



Stereocomplexed 8-armed poly(ethylene glycol)–poly(lactide) star block copolymer hydrogels: Gelation mechanism, mechanical properties and degradation behavior

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ABSTRACT

Mixing aqueous poly(ethylene glycol)–poly(D-lactide) and poly(ethylene glycol)–poly(L-lactide) star block copolymer solutions resulted in the formation of stereocomplexed hydrogels within 1 min. A study towards the mechanism of the temperature dependent formation of stereocomplexes in the hydrogels using rheology and nuclear magnetic resonance experiments revealed that the formation of stereocomplexes is facilitated at higher temperatures, due to rearrangement in the micellar aggregates thereby exposing more PLA units available for stereocomplexation. The formed gels became temperature irreversible due to the presence of highly stable semi-crystalline stereocomplexed PLA domains. An enantiomeric mixture of 8-armed star block copolymers linked by an amide group between the poly(ethylene glycol) core and the poly(lactide) arms (PEG–(NHCO)–(PLA)₈) yielded hydrogels with improved mechanical properties and stability at 37 °C in PBS compared to 8-armed star block copolymers linked by an ester group. The possibility to be formed in situ in combination with their robustness make PEG–(NHCO)–(PLA)₈ hydrogels appealing materials for various biomedical applications.

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1. Introduction

Hydrogels are polymer networks which are able to swell considerably and retain large amounts of water in their swollen structures [1–3]. Biodegradable poly(ethylene glycol)–poly(lactide) (PEG–PLA) type hydrogels generally exhibit excellent biocompatibility and are accordingly of interest for biomedical applications such as tissue engineering and systems for controlled delivery of biologically active agents. Recently, much effort has been devoted to hydrogels that can be formed in situ under physiological conditions. In situ forming hydrogel systems are injectable fluids that can be introduced into any tissue, organ or body cavity in a minimally invasive manner prior to gelation [4]. In situ forming hydrogels offer several advantages over systems that have to be

formed into their final shape before implantation: there is no need for surgical procedures, their initially flowing nature ensures proper shape adaptation as well as a good fit with surrounding tissue, and biologically active species such as cells or growth factors can be incorporated homogeneously in the hydrogel by simple mixing with the precursor polymer solution. Both chemical and physical crosslinking can be used to create three-dimensional networks in situ. Chemical crosslinking in general provides mechanically stable gels, but care has to be taken of health risks associated with reactive macromonomers and crosslink agents, and practical limitations concerning reaction initiation. In the development of physically crosslinked hydrogels stereocomplexation between enantiomeric PDLA and PLLA blocks in amphiphilic copolymers was shown to be a powerful tool to provide in situ forming gels.

The architecture of stereocomplexed PLA–PEG type block copolymers has a large influence on their solution and gel properties [5–7]. Whereas stereocomplexed AB diblock and ABA triblock copolymers (with A the PLA block and B the PEG block) show irreversible sol–gel transitions, BAB triblock copolymers appear thermo-reversible. They also showed that in general stereocomplex formation cannot be directly related to gel formation. Mixing

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enantiomeric AB copolymers affords hydrogels that upon heating to the sol state do not return to the gel state because of formation of a micellar system in which the micelles have cores composed of highly stable stereocomplexed PLA blocks. The ABA copolymers form gels that upon heating reorganize with the formation of stereocomplexed domains with no gel to sol transition at high or low temperatures. A stereocomplexed gel obtained from a BAB copolymer at high concentration, on the other hand, is thermo-reversible. Thermo-reversibility here is possible because of the presence of bridging molecules between stereocomplexed aggregates. Hydrogels based on stereocomplexation of PLA blocks were mainly studied for their application as a drug delivery system. Li et al. prepared stereocomplexed hydrogels from enantiomeric AB diblock and ABA triblock copolymers [8]. From these hydrogels, a controlled release of bovine serum albumin was achieved up to 15 d. Star-shaped block copolymers of PEG and aliphatic polyesters, showing stereocomplex mediated gelation [9], have also been investigated as a drug delivery system. It was shown that the release of the relatively small protein lysozyme followed first order kinetics and approximately 90% was released in 10 d [10]. The relatively large protein immunoglobulin G could be released from stereocomplexed hydrogels with nearly zero order kinetics and 50% was released in 16 d. As a consequence of a labile ester linkage between PEG and PLA these hydrogels showed limited hydrolytic stability. The stereocomplex mediated gelation mechanism was not investigated.

PEG-(NHCO)-(PLLA)₈ star block copolymers, possessing an amide linkage between PEG and PLA, yield thermo-reversible, single enantiomer hydrogels with good mechanical properties and improved stability towards hydrolytic degradation compared to PEG-(OCO)-(PLLA)₈ star block copolymers having an ester linkage between PEG and PLA blocks [11]. Based on these data, stereocomplexed hydrogels prepared from PEG-(NHCO)-(PLA)₈ were expected to have further improved properties. In this paper, the physical, mechanical and degradation properties of stereocomplexed PEG-(NHCO)-(PLA)₈ hydrogels are described. Furthermore, the mechanism of the temperature dependent formation of stereocomplexes is studied in detail by NMR and rheology. To the best of our knowledge, this is the first paper in which a gelation mechanism is presented at a macromolecular level for star-shaped PEG-PLA diblock copolymers that form a hydrogel by stereocomplexation.

2. Experimental section

2.1. Materials

Hydroxyl terminated 8-armed poly(ethylene glycol) (PEG-(OH)₈, M_n , NMR = 20,600 g/mol and 23,700 g/mol) was purchased from Jenkem (Allen, Texas, USA) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. The PEG-(OH)₈ was converted in PEG-(NH₂)₈ as described previously [11]. D-Lactide and L-lactide were obtained from Purac (Gorinchem, The Netherlands) and used as received. Tin(II) 2-ethylhexanoate (stannous octoate), methanesulfonyl chloride (mesyl chloride), triethylamine (TEA) and 25% aqueous ammonia solution were all from Sigma-Aldrich (St Louis, Missouri, USA). Dichloromethane, TEA and toluene were dried over calcium hydride, potassium hydroxide and sodium, respectively, and distilled prior to use.

