Robust & thermosensitive poly(ethylene glycol)-poly(ϵ-caprolactone) star block copolymer hydrogels

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Abstract

Novel 8-armed poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) star block copolymers, possessing an amide or an ester group between the PEG core and the PCL arms (PEG_{20K}-(NHCO)-$(PCL_9)_8$ and PEG_{20K}-(OCO)-$(PCL_9)_8$), are synthesized by ring opening polymerization of ε-caprolactone in toluene at 110 °C initiated by 8-armed star PEG_{20K}-$(NH_2)_8$ and PEG_{20K}-$(OH)_8$, respectively. Compared to linear triblock copolymers with a similar hydrophilic/hydrophobic balance and molecular weight, star block copolymers show better aqueous solubility and yield more homogeneous and transparent hydrogels. PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogels exhibit a significantly higher storage modulus and *in vitro* stability in comparison with PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogels of similar concentration and molecular weight. ^{1}H NMR analysis of degrading hydrogel samples clearly demonstrates different degradation mechanisms for the ester and amide type star block copolymers. Their robust mechanical properties, the possibility to be formed *in situ* and their excellent resistance

against hydrolytic degradation make these PEG-PCL star block copolymer hydrogels, especially those based on PEG_{20K}-(NHCO)-$(PCL_9)_8$, appealing for various biomedical applications.

Keywords: Hydrogel, thermosensitive, PEG, PCL, star block copolymer, degradation.

1. Introduction

Hydrogels are water swollen polymer networks whose properties resemble those of natural soft tissues.[1,2] They generally exhibit excellent biocompatibility and are accordingly widely investigated for use in biomedical applications such as tissue engineering and systems for controlled delivery of biologically active agents. Amphiphilic block copolymers may form thermosensitive hydrogels through physical crosslinks such as hydrophobic interactions. Depending on the nature of the blocks, the molecular architecture and the molecular weight, they provide a sol to gel transition upon a decrease or increase in temperature. Such systems offer the possibility of a simple injection when the sol to gel transition is close to body temperature. *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 can be incorporated homogeneously in the hydrogel by simple mixing with the precursor polymer solution.[3] Amphiphilic poly(ethylene glycol)-polyester block copolymers have demonstrated adequate physicochemical properties for the formation of thermosensitive hydrogels. Various polyesters have been employed as the hydrophobic block, predominantly poly(lactide) (PLA) because of its biocompatibility, biodegradability and facile synthesis via the ring opening polymerization of lactide. The first examples of poly(ethylene glycol)-polyester hydrogels were reported in the late 1990s by Choi et al. who synthesized a number of linear AB diblock and ABA triblock copolymers, with A as a hydrophilic poly(ethylene glycol) (PEG) block and B as a hydrophobic PLA block.[4-6] Diblock copolymers were synthesized by ring opening polymerization of lactide initiated by the hydroxyl group of monomethoxy PEG, while triblock copolymers were prepared

by coupling the diblock copolymers with monomethoxy PEG using hexamethylene diisocyanate. In comparison with diblock copolymers possessing the same PEG content and PEG molecular weight, triblock copolymers generally yielded hydrogels at lower polymer concentrations. The thermoresponsive behavior could be tuned by the hydrophilic/hydrophobic balance, the block length, and the stereoregularity of the PLA block.

Besides linear PEG–PLA copolymers, also a number of star-shaped architectures have been explored for the preparation of thermoresponsive hydrogels. Park et al. synthesized 3-armed PLA centered star block copolymers by coupling monocarboxylated PEG to a 3-armed hydroxyl-terminated PLA using dicyclohexylcarbodiimide as coupling agent.[7] The 3-armed star block copolymer exhibited a lower critical gel concentration (CGC) in comparison with a PEG–PLA–PEG triblock copolymer possessing the same PEG content. More recently it was found that 8-armed PEG-PLA star block copolymers, possessing an amide linkage between PEG and PLA, yield hydrogels with improved mechanical properties and a more controlled hydrolytic degradation compared to 8-armed PEG–PLA star block copolymers having an ester linkage between the PEG and PLA blocks.[8,9]

As an alternative to PEG-PLA type hydrogels, Bae et al. prepared ABA and BAB block copolymers based on poly(ε-caprolactone) (PCL) and PEG.[10,11] Copolymers with an appropriate hydrophilic/hydrophobic balance showed both a lower sol-gel transition and an upper gel-sol transition with increasing temperature, which were ascribed to micellar aggregation through hydrophobic interactions and micellar collapse through increased molecular motion of PCL, respectively. Similar to PEG-PLA based systems, PEG and PCL block lengths as well as block topography were found to influence gelation properties. Solutions of PCL-PEG-PCL exhibited a lower critical gelation temperature and a larger gel window compared to PEG-PCL-PEG, probably due to the possibility of intermicellar PCL bridging leading to more facile aggregation. PEG-PCL copolymers with a star-shaped architecture received relatively little attention in comparison with their PEG-PLA analogs. PEG-PCL star block copolymers have mainly been used for the preparation of micelles[12-15] but rarely for hydrogels. Lu et al. prepared 4-armed PEG-PCL star block copolymers by ring opening polymerization

of ε-caprolactone using hydroxyl-terminated 4-armed PEG as initiator.[16] In aqueous solutions the macromonomers self-assembled into micelles at low concentrations and formed physically crosslinked, thermoreversible hydrogels above a concentration of 10 w/v %. No data were reported concerning the mechanical properties or hydrolytic stability of the hydrogels. The group of Liu reported on thermosensitive hydrogels based on 4-arm PEG–PCL block copolymers with porphyrin covalently incorporated as a fluorescence tag.[17] The polymers were prepared by coupling PEG arms onto the carboxylic acid groups of porphyrin using carbodiimide chemistry, followed by ring opening polymerization of ε-caprolactone initiated by the free hydroxyl groups of the PEG arms. At 40 w/v %, the fluorescent PEG-PCL block copolymers exhibited a sol-gel transition at 32 °C and a gel-sol transition around 50 °C. After subcutaneous injection in mice, hydrogels formed *in situ* and could be clearly visualized by fluorescent imaging. These systems showed a relatively low stability as 40 w/v % hydrogels lost 35 % of their mass after 24 hours of immersion in PBS.

In previous research it was found that 8-armed PEG-PLA star block copolymers yield physically crosslinked hydrogels at much lower concentrations compared to linear PLA-PEG-PLA triblock copolymers.[18] Furthermore it was shown that replacing the ester linkages between PEG and PLA for amide linkages results in more robust mechanical properties and a higher *in vitro* stability.[8] Although these systems showed an *in vitro* stability of up to 16 days, for some hydrogel applications longer degradation times are required. Inspired by the promising results for 8-armed PEG-PLA star block copolymers and by the lower degradation rate of PCL compared to PLA, hydrogels from PEG_{20K}-(NHCO)-$(PCL_n)_8$ possessing an amide linkage between PEG and PCL were expected to have excellent mechanical properties and hydrolytic stability. In this paper, the physical, mechanical and degradation properties of a series of 8-armed PEG-PCL star block copolymers hydrogels are described and compared with hydrogels based on analogous linear PEG-PCL block copolymers.

