by photocrosslinking of OEG-OPG-OEG functionalized precursors, also in the context of thermo-sensitivity. Two different block copolymer were used, with M_n = 8400 g·mol^{-1} or M_n = 14600 g·mol^{-1}, both with comparable hydrophilicity 80 wt% OEG, and aqueous precursors solutions were prepared in concentration 10 to 30 wt%.

Experimental details

Synthesis

The functionalization of OEG-OPG-OEG of M_n = 8400 g·mol^{-1} and M_n = 14600 g·mol^{-1} with 2-isocyanate ethyl methacrylate (IEMA) was performed as described in reference [6]. The hydrogel networks were prepared from OEG-OPG-OEG-IEMA monomer solutions, in concentration 10 wt%, 20 wt% and 30 wt% in water for injection (WFI). A 33.3 mg·mL^{-1} photoinitiator solution (Irgacure 2959, BASF, Germany) was prepared and added in concentration 15 wt% to the monomer solution. The mixture was carefully injected with a syringe between two quartz glass plates (100 mm x 100 mm, separated by a 1 mm thick Teflon spacer) whereby the formation of air bubbles was avoided. The mold was placed under an Excimer Laser (Bluelight PS 30P excimer laser, λ = 308 nm, Heraeus Noblelight, Germany) at a distance of 15 cm on the surface of ice cold water/crushed ice at 5 °C, and irradiated for 5 min on each side. 3 sample pieces were cut from the hydrogel after crosslinking for the determination of gel content (G), the remaining polymer network was carefully detached from the quartz glass plates, extracted 24 hours in 25 vol% Ethanol, and then stored in sterile water in a fridge until further characterization.

Characterization methods

For the determination of G, three sample pieces were extracted in a 25 vol% Ethanol solution for 24h, the mass of the dried samples before extraction (m_d) and after extraction ($m_{d,ext}$) were recorded and G was calculated according to equation 1:

$$G = \frac{m_{d,ext}}{m_d} \cdot 100 \quad (\%) \tag{1}$$

The degree of swelling (Q) was determined in water at 5 °C and 50 °C, as well as in phosphate buffered saline without Ca^{2+} and Mg^{2+} (PBS, Dulbecco). Swelling kinetics were performed in distilled water at 5 °C. 25 mm diameter chips were punched from the hydrogels at 5 °C, and immersed in a water bath at 5 °C or 50 °C. The surface area was determined from photographs by the use of the program ImageJ. Rheological investigations in time sweep mode were performed on a HAAKE rheometer Mars II (Thermo Scientific), with a plate-plate geometry on

punched samples of 2 cm diameter. After verifying that all measurements were performed in the linear viscoelastic region for all samples, a constant deformation of γ = 0.002 was applied for all frequency- and temperature-dependent measurements. Temperature sweeps were performed between 1 and 60 °C with a frequency f = 0.15 Hz and a constant force of F = 0.10 N. Other characterization methods were performed as described in reference [6].

Results and discussion

Samples are denoted as xxK(yy), were xxK refers to the M_n of the selected OEG-OPG-OEG block copolymer in kDa, and (yy) to the monomer concentration in wt% of the precursor solutions. The hydrogels appeared as homogeneous, transparent, and air bubble free films. With the exception of the sample of composition 15K(10), crosslinked films samples could be obtained.

Gel content and swellability

The gel content (G) was measured by gravimetric methods to assess the efficiency of crosslinking. Values ranged between 80% and 95% for 8K(yy) and between 74% and 88% for 15K(yy). G was higher for 8K(yy) samples than for 15K(yy) ones and increased with the monomer concentration. In general, G was lower for these networks, synthesized at 5 °C, than those described in our previous work [6], which were synthesized at room temperature. This difference can be attributed to the thermo-sensitive behavior of OEG-OPG-OEG block copolymers solutions which already form micelles at room temperature.