The 8-armed poly(ethylene glycol)-poly(D-lactide) and poly(ethylene glycol)-poly(L-lactide) star block copolymers (PEG-(NHCO)-(PDLA)₈ and PEG-(NHCO)-(PLLA)₈) were synthesized by ring opening polymerization of D-lactide and L-lactide, respectively, in toluene at 110 °C. Amine terminated 8-armed star

PEG (PEG-(NH₂)₈) and stannous octoate were used as initiator and catalyst, respectively. This procedure was described in detail previously for PEG-(NHCO)-(PLLA)₈ [11].

2.2. Characterization

¹H NMR (300 MHz) spectra were recorded on a Varian Inova 300 NMR spectrometer. Polymers were dissolved in CDCl₃ at a concentration of 15 mg/ml. Thermal properties of polymers were determined using differential scanning calorimetry (DSC). Heating and cooling rates of 20 °C/min were applied. Samples were heated from 25 to 200 °C, kept at 200 °C for 1 min, cooled to -50 °C, kept at -50 °C for 1 min, and finally heated to 200 °C. Crystallization temperatures (T_c) and corresponding enthalpies (ΔH_c) were obtained from the cooling scan, while melting temperatures (T_m) and corresponding enthalpies (ΔH_m) were obtained from the second heating scan.

2.3. Aggregation in aqueous solutions

The critical association concentration (CAC) values of aqueous solutions of the PEG-PLA star block copolymers were determined by a fluorescence technique using pyrene as a probe [12]. In a hydrophobic environment such as a micellar core, pyrene molecules exhibit a much stronger fluorescence than in a hydrophilic environment, leading to a redshift of their excitation spectrum. The onset of this redshift relates to the CAC. Mixed enantiomer solutions (D/L ratio 1/1) of star block copolymers in the concentration range of 0.0025–2 w/v % were prepared using distilled water. An aqueous solution of pyrene was prepared at a concentration of 1.2×10^{-6} M. Approximately 100 μ l of polymer solution was added to a well in a 96-well culture plate, followed by addition of 100 μ l of the pyrene solution. Fluorescence spectra were obtained with a Tecan Safire microplate reader at room temperature. The detection wavelength was set at 383 or 390 nm. The fluorescence intensity ratio (I_{326}/I_{311} or I_{328}/I_{314}) of pyrene excitation spectra was plotted against the logarithm of the polymer concentration and the intercept of the extrapolated straight lines was taken as the CAC.

Dynamic light scattering (DLS) of dilute solutions (0.5 w/v %) of mixed enantiomer PEG-PLA star block copolymers (D/L ratio 1/1) in water was performed to determine aggregate sizes. Experiments were carried out at 25 °C using a Malvern Nano ZS, a laser wavelength of 633 nm and a scattering angle of 173°.

2.4. Gel properties

The vial tilting method was used to determine the critical gel concentration (CGC) at room temperature as well as the gel to sol transition of PEG-PLA star block copolymers. Separate enantiomeric solutions were prepared by adding distilled water to an appropriate amount of D- or L-enantiomer. Repeated heating cycles were necessary to dissolve the polymers. The enantiomeric solutions were combined and the resulting hydrogel was allowed to equilibrate for 24 h. The reversible gel to sol transition of polymer solutions containing the D- and L-enantiomer in equal amounts was studied in the range of 2–80 °C with temperature increments of 2 °C. At each temperature, the sample was allowed to equilibrate for 10 min. If there was no flow after turning the vials 90° for 1 min, the sample was regarded as a gel; otherwise it was regarded as a sol.

¹H NMR experiments on aqueous solutions and gels were carried out on a Bruker AMX-300 WB spectrometer working at 300.13 MHz for proton and 46.04 MHz for deuterium, using a 5 mm probe head. The samples, containing 10 w/v % of PEG-(NHCO)-(PLLA₁₁)₈ or a 1:1 mixture of the two enantiomers, were prepared by dissolving the appropriate amounts of copolymer in D₂O (99.98% D, Eurisotop);

repeated heating cycles were necessary to obtain homogeneous samples. ^1H NMR spectra were recorded on all samples at 25, 40, 55 and 70 °C using a 90° pulse of 5.7 μs and a recycle delay ranging from 5 to 30 s, depending on sample and temperature, and acquiring 64 scans. Relative peak intensities within each spectrum were determined by integration of the peaks and were then calibrated using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) at known concentration as a standard. DSS was also used as reference for the ^1H chemical shifts. Spin-lattice relaxation times (T_1) of polymer protons were determined in the 10 w/v % samples at 25, 40, 55 and 70 °C, using the inversion recovery pulse sequence with a recycle delay ranging from 5 to 30 s, depending on sample and temperature, and acquiring 32 scans. The baseline corrected areas of the peaks ($I(t)$) at different values of the characteristic delay of the pulse sequence were measured and T_1 values were determined by fitting the inversion recovery curve using the equation:

$$I(t) = I_{\infty} (1 - \alpha \exp(-t/T_1)) \quad (1)$$

where I_{∞} is the signal intensity at time ∞ and α is a parameter accounting for non-complete magnetization inversion [13]. ^2H inversion recovery experiments were also performed on 10 w/v % samples at 25, 40, 55 and 70 °C, using a 9.5 μs 90° pulse, a recycle delay of 8 s, and acquiring 8 scans. The area of the baseline corrected D_2O peak was measured in the spectra and used to determine water ^2H T_1 values through equation (1). For all experiments the temperature was controlled within 0.1 °C.

Oscillatory rheology experiments were performed to determine the mechanical properties of stereocomplexed hydrogels. The storage (G') and loss (G'') modulus of hydrogels were monitored at temperatures ranging from 20 to 60 °C on an Anton-Paar Physica MCR 301 rheometer. To determine the kinetics of gel formation, separate solutions of D-enantiomer and L-enantiomer (D/L ratio 1/1) were mixed, homogenized and quickly applied to the rheometer. Experiments were performed using a flat plate measuring geometry (diameter 25 mm, gap 0.3 mm) utilizing a strain of 0.5% and a frequency of 1 Hz. To prevent water evaporation, a solvent trap was placed over the geometry. To confirm that the strain is within the viscoelastic regime, an amplitude sweep from 0.1 to 10% was performed at 1 Hz.