2. Materials and methods

2.1 Materials

Hydroxyl-terminated 8-armed poly(ethylene glycol) (PEG_{20K}-$(OH)_8$, M_n = 20 kg mol^{-1}, degree of polymerization (DP) of 57 ethylene glycol units in each of the 8 PEG arms, Đ = 1.06) was purchased from Jenkem (Allen, Texas, USA) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. ε-caprolactone was obtained from Acros (Geel, Belgium). PEG_{4K}-$(OH)_2$ and PEG_{20K}-$(OH)_2$ (M_n = 4 and 20 kg mol^{-1}; DP = 91 and 454, Đ = 1.06 and 1.11 respectively), 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, toluene and ε-caprolactone were dried over calcium hydride, whereas TEA was dried over potassium hydroxide. All were distilled prior to use.

2.2 Synthesis

The 8-armed poly(ethylene glycol)-poly(ε-caprolactone) star block copolymers with an amide linkage between the PEG and PCL blocks (PEG_{20K}-(NHCO)-$(PCL_n)_8$) were synthesized by ring opening polymerization of ε-caprolactone in toluene at 110 °C. Amine-terminated 8-armed star PEG (PEG_{20K}-$(NH_2)_8$) and stannous octoate were used as initiator and catalyst, respectively.

PEG_{20K}-$(NH_2)_8$ was synthesized starting from PEG_{20K}-$(OH)_8$ using a two-step procedure analogous to that described by Elbert and Hubbell for linear hydroxyl-terminated PEGs.[19] Typically, PEG_{20K}-$(OH)_8$ (45 g, 2.3 mmol) dissolved in 250 ml of toluene was dried by azeotropic distillation of approximately 225 ml of toluene in a nitrogen atmosphere. After cooling the solution with an ice bath, 125 ml of dichloromethane was added. Subsequently, TEA (6.0 ml, 43 mmol) and mesyl chloride (3.3 ml, 43 mmol) were added dropwise under stirring. The reaction mixture was allowed to warm to room temperature and stirred for 18 hours in a nitrogen atmosphere. The mixture was filtered, concentrated and precipitated in a large excess of cold diethyl ether. The product was collected by filtration and dried overnight in vacuo. The PEG_{20K}-$(mesylate)_8$ was added to 600 ml of a 25 % aqueous ammonia solution

in a tightly closed bottle. The mixture was vigorously stirred for 5 days at room temperature, after which the ammonia was allowed to evaporate for 3 days. The pH of the solution was adjusted to 13 with 60 ml of a 1 M sodium hydroxide solution, and the resulting solution was extracted with dichloromethane. The extract was dried with magnesium sulfate, concentrated in vacuo and precipitated in a large excess of cold diethyl ether. PEG_{20K}-$(NH_2)_8$ was obtained by filtration and dried in vacuo. Conversion: >97 %, yield: 70 %. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.63 (m, PEG protons), 2.94 (t, $CH_2CH_2NH_2$). SEC: M_n 15700 g mol^{-1}, Đ = 1.11.

A typical procedure for the synthesis of PEG_{20K}-(NHCO)-$(PCL_9)_8$ star block copolymer with a degree of polymerization of the PCL arms of 9 was as follows. To a solution of PEG_{20K}-$(NH_2)_8$ (1.0 g, $5.0 \cdot 10^{-2}$ mmol) in 15 ml of toluene, ε-caprolactone (0.357 ml, 3.3 mmol) and stannous octoate (0.3 ml, 0.92 mmol) were added. The reaction was allowed to proceed for 4 hours at 110 °C in a nitrogen atmosphere. The product was purified by precipitation in a large excess of cold diethyl ether. PEG_{20K}-(NHCO)-$(PCL_9)_8$ was obtained by filtration and dried overnight in vacuo at room temperature. Yield: 1.27 g (92 %). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.11 (s, CH_2NHCO), 4.06 (t, CH_2CH_2O), 3.64 (m, PEG protons), 2.30 (t, $COCH_2CH_2$), 2.18 (t, $NHCOCH_2CH_2$), 1.65 (m, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.39 (m, $CH_2CH_2CH_2CH_2CH_2$). SEC: M_n 24700 g mol^{-1}, Đ = 1.23.

Linear PEG_{4K}-(OCO)-$(PCL_8)_2$ and PEG_{20K}-(OCO)-$(PCL_{35})_2$ triblock copolymers as well as 8-armed PEG_{20K}-(OCO)-$(PCL_9)_8$ star block copolymer were prepared similarly, using PEG_{4K}-$(OH)_2$, PEG_{20K}-$(OH)_2$ and PEG_{20K}-$(OH)_8$ as the initiator, respectively. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 4.22 (t, OCH_2CH_2OCO), 4.06 (t, CH_2CH_2O), 3.64 (m, PEG protons), 2.30 (t, $COCH_2CH_2$), 1.65 (m, $CH_2CH_2CH_2CH_2CH_2CH_2$), 1.39 (m, $CH_2CH_2CH_2CH_2CH_2$). SEC: M_n 9000 g mol^{-1}, Đ = 1.10 (PEG_{4K}-(OCO)-$(PCL_8)_2$); M_n 29000 g mol^{-1}, Đ = 1.39 (PEG_{20K}-(OCO)-$(PCL_{35})_2$); M_n 25600 g mol^{-1}, Đ = 1.23 (PEG_{20K}-(OCO)-$(PCL_9)_8$).

2.3 Characterization

^{1}H NMR (300 MHz) spectra were recorded on a Bruker AMX300 spectrometer at 25 °C. Polymers were dissolved in $CDCl_3$ at a concentration of 15 mg ml^{-1}. As a standard, residual internal $CHCl_3$ (δ 7.26) was used.

The number-average and weight-average molar masses (M_n and M_w, respectively) and dispersity (Đ, M_w/M_n) of the polymers were determined by size exclusion chromatography (SEC) using a Viscotek GPCmax VE2100 liquid chromatograph equipped with a Viscotek VE3580 refractive index detector operating at 35 °C. Tetrahydrofuran was used as the eluent and the flow rate was set up at 1.0 ml min^{-1}. Two LT5000L 300 x 7.8 mm columns operating at 29 °C were used. Calibrations were performed with polystyrene standards (600-1·10^6 g mol^{-1}).

Thermal properties of the polymers were determined using a Perkin-Elmer DSC 6000 differential scanning calorimeter. Heating and cooling rates of 20 °C min^{-1} were applied. Samples were heated from 25 to 200 °C, kept at 200 °C for 1 min, cooled to -40 °C, kept at -40 °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 enthalpies (ΔH_m) were obtained from the second heating scan.

2.4 Aqueous solution properties

The critical association concentration (CAC) values of aqueous solutions of the PEG-PCL block copolymers were determined using a dye solubilization method.[20] Aqueous polymer solutions in the concentration range of 0.001 to 5 w/v % were prepared using distilled water. A solution of the hydrophobic dye 1,6-diphenyl-1,3,5-hexatriene (DPH) was prepared in methanol at a concentration of 0.5 mM. Approximately 250 µl of polymer solution was added to a 96-wells plate, followed by addition of 2.5 µl of the DPH solution. The samples were allowed to equilibrate for at least 3 hours in the dark, after which the absorption at 357 nm was measured using a BMG Labtech Clariostar plate reader. The

absorption was plotted against 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 PEG-PCL block copolymers in water was performed to determine aggregate sizes. Experiments were carried out at 25 °C using a Malvern Zetasizer Nano ZS, a laser wavelength of 633 nm and a scattering angle of 173°. The sample positioning, attenuation selection and the measurement duration were run in automatic mode. Each measurement was the result of 10-15 test runs.