Table 1: Composition of the hydrogels and properties

Sample Id [a]	G [b]	in water		in PBS		Contraction
		Q 5 °C [c]	Q 50 °C [d]	Q 5 °C [e]	Q 50 °C [f]	5↔50 °C [g]
	[%]	[%]	[%]	[%]	[%]	[%]
8K(10)	80 ± 1	1740 ± 30	830 ± 50	1280 ± 60	730 ± 20	51
8K(20)	94 ± 1	1270 ± 40	600 ± 20	1230 ± 60	540 ± 10	44
8K(30)	95 ± 2	1000 ± 50	530 ± 10	1040 ± 60	490 ± 10	35
15K(10)	n.c.	n.m.	n.m.	n.m.	n.m.	n.m.
15K(20)	74 ± 2	3390 ± 50	1150 ± 70	3130 ± 60	1100 ± 200	55
15K(30)	88 ± 1	2300 ± 120	770 ± 20	2200 ± 130	760 ± 20	51

a) Samples are denoted as xxK(yy), were xx refers to the M_n of the selected OEG-OPG-OEG block copolymer, and (yy) to the monomer concentration in wt% of the precursor solutions, b) gel content G, n.c. = not crosslinked, c) Degree of swelling Q, measured in distilled water at 5 °C, d) Q at 50 °C, e) Q in PBS at 5 °C, f) Q in PBS at 50 °C, g) volumetric shrinking calculated from the change in surface area between 5 °C and 50 °C in water, n.m. = not measurable

The swelling kinetics was calculated by determining Q at 5 °C at different time intervals (see Figure 1). The equilibrium degree of swelling was determined at 5 °C and 50 °C in distilled water as well as in PBS (See Table 1). 8K(yy) networks reached their equilibrium Q within 2 h and 15K(yy) in 3 h, probably due to the greater volume of water absorbed. 15K(yy) samples could swell up to 3400% and to a higher extent than 8K(yy) ones, up to 1740%. The swellability in PBS was slightly lower than in water, but in a comparable range. However, an increasing monomer concentration of the precursor solution directly influenced the swellability, with decreasing

values, due to the resulting tightening of the networks. In addition, at 5 °C, the value of *Q* was for all samples twice as high as the value at 50 °C.

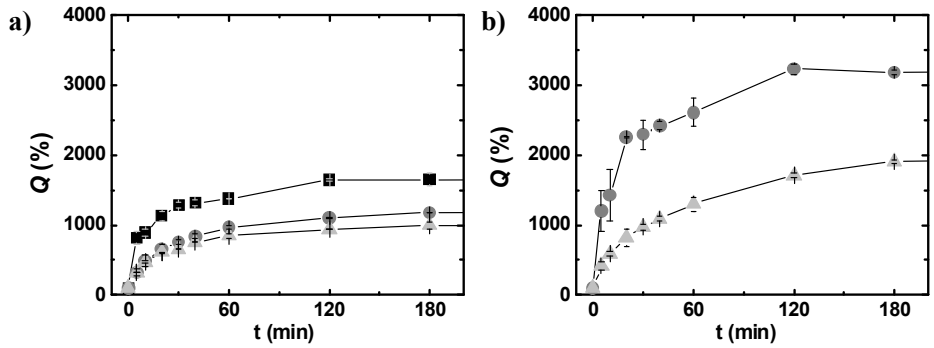


Figure 1: Swelling kinetics of dried OEG-OPG-OEG based hydrogels in distilled water at 5 °C. a) 8K(yy), ■ 8K(10), ● 8K(20), ▲ 8K(30), b) 15K(yy), ● 15K(20), ▲ 15K(30).