2.5. Degradation

Gravimetric degradation/dissolution experiments were performed to determine the stability of stereocomplexed hydrogels

which had been subjected to repeated heating cycles. On top of 0.5 ml of hydrogel 3 ml of PBS was placed and the samples were kept at 37 °C. At regular time intervals, 2.5 ml of the supernatant was removed and replaced by fresh buffer. The supernatant samples, taken at various time points, were freeze-dried and the mass of solubilized polymer was determined gravimetrically by subtracting the mass of the PBS salts from the mass of the sample. Degradation/dissolution experiments were performed in duplo. The structure of the remaining polymers in hydrogel samples was analyzed by ^1H NMR spectroscopy. After freeze-drying of a sample, polymers were dissolved in a minimal amount of dichloromethane and the solution was precipitated in an excess of a mixture of cold diethyl ether and methanol (20/1 v/v). The precipitate was collected by filtration, dried under a nitrogen flow and analyzed by ^1H NMR. Oscillatory rheology was used to investigate the mechanical properties of the hydrogels upon degradation by determining G' and G'' of hydrogels as described above.

3. Results and discussion

3.1. Synthesis and characterization of PEG–PLA star block copolymers

Enantiomeric PEG–(NHCO)–(PDLA) $_8$ and PEG–(NHCO)–(PLLA) $_8$ star block copolymers were prepared by the stannous octoate catalyzed ring opening polymerization of D-lactide and L-lactide, respectively, initiated by PEG–(NH $_2$) $_8$ (M_n , NMR = 23,700 g/mol) (Fig. 1). The 8-armed PEG–(OCO)–(PDLA) $_8$ and PEG–(OCO)–(PLLA) $_8$ star block copolymers were prepared analogously, using PEG–(OH) $_8$ (M_n , NMR = 20,600 g/mol) as the initiator (Fig. 1) [9].

Star block copolymers with a degree of polymerization (DP) of 11 or 13 lactyl units per PLA arm were selected (Table 1), because higher DPs of the PLA arms led to a decreased aqueous solubility whereas lower DPs would hamper stereocomplex formation [14]. The aqueous solubility of the 8-armed PEG–(NHCO)–(PLA) $_8$ block copolymers appeared higher than that of the 8-armed PEG–(OCO)–(PLA) $_8$ block copolymers. Whereas the former gave transparent solutions and gels at room temperature up to high concentrations (<20 w/v %) the latter type copolymer solutions and gels always appeared opaque.

The thermal properties of enantiomeric mixtures of the block copolymers as determined with differential scanning calorimetry (DSC) revealed major transitions in both second heating and

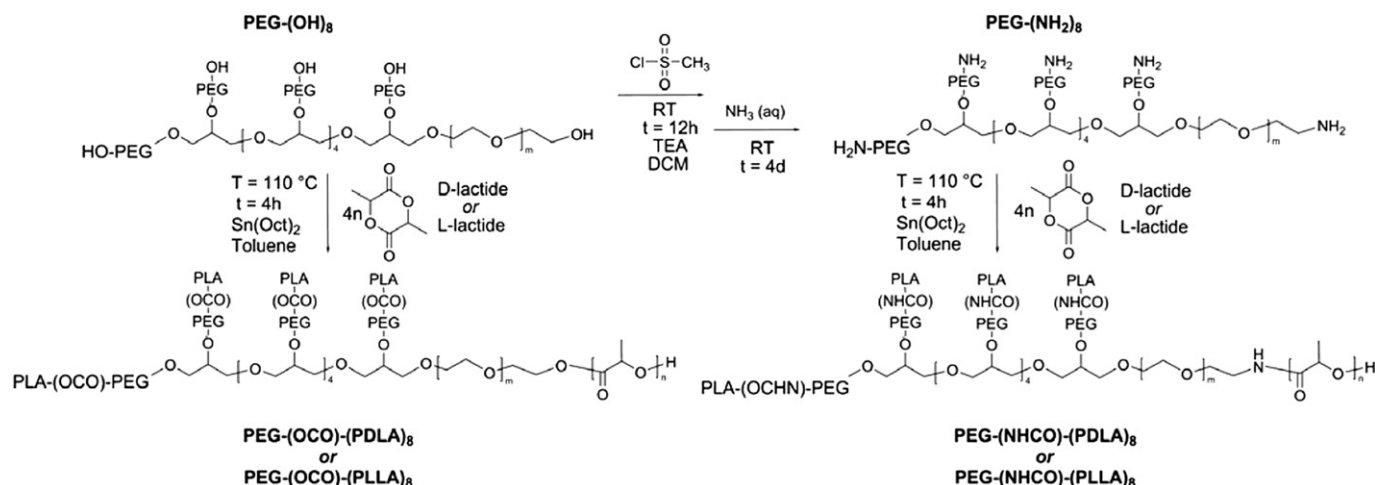


Fig. 1. Synthesis scheme for the preparation of PEG–(NHCO)–(PLA) $_8$ and PEG–(OCO)–(PLA) $_8$ star block copolymers.

Table 1
Molecular weights and thermal properties of PEG initiators and PEG-PLA star block copolymers.

Polymer	¹ H NMR		<i>T_m</i> (°C)	ΔH_m (J/g)	<i>T_c</i> (°C)	ΔH_c (J/g)	CAC (w/v %)	CGC (w/v %)
	DP ^a	<i>M_n</i> (kg/mol)						
PEG-(NH ₂) ₈	—	23.7	53	126	28	126	—	—
PEG-(NHCO)-(PDLA ₁₁) ₈ + PEG-(NHCO)-(PLLA ₁₁) ₈	10.8	29.9	41	56	13	57	0.20	5
	12.0	30.6						
PEG-(NHCO)-(PDLA ₁₃) ₈ + PEG-(NHCO)-(PLLA ₁₃) ₈	13.1	31.2	40	55	3	55	0.10	4
	13.3	31.4						
PEG-(OH) ₈	—	20.6	54	141	31	141	—	—
PEG-(OCO)-(PDLA ₁₃) ₈ + PEG-(OCO)-(PLLA ₁₃) ₈	13.3	28.3	40	56	6	56	0.06	14
	13.3	28.3						

^a Degree of polymerization of the PLA blocks, expressed in lactyl units per arm.

cooling scans, corresponding to melting and crystallization of the PEG domains, respectively (Table 1). All enantiomeric mixtures of the PEG-PLA star block copolymers exhibit lower melting transitions and accompanying enthalpies than their PEG precursors. Apparently, the crystallization of PEG is hampered by the presence of PLA blocks. Only in case of the stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ copolymers, a small endothermic peak was observed at ~165 °C. This transition, which can be attributed to melting of stereocomplex crystals, was not observed for stereocomplexed PEG-(OCO)-(PLA)₈. Transitions of stereocomplexes of low molecular weight polyester blocks with a DP up to 13 are well below 200 °C. Only moderate to high molecular weight PLA stereocomplexes show melting above 200 °C. Also preliminary scans up to 230 °C showed no transitions.