2.5 Gel properties

The vial tilting method was used to determine the critical gel concentration (CGC) at room temperature, as well as the temperature dependent gelation behavior of aqueous solutions of PEG-PCL block copolymers. Samples were prepared by adding the appropriate amount of polymer to distilled water in tightly capped vials, followed by equilibration overnight under mild shaking at 37 °C. For determination of the CGC, a range of samples was prepared with concentration increments of 1 w/v % (5 w/v % for the more soluble PEG_{4K}-(OCO)-$(PCL_8)_2$ polymer). The temperature dependent gelation behavior was studied in the range of 5 to 85 °C with temperature increments of 5 °C. At each temperature, the sample was allowed to equilibrate for 10 minutes. If there was no flow after tilting the vials 90° for 1 min, the sample was regarded as a gel; otherwise it was regarded as a sol.

Oscillatory rheology experiments were performed to determine the mechanical properties of the hydrogels. The storage (G') and loss (G'') modulus of hydrogels were monitored for 1 minute at 25 °C on a TA Instruments AR1000 rheometer. Experiments were performed using a parallel plate geometry (diameter 20 mm, gap 0.05 mm) utilizing a strain of 1 % and a frequency of 1 Hz. To confirm that this strain and frequency are within the viscoelastic regime, an amplitude sweep from 0.1 to 10 % was performed at 1 Hz followed by a frequency sweep from 0.1 to 10 Hz at 1 % at the end of selected rheological experiments. Rheological experiments were performed in duplicate.

2.6 Degradation

Degradation/dissolution experiments were performed to determine the stability of the hydrogels. 4 ml of PBS (pH 7.4) or $NaHCO_3/Na_2CO_3$ buffer (pH 10) was placed on top of 0.25 ml of hydrogel and the samples were kept at 37 °C. At regular time intervals, the vials were tilted 90°. The degradation/dissolution time was defined as the number of days until the sample became fully solubilized.[21] Degradation/dissolution experiments were performed in duplicate. The structure of the remaining polymers in degrading PEG_{20K}-(OCO)-$(PCL_9)_8$ and PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogel samples was analyzed by ^{1}H NMR spectroscopy. After freeze-drying of a sample, polymers were dissolved in a minimal amount of dichloromethane. The solution was filtered and precipitated in an excess of cold diethyl ether. The precipitate was collected by filtration, dried in vacuo and analyzed by ^{1}H NMR in $CDCl_3$. Prior to ^{1}H NMR measurements of the degrading PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel samples, 0.1 ml trifluoroacetic anhydride was added to the NMR tube.

3. Results and discussion

3.1 Synthesis and characterization of linear and star PEG-PCL block copolymers

A series of PEG-PCL block copolymers was synthesized to systematically investigate the effect of the hydrophobic block length, the polymer architecture and the PEG-PCL linking unit on various polymer and aqueous solution properties. PEG_{20K}-(NHCO)-$(PCL_n)_8$ and PEG_{20K}-(OCO)-$(PCL_9)_8$ star block copolymers were prepared by the stannous octoate catalyzed ring opening polymerization of ε-caprolactone in toluene at 110 °C, initiated by PEG_{20K}-$(NH_2)_8$ and PEG_{20K}-$(OH)_8$, respectively (Figure 1). PEG_{20K}-$(NH_2)_8$ was prepared in high yield from PEG_{20K}-$(OH)_8$ by first converting the hydroxyl groups in their mesylate esters followed by a reaction with ammonia.[19] The average PCL block length was calculated from the ^{1}H NMR spectrum using the integrals of peaks corresponding to the methylene protons of the caprolactone units and the main chain protons of PEG (Figure 2). A signal at 6.11 ppm in the spectrum of PEG_{20K}-(NHCO)-$(PCL_9)_8$ confirmed the presence of amide groups, whereas no peaks were found relating to unreacted amine groups, indicating that each of the 8 arms initiated the ROP of ε-

caprolactone. A signal of the PCL methylene protons next to the amide group appeared at 2.18 ppm. The ^{1}H NMR spectrum of PEG_{20K}-(OCO)-$(PCL_9)_8$ showed a triplet at 4.22 ppm corresponding to the terminal PEG methylene protons next to the linking ester unit. Linear PEG_{4K}-(OCO)-$(PCL_8)_2$ and PEG_{20K}-(OCO)-$(PCL_{35})_2$ triblock copolymers were prepared analogously to PEG_{20K}-(OCO)-$(PCL_9)_8$, using PEG_{4K}-$(OH)_2$ and PEG_{20K}-$(OH)_2$ as the initiator, respectively (Figure 1).