The difference of swellability of factor 2 measured by *Q* was also translated in a volumetric contraction (Figure 2). The relative contractions calculated from the surface area are recorded in Table 1. As this phenomenon has been reported for thermo-sensitive hydrogels, e.g based on PNIPAm [7], it is assumed that the volumetric contraction is the result of loss of hydrophilicity of OPG-blocks at increased temperatures, inducing the formation of hydrophobic domains and tightening the network. It could be observed that the volumetric shrinking was higher for polymer networks or hydrogels prepared from lower monomer concentration and for 15K based hydrogels. The hydrogels from precursor with a higher M_n resulted in a loose network with higher flexibility in terms of swellability.

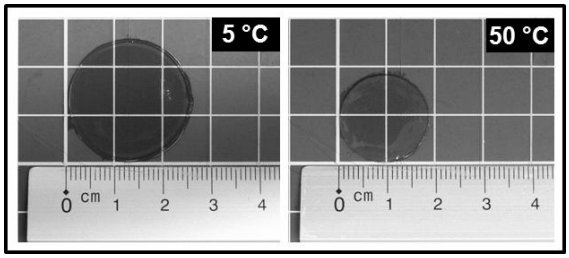


Figure 2: Photographs of a hydrogel of composition 8K(10), after immersing in a water bath at 5 °C or 50 °C.

Mechanical properties

The mechanical strength of the hydrogels was investigated by rheology, from which the storage modulus *G'* and loss modulus *G''* were determined as a function of frequency and temperature. Figure 3 shows that all hydrogels exhibited a typical behavior of covalently crosslinked hydrogels, with *G'* > *G''* and both moduli showing rather independent values from the frequency. In addition, a systematic correlation between mechanical properties and monomer concentration with increasing moduli with increasing precursor content could be observed, e.g. for 15K(30) *G'* appeared in the range of 1 kPa while for 15K(20), values were found at ~0.2 kPa. The same

tendency was observed for 8K(yy) networks. A systematic comparison between *G'* and *G''* of hydrogels prepared from OEG-OPG-OEG block copolymer from different M_n but with similar precursors concentration showed that 15K(yy) samples were of lower mechanical strength than 8K(yy) gels, which is in good correlation with its higher swellability as well as with our previous study [6].

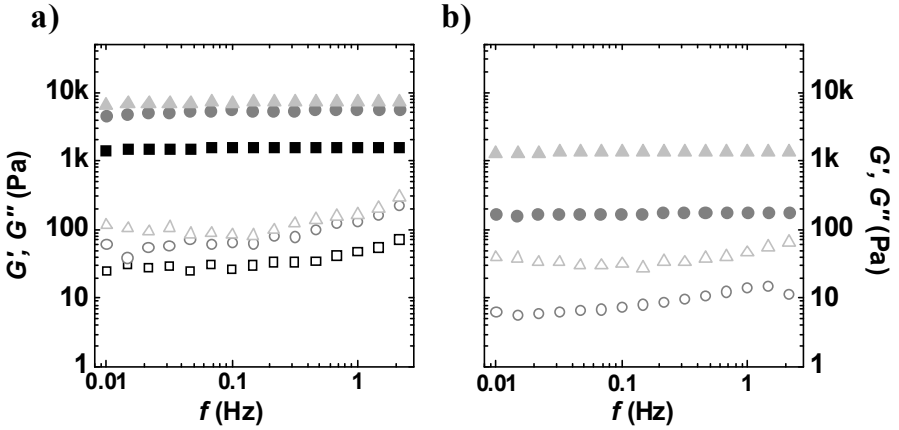
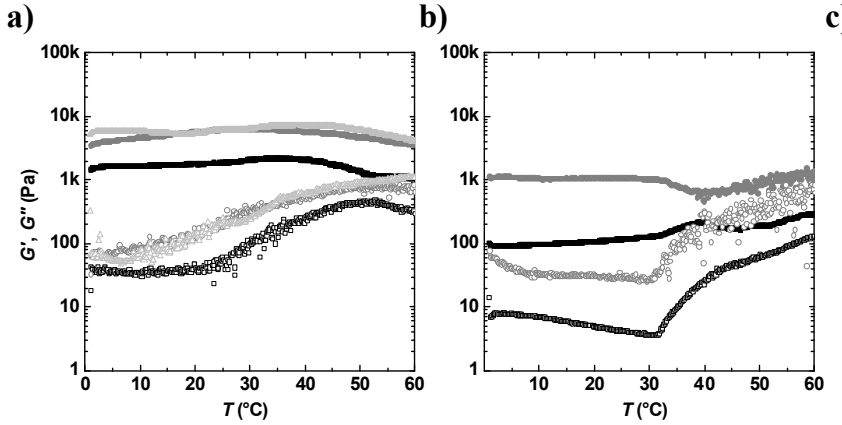