3.2. Gelation behavior

The single enantiomer block copolymers show critical gel concentrations at 10–12 w/v % for PEG-(NHCO)-(PLA_n)₈ (DP 11–13 lactyl units) and 20 w/v % for PEG-(OCO)-(PLA₁₃)₈. At these concentrations, transitions to the sol state upon temperature increase take place at 30–40 °C [11]. To study the gel to sol transitions of the stereocomplexed 8-armed block copolymers, D- and L-enantiomers were dissolved separately in distilled water in tightly capped vials. The enantiomeric solutions were combined and the resulting hydrogel was allowed to equilibrate for 24 h. Subsequently the gel to sol phase transitions were determined with the vial tilting method in the range of 2–80 °C (Fig. 2).

The mixed enantiomer solutions of PEG-(NHCO)-(PLA_n)₈ formed a hydrogel almost instantaneously at low concentrations whereas the gelation times for mixed enantiomer solutions of PEG-(OCO)-(PLA₁₃)₈ were approximately 30 min at higher concentrations. Upon heating, a gel to sol transition could be observed but it was limited to a narrow concentration range of ~4–8 w/v % for stereocomplexed PEG-(NHCO)-(PLA_n)₈ and ~14–18 w/v % for stereocomplexed PEG-(OCO)-(PLA₁₃)₈. At higher concentrations no gel to sol transition was observed up to the boiling temperature of water, whereas at lower concentrations no gels were formed. Repeating the heating and cooling cycle revealed a progressive increase in temperature of the gel to sol phase transition leading eventually to an irreversible gel.

Combining all the above results some general conclusions can be drawn: i) stereocomplexation leads to gel formation at lower concentrations and gel to sol transitions at higher temperatures compared to single enantiomer 8-armed PEG-PLA star block copolymers, ii) an increase in polymer concentration shifts the gel to sol transition to higher temperatures as a result of an increased physical crosslink density, iii) increasing the DP of the hydrophobic block shifts the gel to sol transition to higher temperatures, which is likely a result of the more facile formation of stereocomplexed

crystals at higher DP, iv) replacing the ester group with an amide group as linking unit between the hydrophilic PEG and hydrophobic PLA block gives a shift of the boundary curve to lower concentrations indicating an even more efficient interaction between PLA chains, v) hydrogels formed from stereocomplexed 8-armed block copolymers become irreversible after repeated heating and cooling cycles due to the slow formation of stereocomplexed crystals.

3.3. Aggregate size and size distribution in dilute aqueous solution

Depending on the block lengths, solvent applied and charges incorporated, block copolymers may form different associates like micelles, micellar aggregates and polymersomes [15–20]. The critical association concentration (CAC) values of aqueous solutions of the PEG-PLA star block copolymers were determined using pyrene as a fluorescent probe [12]. The CAC values of the single enantiomers of the 8-armed block copolymers (0.20–0.50 w/v %) are in general somewhat higher than the values of the corresponding stereocomplexed PEG-(PLA)₈ (Table 1). The CAC value decreases at higher PLA block length as a result of increased hydrophobic interactions. Dynamic light scattering (DLS) experiments revealed that two size distributions are present in dilute aqueous solutions of mixed enantiomers (Fig. 3). The results show that above the CAC these materials form small aggregates with

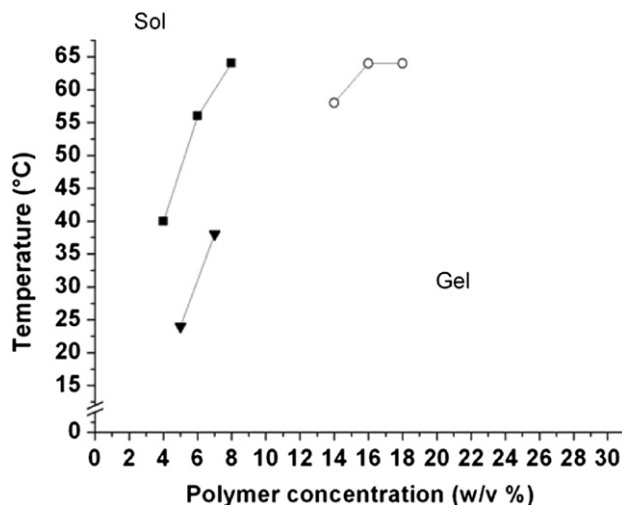


Fig. 2. Gel to sol transition temperatures of stereocomplexed PEG-PLA hydrogels (D/L = 1/1). PEG-(NHCO)-(PLA₁₁)₈ (▼), PEG-(NHCO)-(PLA₁₃)₈ (■), PEG-(OCO)-(PLA₁₃)₈ (○). The transition temperatures were determined after combining enantiomeric solutions and equilibration for 24 h at room temperature.

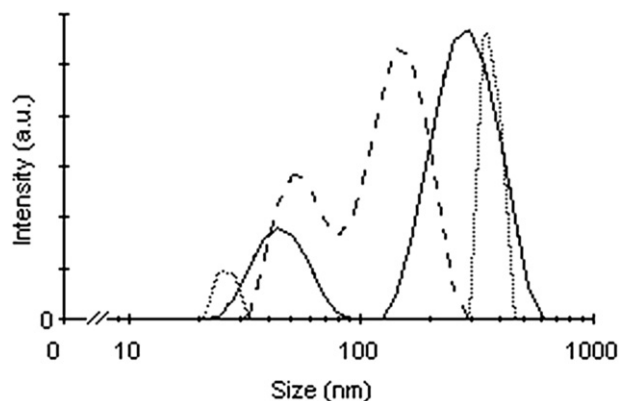


Fig. 3. Aggregate size distributions in 0.5 w/v % aqueous solutions containing mixed enantiomers at room temperature. PEG-(NHCO)-(PLA₁₁)₈ (solid line), PEG-(NHCO)-(PLA₁₃)₈ (dashed line), PEG-(OCO)-(PLA₁₃)₈ (dotted line).

diameters ranging from 20 to 50 nm, indicative of micellar aggregate type structures, as well as larger aggregates with a diameter between 100 and 400 nm. For all stereocomplexed materials the size distribution is shifted to larger aggregates compared to the single enantiomers [11]. The enhanced tendency of these stereocomplexed block copolymers to form aggregates compared to single enantiomer copolymers, as shown by the lower CAC values and the larger aggregate sizes, may be ascribed to the strong interaction between PLLA and PDLA blocks [21].