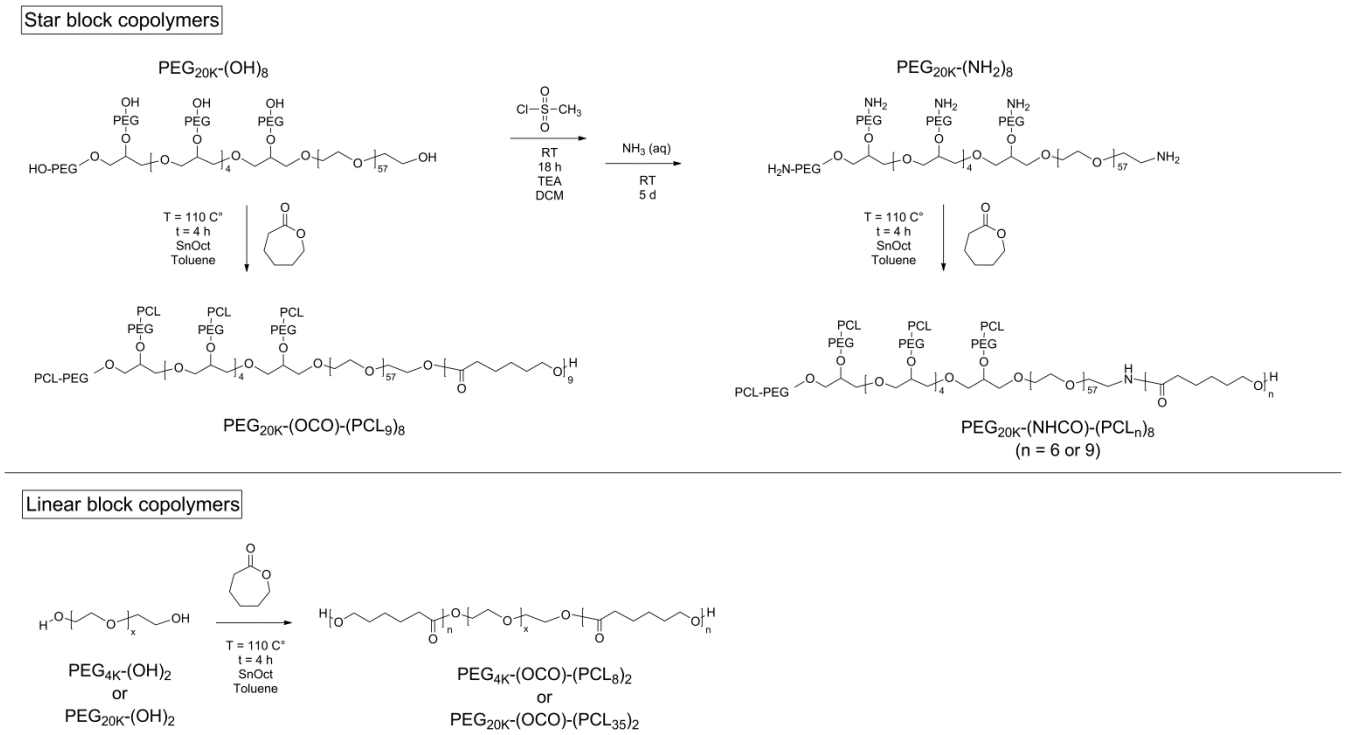

Figure 1. Synthesis scheme for the preparation of linear and star PEG-PCL block copolymers.

^{1}H NMR analysis showed M_n values and copolymer compositions close to the expected values based on the feed rates. SEC analysis indicated a good control of the polymerization with relatively narrow molecular weight distributions for most copolymers ($Đ \leq 1.25$, Table 1). The higher Đ of 1.39 for PEG_{20K}-(OCO)-$(PCL_{35})_2$ is probably the result of the relatively long PCL chains which are more prone to transesterification.

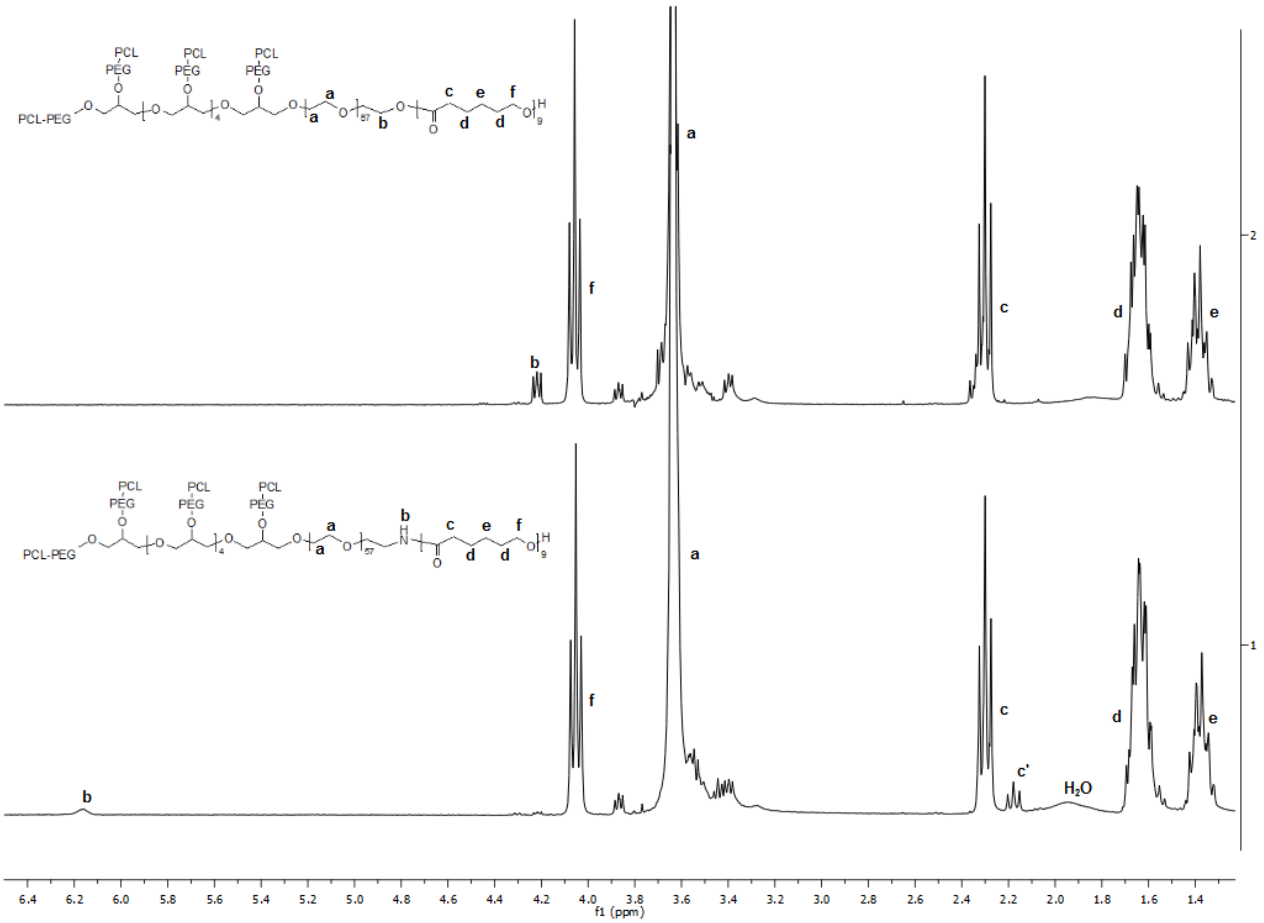

Figure 2. ^{1}H NMR spectra of PEG_{20K}-(OCO)-$(PCL_9)_8$ (top) and PEG_{20K}-(NHCO)-$(PCL_9)_8$ (bottom) star block copolymers. Solvent: $CDCl_3$.

Table 1. Characterization of linear and star PEG-PCL block copolymers.

Polymer	^{1}H NMR			Đ	T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)
	DP[a]	wt% PEG	M_n (kg/mol)					
Star block copolymers								
PEG_{20K}-(NHCO)-$(PCL_6)_8$	6.3	78	25.7	1.22	43	50	10	60
PEG_{20K}-(NHCO)-$(PCL_9)_8$	8.7	72	27.9	1.23	42	51	8	66
PEG_{20K}-(OCO)-$(PCL_9)_8$	9.3	70	28.5	1.23	44	39	22	37
Linear block copolymers								
PEG_{4K}-(OCO)-$(PCL_8)_2$	8.0	69	5.8	1.10	48	86	14	91
PEG_{20K}-(OCO)-$(PCL_{35})_2$	35.4	71	28.1	1.39	59	100	31	116

[a] Degree of polymerization of the PCL blocks, expressed in ε-caprolactone units per arm.

The thermal properties of all block copolymers as determined with DSC revealed a transition in both the second heating and the cooling scan, corresponding to melting and crystallization of the PEG domains, respectively (Table 1 and Figure S1). No other melting endotherms were detected, indicating that no crystallization of the PCL phase takes place. All PEG-PCL star block copolymers exhibit lower melting transitions and accompanying enthalpies than their PEG precursor,[8,22] indicating that the crystallization of PEG is hampered by the presence of PCL blocks. The lower transition temperatures and enthalpies of the star block copolymers compared to the linear block copolymers can be ascribed to their branched structure, which impedes crystallization. No large differences were observed between the melting transitions of the PEG_{20K}-(NHCO)-$(PCL_9)_8$ and PEG_{20K}-(OCO)-$(PCL_9)_8$ polymers. However, PEG_{20K}-(OCO)-$(PCL_9)_8$ crystallizes at a significantly higher temperature. Possibly the rigidity of the amide linkages, which are preferably in a trans configuration, hampers crystallization of the PEG chains.