Figure 3: Rheological investigation, frequency dependencies of *G'* and *G''* of a) 8K and b) 15K-based hydrogels, measured at 5 °C. Solid symbols: *G'*, open symbols: *G''*, ■ 8K(10), ● 8K(20), ▲ 8K(30), b) 15K(yy), ● 15K(20), ▲ 15K(30).

As the linear oligomers are thermo-sensitive, it was explored whether this thermo-sensitivity could also influence the mechanical properties of the crosslinked hydrogel networks (Figure 4). All samples exhibited an increase of *G''* with augmenting temperature. On the other hand, the transition of *G'* with temperature could not be so clearly observed. This transition corresponds to the formation of hydrophobic domains when the temperature is increasing, thus inducing a deswelling and a reduction of flexibility of the networks, as described previously [8]. Such behavior has also been observed in PNIPAm-based hydrogels [9]. All samples displayed a transition of *G'* with augmenting temperature of about one order of magnitude, e.g. for 8K-based hydrogels, *G''* went from a 10-100 Pa range to approximately 1 kPa. In general, compared to 8K(yy) hydrogels, a sharper transition at higher temperatures could be observed for 15K(yy) gels, which can be correlated to a higher degree of flexibility in the material, also noticed from the more significant volumetric contractions of those networks.



c)

Sample ID	$T^{beg}_{trans\ (G'')}$	$T^{end}_{trans\ (G')}$
	[°C]	[°C]
8K(10)	20 ± 3	45 ± 2
8K(20)	10 ± 2	46 ± 3
8K(30)	9 ± 2	46 ± 2
15K(10)	n.m	n.m
15K(20)	32 ± 1	n.m
15K(30)	31 ± 1	n.m

Figure 4: Rheological investigation as temperature dependency of *G'* (solid symbols) and *G''* (open symbols). a) 8K(yy) networks, ■ 8K(10), ● 8K(20), ▲ 8K(30), b) 15K(yy), ● 15K(20), ▲ 15K(30). c) Table of the temperatures corresponding to the beginning and the end of the transition of *G''*, n.m. = not measurable.

Conclusions

Hydrogels networks based on thermo-sensitive OEG-OPG-OEG block copolymers of two different M_n and similar hydrophilicity by a OEG content of 80 wt% were successfully synthesized in several concentrations via photo-irradiation of the respective dimethacrylate telechelic oligomers. The resulting samples were homogeneous and translucent, with storage moduli in the range of $G' \sim 1$ kPa. Hydrogels prepared from lower M_n and/or more concentrated precursor solutions displayed a lower swellability and greater mechanical stability (1 to 10 kPa). All samples exhibited thermo-sensitivity with volumetric shrinking and improved mechanical stability with increasing temperature. However, hydrogel networks prepared from M_n = 14600 g·mol^{-1} showed larger and sharper transitions in terms of swellability, volume shrinking and loss modulus. These observations can be attributed to the higher mechanical flexibility of those hydrogels, and therefore to a higher degree of freedom of the crosslinked monomers to form hydrophobic domains when the temperature is increased, which complements the results of our previous study [6]. Such hydrogels fabricated from biocompatible monomers and with demonstrated tunable properties and thermo-sensitivity at physiological temperatures can be considered as relevant candidates for thermo-sensitive drug delivery system.

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