3.4. NMR spectroscopy on aqueous solutions and hydrogels

¹H NMR spectroscopy was employed to investigate the aggregation behavior of stereocomplexes in water in comparison with that of the single enantiomers. To this aim ¹H NMR spectra were recorded at different temperatures (i.e. 25, 40, 55, and 70 °C) on 10 w/v % samples of PEG-(NHCO)-(PLLA₁₁)₈ and 1:1 mixtures of the two copolymers in D₂O (Fig. 4). It should be noted that this concentration is below the CGC for the single enantiomer and above the CGC for the mixed enantiomers.

The spectra showed a peak ascribable to the PEG methylene protons at 3.70 ppm, several signals ascribable to the PLA methyl protons in the spectral region between 1.3 and 1.6 ppm (Fig. 4) and signals arising from the PLA methine protons in the regions 4.2–4.5 and 5.0–5.3 ppm, as well as the signal of HDO at approximately 4.8 ppm. At 25 °C the ¹H NMR spectrum of the 1:1 PEG-(NHCO)-(PDLA₁₁)₈/PEG-(NHCO)-(PLLA₁₁)₈ mixture in D₂O was characterized by broader lines in comparison with the single enantiomer due to the formation of the gel phase. For both the single enantiomer and the mixed enantiomers all PEG methylene protons were detected at all temperatures, whereas the fraction of detected PLA protons was always lower than that expected on the basis of stoichiometry with respect to the PEG methylene protons. This behavior is due to the formation of solid-like aggregates of PLA chains which prevents the observation of all PLA protons in solution state NMR experiments. The PLA proton signals detected at room temperature were attributed, on the basis of their chemical shift and trend with temperature, to chains dangling in water and to chains in micellar aggregates; the increase in intensity with temperature observed for the single enantiomers could be ascribed to a progressive increase in PLA chain mobility within intermicellar aggregates [22], which were also observed in DLS experiments (vide supra). In the case of the single enantiomers at concentrations below (Fig. 4a) and above

[11,22] the CGC, the amount of detected PLA protons increased from 23 to 73% and from 31 to 86%, respectively, on raising the temperature from 25 to 70 °C. In contrast, the effect of temperature on the amount of observed PLA protons was quite small for the mixed enantiomers (see Fig. 4b). In fact, the fraction of observed PLA units increased from 20 ± 2 to only 28 ± 2% on going from 25 to 70 °C, indicating that in this case the intermolecular PLA aggregates remain solid-like also at 70 °C. The different behavior observed for single enantiomers and stereocomplexes can be ascribed to the different state of PLA chains in the hydrophobic aggregates. For single enantiomers, the PLA domains are mainly amorphous, with a glass transition in the temperature range investigated [23], whereas for the stereocomplex, also crystalline PLA domains are present, with a melting temperature of approximately 165 °C.

Importantly, since no spectral differences were detected for PEG methylene protons between the single enantiomer samples and the mixtures, the formation of a stronger network does not seem to influence the dynamics of the PEG chains. Moreover, the fact that the PEG signal did not show any temperature dependence is a strong indication that PEG entanglements or deswelling do not occur at least in the temperature range investigated. The trends with temperature (25–70 °C) of the PEG ¹H spin-lattice relaxation times (*T*₁) measured on the 10 w/v % samples of the single enantiomers and of the 1:1 mixture in D₂O confirmed that the PEG chains are in all cases well swollen in water and quite mobile. In fact, essentially the same *T*₁ values were determined for PEG methylene protons in all samples at the different temperatures (*T*₁ ranging from 550 ± 10 ms to 1350 ± 10 ms on going from 25 °C to 70 °C), and these values were quite similar to those reported for pure PEG in water [24,25], where the PEG chains undergo fast motions. Finally, water ²H *T*₁ values measured for the same samples in the 25–70 °C temperature range (*T*₁ ranging from 350 ± 10 ms to 990 ± 10 ms on heating) were indicative of a bulk-like behavior of water [22], irrespective of the network formed from a single enantiomer or a mixture of enantiomers.

3.5. Rheology

The storage (*G'*) and loss (*G''*) moduli of stereocomplexed PEG-(NHCO)-(PLA₁₁)₈ and PEG-(OCO)-(PLA₁₃)₈ hydrogels were determined with oscillatory rheology measurements at 20 °C. For all samples, *G'* exceeded *G''*, confirming that the systems were in the gel state [26]. The dependence of *G'* on polymer concentration (Fig. 5) was almost linear, but is shifted to lower concentrations for the stereocomplexed PEG-(NHCO)-(PLA₁₁)₈.

It is apparent from Fig. 5 that at the same concentration hydrogels based on a racemic mixture of PEG-(NHCO)-(PLA₁₁)₈ exhibit a much higher gel stiffness than hydrogels based on a racemic mixture of PEG-(OCO)-(PLA₁₃)₈. As an example, a 16 w/v % stereocomplexed PEG-(OCO)-(PLA₁₃)₈ hydrogel has a storage modulus of 6 kPa, whereas a 16 w/v % stereocomplexed PEG-(NHCO)-(PLA₁₁)₈ hydrogel has a storage modulus of 21 kPa. These results show that replacing an ester bond with an amide bond in the PEG-(PLA)₈ leads to more efficient crosslinking. In comparison with single enantiomer hydrogels at similar polymer concentrations [11], stereocomplexed hydrogels exhibit a 10–15 kPa higher gel stiffness, suggesting a higher effective crosslink density. The increase in *G'* with concentration can be ascribed to the formation of a more densely physically crosslinked network.