3.2 Aqueous solution properties

The linear and star PEG-PCL block copolymers self-assembled at low concentrations in water to form micelles and aggregates with a hydrophobic PCL core and a hydrophilic PEG shell, as indicated by DLS measurements (vide infra). Since the polymers possess a central PEG moiety (Figure 1), they most likely form flower-like micelles with bending PEG blocks that point outwards and terminal PCL blocks that assemble into the hydrophobic core.[23,24] Critical association concentration (CAC) values of the linear and star PEG-PCL block copolymers in aqueous solution were determined with the 1,6-diphenyl-1,3,5-hexatriene dye solubilization method (Table 2 and Figure S2). A relative error of 5 % is estimated in the value taken as the intercept of the extrapolated straight lines.[25] The CAC values increase with increasing PCL block length, possibly as a result of increased steric hindrance between PCL blocks during their self-assembly into micellar cores. The CAC values of PEG_{20K}-(NHCO)-$(PCL_9)_8$ and PEG_{20K}-(OCO)-$(PCL_9)_8$ are approximately the same, indicating that the linkage between the PEG and

PCL blocks does not have a large influence on the tendency of the polymers to aggregate. By comparing PEG_{20K}-(OCO)-$(PCL_9)_8$ and PEG_{4K}-(OCO)-$(PCL_8)_2$ it can be concluded that the CAC increases with increasing arm number, as observed previously for PEG-PCL (2000-2000 g mol^{-1}) multi-arm block copolymers.[12] This was explained in terms of the translational entropic change associated with the formation of micellar aggregates, which increases with increasing arm number.

Table 2. Critical association concentrations (CAC) and critical gel concentrations (CGC) of linear and star PEG-PCL block copolymers at room temperature.

Polymer	CAC (w/v %)	CGC (w/v %)
Star block copolymers		
PEG_{20K}-(NHCO)-$(PCL_6)_8$	0.30	10
PEG_{20K}-(NHCO)-$(PCL_9)_8$	0.40	7
PEG_{20K}-(OCO)-$(PCL_9)_8$	0.43	8
Linear block copolymers		
PEG_{4K}-(OCO)-$(PCL_8)_2$	0.06	30
PEG_{20K}-(OCO)-$(PCL_{35})_2$	0.30	8

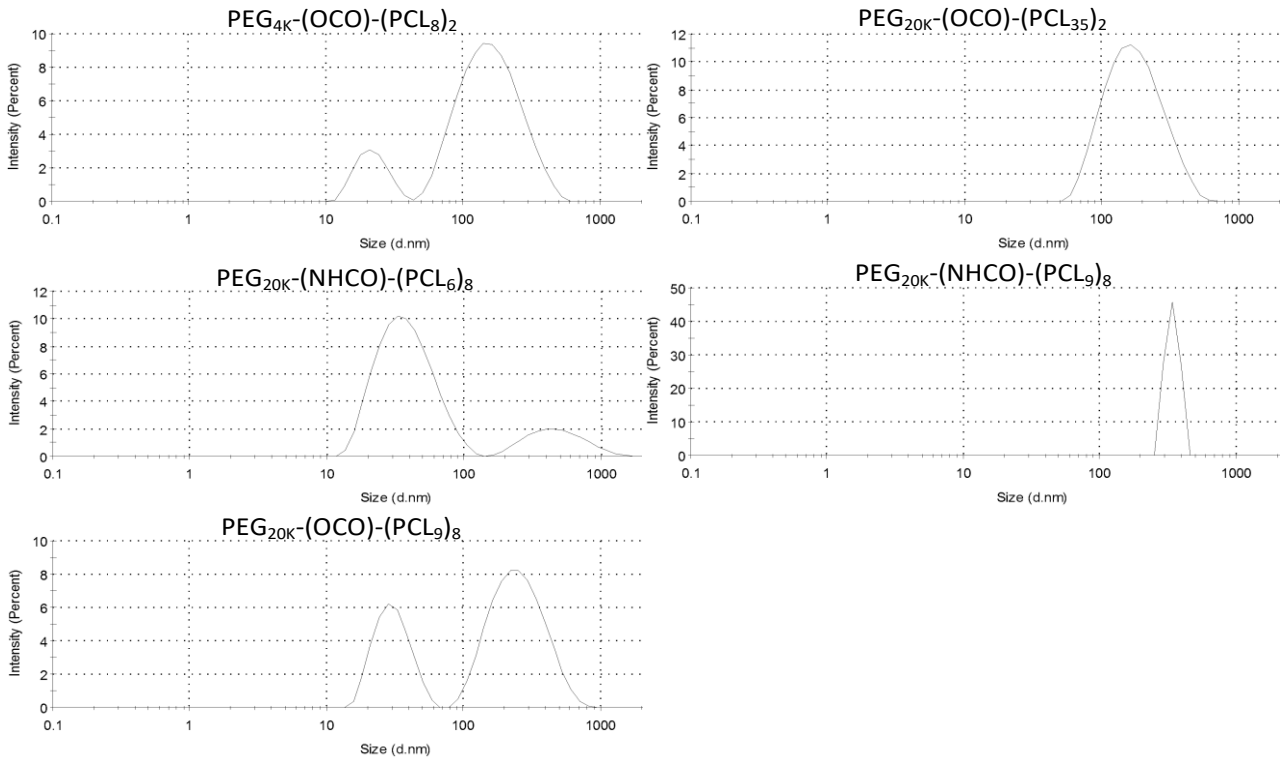

Figure 3. Aggregate size distributions of 0.5 w/v % aqueous solutions of linear and star PEG-PCL block copolymers at 25 °C.

Using dynamic light scattering (DLS) the aggregate size and aggregate size distributions of the linear and star PEG-PCL block copolymers in 0.5 w/v % aqueous solutions were determined (Figure 3). The intensity plots show that at concentrations above the CAC these materials form micelles with a diameter of 20-40 nm and/or larger aggregates, depending on the copolymer composition. The larger aggregates with a diameter of 200-500 nm may result from secondary aggregation of micelles via intermicellar bridging of hydrophobic chains.[10,26] Replacing the ester linkage between the PEG and PCL blocks for an amide linkage shifts the aggregate size distribution to larger aggregates. The increased rigidity of a linking amide group as compared to a linking ester group may hamper individual molecules to adapt their micellar conformation, resulting in larger aggregates. An increase in the hydrophobic block length also shifts the distribution to larger aggregates, possibly because longer chains facilitate intermicellar bridging. It should be emphasized that the intensity of scattered light cannot directly be related to the

number of particles, since the intensity of light scattered by larger particles is larger than that of smaller particles.