Gel formation kinetics were studied by monitoring the storage modulus (*G'*) and loss modulus (*G''*) in time after mixing solutions of macromonomers with opposite chirality. When 6 w/v % PEG-(OCO)-(PDLA₁₃)₈ and PEG-(OCO)-(PLLA₁₃)₈ solutions were

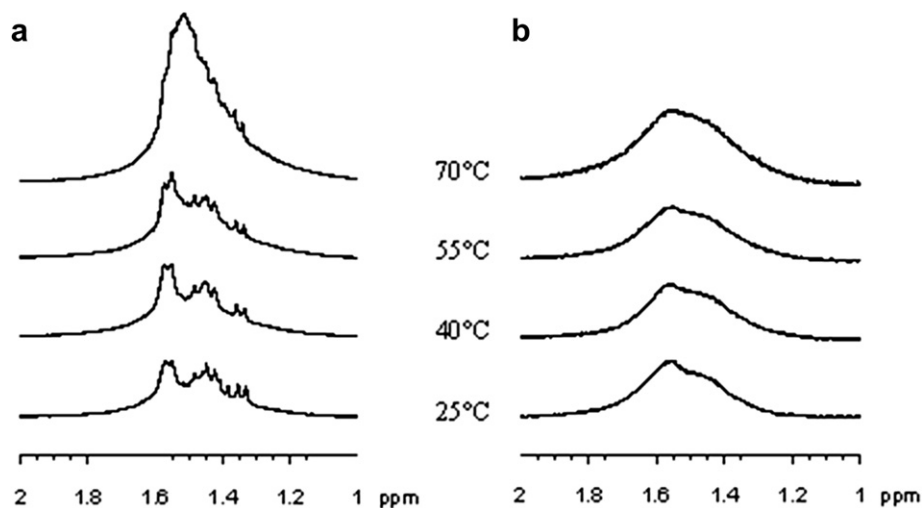


Fig. 4. ^1H NMR methyl spectral region of 10 w/v % samples of PEG-(NHCO)-(PLLA₁₁)₈ (a) and PEG-(NHCO)-(PDLA₁₁)₈ + PEG-(NHCO)-(PLLA₁₁)₈ (b) in D₂O at the indicated temperatures.

mixed, the crossover point of G' and G'' , which can be regarded as the gel point, was observed after 33 min (Fig. 6A and B). The storage modulus levels off after approximately 48 h, showing that gelation is complete. When 6 w/v % PEG-(NHCO)-(PDLA₁₃)₈ and PEG-(NHCO)-(PLLA₁₃)₈ solutions were mixed, G' crossed G'' within 1 min (Fig. 6C and D). Importantly, the stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ hydrogels show much lower damping factors ($\tan \delta = G''/G'$) compared to the stereocomplexed PEG-(OCO)-(PLA₁₃)₈ hydrogels. This indicates a more elastic, i.e. gel-like, behavior of the stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ hydrogels [27]. The faster gelation kinetics and lower damping factor of stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ hydrogels (Fig. 6) and their higher storage moduli (Fig. 5) may be explained by the conformational rigidity of the amide groups, which may result in better phase separation between the hydrophilic and hydrophobic blocks. This enhanced phase separation allows for easier crystallization of PLA stereocomplexes as indicated by the DSC results and thereby a better physically crosslinked network. For all samples G' and G'' showed little variation when an amplitude sweep from 0.1

to 10% was performed at the end of each rheological experiment, indicating that a strain of 0.5% is within the linear viscoelastic range of the hydrogels [28].

To investigate the effect of repeated heating cycles on the gel stiffness, PEG-(NHCO)-(PDLA₁₃)₈ and PEG-(NHCO)-(PLLA₁₃)₈ solutions were mixed at a low concentration of 7 w/v %, equilibrated for 24 h at room temperature and subjected to rheological measurements with temperature profiles as specified in Fig. 7A. During the entire measurement G' exceeded G'' (G' values not shown), showing that the system retained the gel state. After reaching equilibrium, as shown in interval I, G' is constant. Upon heating the gel from 20 to 60 °C (interval II) G' decreases rapidly because physical crosslinks are lost due to an increased mobility of the PLA chains. Keeping the temperature constant at 60 °C for 15 min (interval III), a large increase in G' is observed. This indicates that new crosslinks are formed which are stable even at 60 °C. During cooling from 60 to 18 °C (interval IV) G' increased likely due to crystallization of the PLA domains. Bringing the system to equilibrium a small decrease of G' can be seen which is mainly due to a shift in temperature up to 20 °C. In this temperature cycle G' thus increases to a value 4 times higher than its initial value. Repeating this temperatures cycle results in consecutive increments in G' and the final value is one order of magnitude higher than the initial value (Fig. 7B).

The temperature dependent mechanical properties observed can be illustrated by a mechanism depicted in Fig. 8. In aqueous solution the PEG-(NHCO)-(PDLA)₈ and PEG-(NHCO)-(PLLA)₈ star block copolymers form micellar aggregates consisting of a PLA core and a PEG shell. These aggregates do contain PLA blocks dangling in water [22]. After mixing PEG-(NHCO)-(PDLA)₈ and PEG-(NHCO)-(PLLA)₈ solutions, stereocomplexation of these dangling PLA chains results in interaggregate bridges. The resulting stereocomplexes lead to spontaneous gel formation at room temperature. Upon heating, the increased mobility of the PLA units facilitates interaggregate bridging to a greater extent resulting in the formation of stereocomplexes which are irreversible. Consequently, the increase in the number of crosslinks results in a stiffer gel as shown by the increased storage modulus. Repeated heating cycles result in higher G' values, but the effect becomes progressively smaller (Fig. 7B). Because of the high stability of the stereocomplexes formed a temperature irreversible system is established.

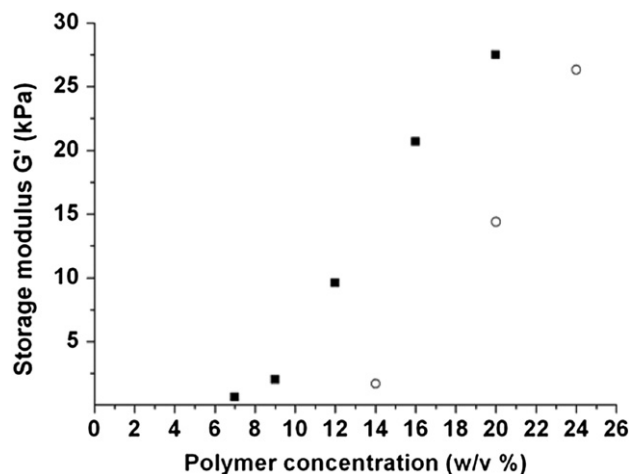


Fig. 5. Storage modulus (G') as a function of the polymer concentration at 20 °C for stereocomplexed PEG-PLA hydrogels. PEG-(NHCO)-(PLA₁₁)₈ (■), PEG-(OCO)-(PLA₁₃)₈ (○).