3.3 Gelation behavior

In water all PEG-PCL block copolymers formed hydrogels above the CGC, probably as a result of micellar aggregation.[8] As the polymer concentration increases, the number and/or size of the aggregates becomes larger, leading to an increase in the aggregate volume fraction. When the volume fraction of the aggregates exceeds the maximum packing fraction, a gel is formed. The dense aggregate packing may also be accompanied by interaggregate bridging of polymer chains. All star PEG-PCL block copolymers formed homogeneous, transparent hydrogels that could easily be manipulated, whereas the linear PEG_{20K}-(OCO)-$(PCL_{35})_2$ yielded white, opaque, brittle hydrogels that lost their consistency upon handling. This reflects the increased solubility of star block copolymers in aqueous media compared to linear block copolymers of similar hydrophilic/hydrophobic ratio and molecular weight.[27] Due to the poor quality of the hydrogels constituted by PEG_{20K}-(OCO)-$(PCL_{35})_2$, they were excluded from further gel experiments. The low molecular weight PEG_{4K}-(OCO)-$(PCL_8)_2$ formed homogenous hydrogels, but at a significantly higher CGC (30 w/v %) in comparison with the other copolymers (7-10 w/v %) (Table 2). This may be due to the low number of PCL arms in combination with short hydrophobic chains in PEG_{4K}-(OCO)-$(PCL_8)_2$, leading to less facile interaggregate bridging and fewer hydrophobic interactions.

The temperature dependent gelation behavior, determined with the vial tilting method in the range of 5 to 85 °C (Figure 4), shows that PEG_{4K}-(OCO)-$(PCL_8)_2$ and all star PEG-PCL block copolymers exhibit a reversible phase transition from the gel to the sol state. The gel-sol transition may be explained by disruption of micellar or aggregate interactions because of partial dehydration and shrinkage of the PEG chains.[4] The transition is limited to a narrow concentration range of 8-12 w/v % for PEG_{20K}-(NHCO)-$(PCL_9)_8$ and PEG_{20K}-(OCO)-$(PCL_9)_8$. 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. Changing the

linkage between PEG and PCL from an ester to an amide group results in a shift of the boundary curve to higher temperatures. Compared to PEG_{20K}-(NHCO)-$(PCL_9)_8$ and PEG_{20K}-(OCO)-$(PCL_9)_8$, the transition for PEG_{20K}-(NHCO)-$(PCL_6)_8$ is shifted to higher concentrations and lower temperatures as a result of decreased hydrophobic interactions. The gel-sol transition for PEG_{4K}-(OCO)-$(PCL_8)_2$ occurs at high concentrations and low temperatures as a result of the linear structure in combination with the short PCL chains leading to relatively few hydrophobic interactions. At higher concentrations, the gel-sol transition shifts to higher temperatures due to an increased number of physical crosslinks between micelles. It follows from Figure 4 that the transition from the sol to the gel state upon temperature decrease can be tuned closely to body temperature by adjusting the concentration of the solution or the block copolymer composition. Therefore, these hydrogels are of interest as injectable systems for biomedical applications.

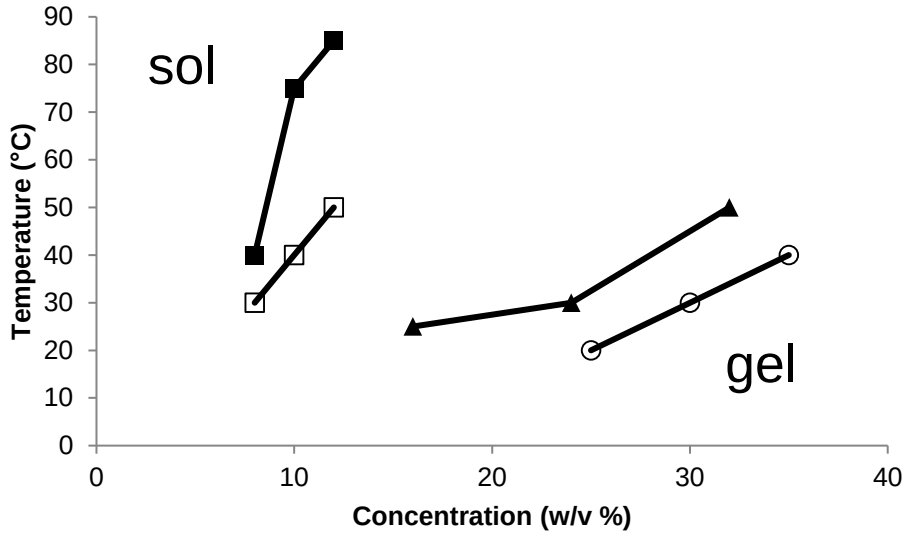

Figure 4. Gel-sol transition temperatures of linear and star PEG-PCL block copolymers in water determined with the vial tilting method. PEG_{20K}-(NHCO)-$(PCL_6)_8$ (▲), PEG_{20K}-(NHCO)-$(PCL_9)_8$ (■), PEG_{20K}-(OCO)-$(PCL_9)_8$ (□) and PEG_{4K}-(OCO)-$(PCL_8)_2$ (○).

The storage (G') and loss (G'') modulus of hydrogels at various polymer concentrations were determined with oscillatory rheology measurements at 25 °C. For PEG_{20K}-(NHCO)-$(PCL_9)_8$, PEG_{20K}-

(OCO)-$(PCL_9)_8$ and PEG_{4K}-(OCO)-$(PCL_8)_2$, G' exceeded G'' for all investigated concentrations (G'' values not shown), confirming that these systems were in the gel state.[28] For PEG_{20K}-(NHCO)-$(PCL_6)_8$ G'' was slightly higher than G' for concentrations between 12 and 20 w/v %, even though no flow was observed after tilting the vials 90° for 1 minute during the CGC determination. Aqueous solutions of PEG_{20K}-(NHCO)-$(PCL_6)_8$ above 12 w/v % should therefore be considered as very viscous liquids with gel-like properties. It is apparent from Figure 5 that replacing the ester group between the PEG and PCL blocks with an amide group results in a marked increase in the storage modulus. For example, a 12 w/v % PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel has a storage modulus of 1.6 kPa whereas a 12 w/v % PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogel has a significantly higher storage modulus of 13 kPa. Possibly, the rigidity of the amide groups results in restricted conformational freedom leading to more effective physical crosslinks between hydrophobic domains. This is supported by the presence of larger aggregates in PEG_{20K}-(NHCO)-$(PCL_9)_8$ star block copolymer solutions as compared to PEG_{20K}-(OCO)-$(PCL_9)_8$ star block copolymer solutions (Figure 3) and may be an explanation for the enhanced mechanical properties. The higher G' may also be due to the formation of intermolecular H-bonds between amide groups, resulting in a higher crosslink density. Figure 5 further shows that the gel stiffness is strongly dependent on the hydrophobic block length. Increasing the number of caprolactone units from n = 6 to 9 results in an increase in G' from 0.5 to 14 kPa for 16 w/v % PEG_{20K}-(NHCO)-$(PCL_n)_8$ hydrogels, likely due to increased hydrophobic interactions between the PCL arms. The increase in G' with concentration can be ascribed to the formation of a more densely physically crosslinked network. PEG_{4K}-(OCO)-$(PCL_8)_2$ constitutes relatively stiff hydrogels in accordance with the location of its gel region at high polymer concentrations (Figure 4). G' and G'' showed little variation when amplitude and frequency sweeps were performed at the end of selected rheological experiments, showing that a strain of 1 % and a frequency of 1 Hz are within the linear viscoelastic range of the hydrogels. These rheological experiments indicate that PEG-PCL block copolymer hydrogels can be prepared with storage moduli up to 36 kPa by adjusting the concentration and the copolymer composition.