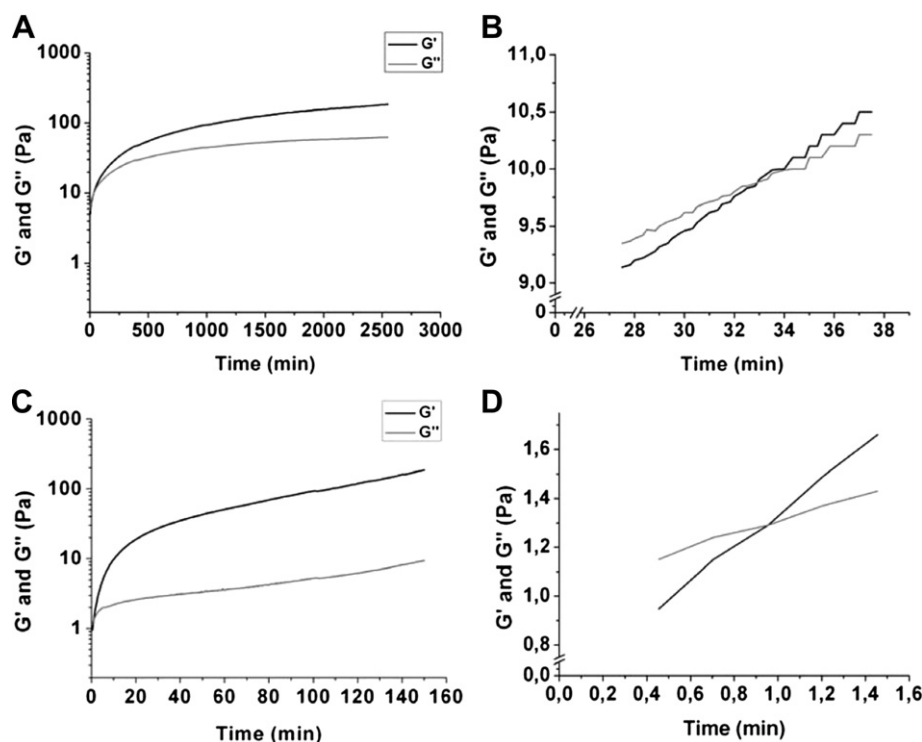


Fig. 6. Storage modulus (G') and loss modulus (G'') as a function of time after mixing 6 w/v % macromonomer solutions of opposite chirality at 20 °C. PEG-(OCO)-(PDLA₁₃)₈ + PEG-(OCO)-(PLLA₁₃)₈ (A, B), PEG-(NHCO)-(PDLA₁₃)₈ + PEG-(NHCO)-(PLLA₁₃)₈ (C, D). Graphs B and D show the crossover points of G' and G'' in graphs A and C, respectively.

3.6. Hydrogel degradation/dissolution

Stereocomplexed hydrogels were prepared by mixing single enantiomer aqueous solutions and a PBS solution was put on top of the gels. The stereocomplexed PEG-(OCO)-(PLA₁₃)₈ hydrogel was completely dissolved within 12 d (Fig. 9). The stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ hydrogel exhibits a significantly higher stability and complete degradation/dissolution is not observed until day 29. Comparing these data to 22 w/v % single enantiomer PEG-(OCO)-(PLA₁₃)₈ and PEG-(NHCO)-(PLA₁₃)₈ hydrogels, which are stable at 37 °C in PBS for 5 and 15 d respectively [11], shows that stereocomplexation prolongs the stability considerably. In general, physically crosslinked hydrogels prepared from amphiphilic block copolymers eventually dissolve because the aggregate packing is disrupted as a result of the concentration difference of the

copolymer in the hydrogel and the surrounding medium. The higher stability of stereocomplex crystallites in comparison to PLLA amorphous aggregates, which act as crosslinks, most likely leads to less facile disruption of the aggregate packing.

Hydrolytic degradation in the PLA arms causes removal of physical crosslinks, leading to increased swelling and eventual dissolution of the hydrogel. ¹H NMR analysis of remaining polymers in degrading hydrogel samples revealed that degradation in stereocomplexed PEG-(OCO)-(PLA₁₃)₈ hydrogels occurs through preferential hydrolysis of the linking PEG-PLA ester groups. In contrast, the linking amide groups in the stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ were found unaffected during the degradation/dissolution experiments. Degradation in these systems occurs by hydrolysis of PLA ester groups as the DP in the PLA arms of the PEG-(NHCO)-(PLA₁₃)₈ hydrogel decreased from 13.3 lactyl

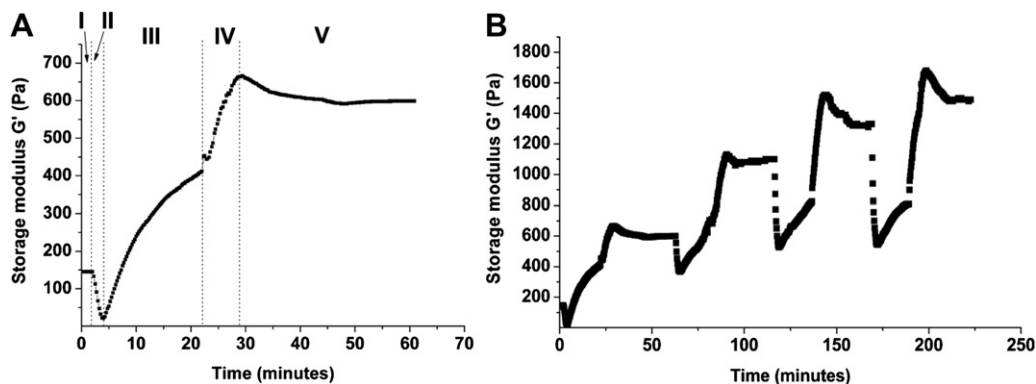


Fig. 7. Storage modulus (G') as a function of time for a 7 w/v % stereocomplexed PEG-(NHCO)-(PLA₁₃)₈ hydrogel. After equilibration at 20 °C (I). Increasing the temperature from 20 to 60 °C (II). Equilibration at 60 °C (III). Cooling to 18 °C (IV). Equilibration at 20 °C (V). A single temperature cycle (A). Four consecutive temperature cycles (B).