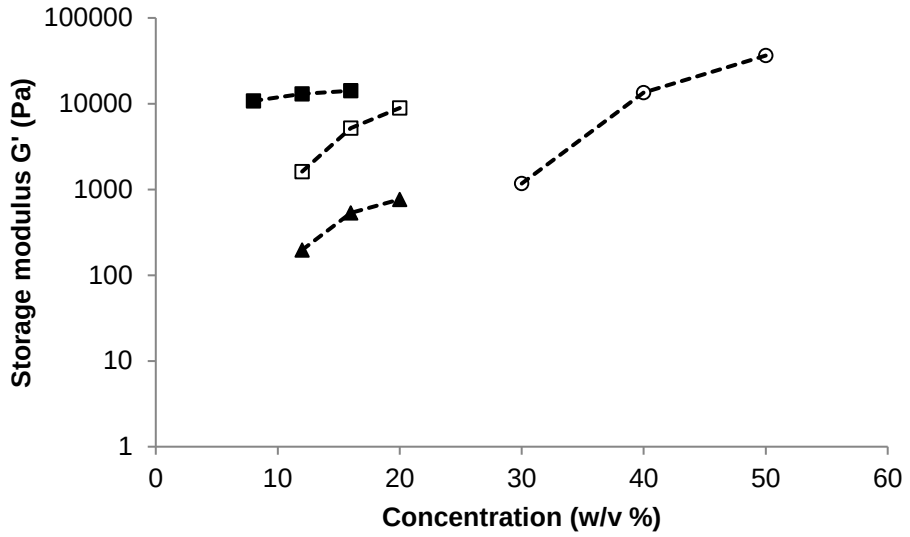

Figure 5. Storage modulus (G') at 25 °C versus concentration for hydrogels prepared from various linear and star PEG-PCL block copolymers. PEG_{20K}-(NHCO)-$(PCL_6)_8$ (▲), PEG_{20K}-(NHCO)-$(PCL_9)_8$ (■), PEG_{20K}-(OCO)-$(PCL_9)_8$ (□) and PEG_{4K}-(OCO)-$(PCL_8)_2$ (○).

3.4 Hydrogel degradation/dissolution

The *in vitro* stability of the PEG-PCL hydrogels was investigated by placing PBS (pH 7.4) or $NaHCO_3/Na_2CO_3$ buffer (pH 10) on top of the hydrogel samples at 37 °C and determining the number of days until the samples became fully solubilized.[21] Experiments at pH 7.4 mimic physiological conditions while accelerated degradation conditions at pH 10 allow for the prediction of the long-term degradation/dissolution behavior of the hydrogels within a shorter time frame, due to the higher concentration of OH^- ions resulting in faster ester group hydrolysis.[29] Under the accelerated, basic degradation conditions a 20 w/v % PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel completely solubilized within 48 h, whereas a 20 w/v % PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogel exhibited a significantly improved stability with complete dissolution around day 7 (Figure 6). These physically crosslinked hydrogels probably lose their structural integrity because the micellar and aggregate packing is disrupted due to the concentration difference of the copolymer in the hydrogel and in the supernatant buffer. Lowering the

number of caprolactone units per PCL arm from 9 to 6 in the PEG_{20K}-(NHCO)-$(PCL_n)_8$ hydrogels results in a faster dissolution (Figure 6), most likely as a result of decreased hydrophobic interactions. PEG_{4K}-(OCO)-$(PCL_8)_2$ hydrogels were tested at a higher concentration than the star block polymer hydrogels (30 versus 20 w/v %) because of the higher CGC of PEG_{4K}-(OCO)-$(PCL_8)_2$. Nevertheless 30 w/v % PEG_{4K}-(OCO)-$(PCL_8)_2$ hydrogels exhibited a fast dissolution within 24 hours, probably due to the low number of short hydrophobic PCL blocks in these polymers.

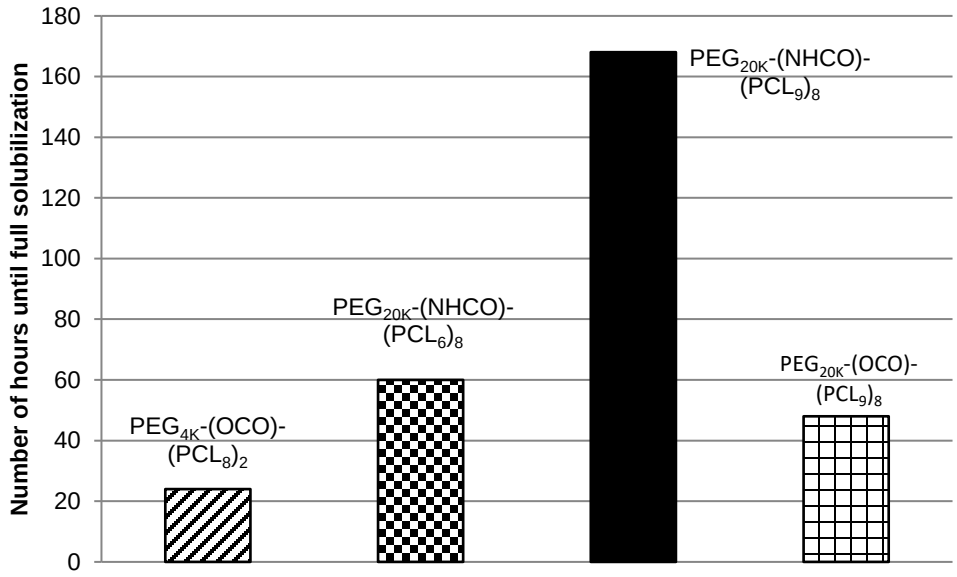

Figure 6. Stability of hydrogels prepared from various linear and star PEG-PCL block copolymers under accelerated degradation conditions ($NaHCO_3/Na_2CO_3$ buffer, pH 10, 37 °C).

In PBS buffer at pH 7.4, all 20 w/v % star block copolymer hydrogels proved highly stable and maintained their integrity after 6 months. The PEG-PCL star block copolymer hydrogels exhibit a significantly higher *in vitro* stability compared to hydrogels based on PEG-PLA star block copolymers with a similar M_n and hydrophilic/hydrophobic ratio, which were stable up to 16 days.[8] This is most likely the result of the lower hydrolytic degradation rate of PCL compared to PLA. Contrary to the star block copolymer hydrogels, the 30 w/v % PEG_{4K}-(OCO)-$(PCL_8)_2$ hydrogel was only marginally more stable at pH 7.4 than at pH 10 and completely solubilized after 48 hours.