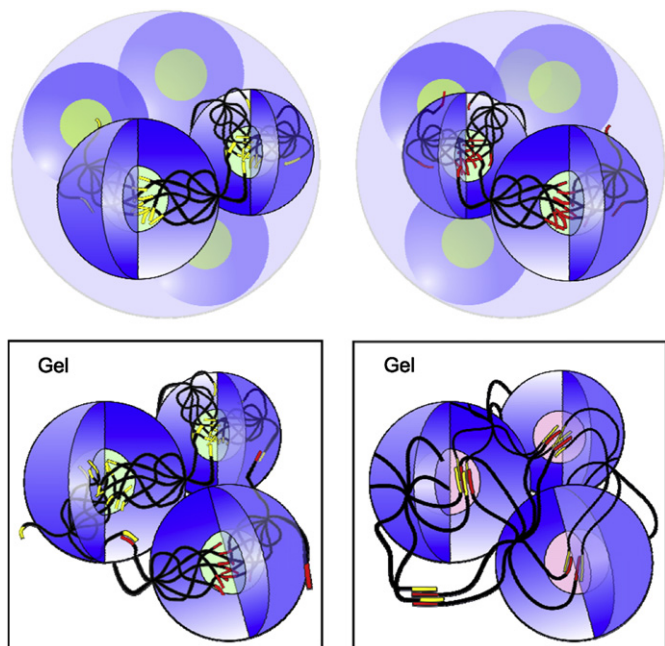


Fig. 8. Schematic representation of micellar aggregates and formation of stereo-complexed gels. In all pictures only a few micelles and molecules are shown for clarity. Top: enantiomeric micellar aggregates of 8-armed PEG–PLA copolymers. Bottom left: after mixing, gelation is caused by stereocomplexation of PLA blocks present in the corona of the micelles. Bottom right: gel with stereo-complexed cores and bridging PEG–PLA molecules.

units at day 0–7.5 lactyl units at day 29. These observations are most likely the reason for the enhanced stability of stereo-complexed PEG–(NHCO)–(PLA)₁₃ hydrogels over stereo-complexed PEG–(OCO)–(PLA)₁₃ hydrogels [11].

Fig. 10 shows that during the degradation/dissolution experiments the storage modulus G' decreases as a result of the degradation and dissolution of the star block copolymer. It should be noted that for some hydrogel samples, additional rheological measurements at later time points could not be performed because of severe hydrogel degradation. The enhanced mechanical properties of the stereo-complexed PEG–(NHCO)–(PLA)₁₃ hydrogels in comparison with PEG–(OCO)–(PLA)₁₃ hydrogels (Fig. 5) are maintained throughout the degradation period.

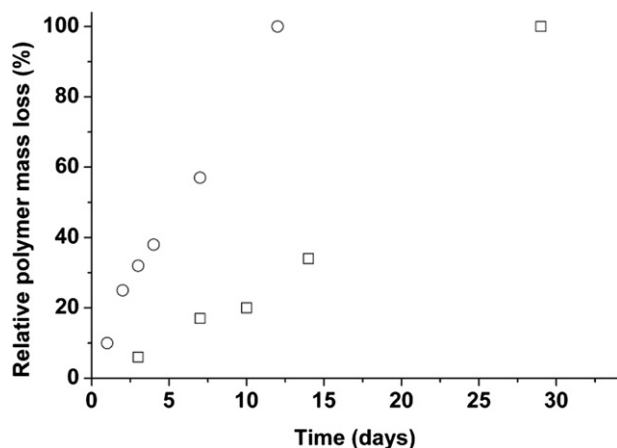


Fig. 9. Relative polymer mass loss from 14 w/v % stereo-complexed PEG–(NHCO)–(PLA)₁₃ (□) and stereo-complexed PEG–(OCO)–(PLA)₁₃ (○) hydrogels in PBS at 37 °C.

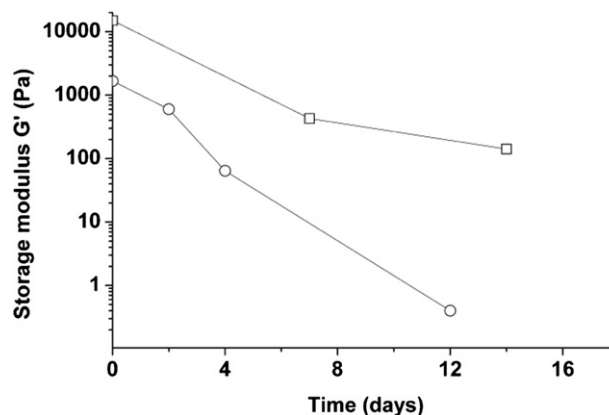


Fig. 10. Storage modulus (G') as a function of degradation time for stereo-complexed PEG–(NHCO)–(PLA)₁₃ hydrogels (□) and stereo-complexed PEG–(OCO)–(PLA)₁₃ hydrogels (○) in PBS at 37 °C.

4. Conclusions

In the present study, the mechanism of the temperature dependent formation of stereo-complexes was elucidated for physically crosslinked hydrogels starting from enantiomeric PEG–PLA star block copolymer solutions. Upon mixing PEG–(PDLA)₈ and PEG–(PLLA)₈ solutions, interaggregate bridging of dangling PLA chains by the formation of stereo-complexes leads to spontaneous gelation. When these gels are heated, rearrangements leading to an increase in the amount of stereo-complexes take place. ¹H NMR experiments revealed that stereo-complexes in the mixed enantiomer PEG–(NHCO)–(PLA)₈ hydrogels are rigid and highly stable up to 70 °C. Stereocomplexation in these systems thus results in a temperature irreversible gel system. Compared to single enantiomer hydrogels of similar concentration, stereo-complexed PEG–(PLA)₈ hydrogels exhibited enhanced mechanical properties and stability at 37 °C in PBS. By changing the linking unit between the hydrophobic and hydrophilic block from an ester to an amide group in the PEG–(PLA)₈ copolymers, hydrogels formed at lower polymer concentrations and showed a larger gel window, improved mechanical properties and a higher stability against hydrolysis. The robust mechanical properties, the possibility to be formed in situ and the stability towards hydrolytic degradation make the stereo-complexed PEG–(NHCO)–(PLA)₈ hydrogels interesting for biomedical applications such as controlled drug delivery systems and matrices for tissue engineering.

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