The significant difference in stability of hydrogels having an ester or amide linkage between the PEG and PCL blocks led us to investigate the degradation mechanism in more detail by analyzing the degraded material via ^{1}H NMR spectroscopy, analogous to the procedure reported previously for PEG-PLA star block copolymer hydrogels.[8] In Figure 7 the ^{1}H NMR spectrum of a fully dissolved 20 w/v % PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel after 5 days at 37 °C in $NaHCO_3/Na_2CO_3$ buffer (pH 10) is presented together with the spectra of the starting star block copolymer and the PEG_{20K}-$(OH)_8$ macroinitiator. Since signals of PEG methylene protons next to a terminal hydroxyl group overlap with the signals of main chain PEG protons, the ^{1}H NMR spectra in Figure 7 were recorded in the presence of trifluoroacetic anhydride. Trifluoroacetylation at free hydroxyl groups results in a downfield shift of the terminal methylene PEG protons, making them well visible in ^{1}H NMR spectra. It should be noted that the hydrogels used in the ^{1}H NMR study kept their integrity for a longer time than the hydrogels used for the qualitative degradation experiment (Figure 6) because the ^{1}H NMR experiments were conducted with a larger quantity of hydrogel to achieve an adequate signal-to-noise ratio. A PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel that became fully solubilized upon degradation (Figure 7B) revealed signals typical of a trifluoroacetyl group linked to PEG at 4.48 and 3.80 ppm. Furthermore the relative intensity of signal **b**, corresponding to the terminal methylene protons of PEG linked to PCL, decreases. By comparing the relative peak integral values of the terminal PEG methylene protons (**y**) and the main chain PEG protons, it was found that on average 4 PCL arms per polymer were completely removed (Figure 9). Because the ratio of the integrals of the PCL methylene protons and the main chain PEG protons (Figure 7A and 7B) remained almost constant, only minor degradation took place in the PCL arms. The M_n of the PEG_{20K}-(OCO)-$(PCL_9)_8$ star block copolymer decreased from 28.5 kg mol^{-1} in a freshly prepared hydrogel to 23.5 kg mol^{-1} in a fully solubilized hydrogel sample after 5 days. The loss of CL units occurs through hydrolysis of ester groups in the PCL arms, most probably by intramolecular transesterification (back-biting).[30]

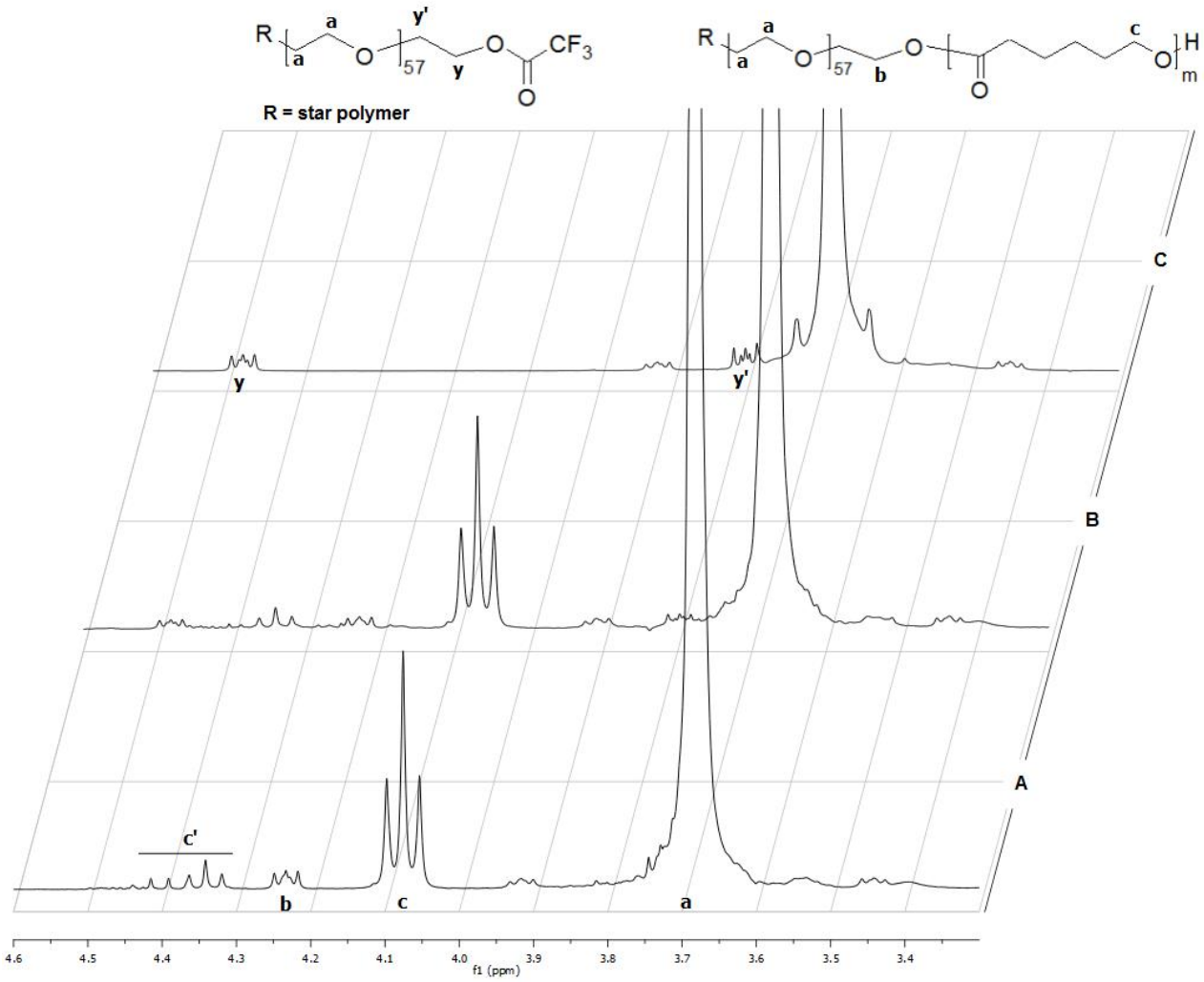

Figure 7. ^{1}H NMR spectra of PEG_{20K}-(OCO)-$(PCL_9)_8$ (A), a fully solubilized PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel after 5 days at 37 °C in $NaHCO_3/Na_2CO_3$ buffer (pH 10) (B) and PEG_{20K}-$(OH)_8$ (C). Solvent: $CDCl_3$ in the presence of trifluoroacetic anhydride. The signals between 4.32 and 4.42 ppm (**c'**) result from terminal PCL methylene units.

Figure 8 shows the ^{1}H NMR spectra of a PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogel after 5 days at 37 °C in $NaHCO_3/Na_2CO_3$ buffer (pH 10) together with the spectra of the starting star block copolymer and the PEG_{20K}-$(NH_2)_8$ macroinitiator. The PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogel still maintained its integrity after 5 days in buffer at pH 10, in contrast to the PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel which completely dissolved in this timeframe. Signals typical of PEG methylene protons next to an amine group at 2.86 and 3.59 ppm were not observed in the degrading block copolymer, showing that the amide groups were

not affected in the hydrolysis reaction and that no PCL arms were removed (Figure 9). By comparing the integrals of peaks corresponding to PCL methylene protons and the PEG main chain protons, it was found that the M_n of the PEG_{20K}-(NHCO)-$(PCL_9)_8$ star block copolymer decreased slightly from 27.9 kg mol^{-1} in a freshly prepared hydrogel to 27.5 kg mol^{-1} after 5 days due to backbiting in the PCL arms.

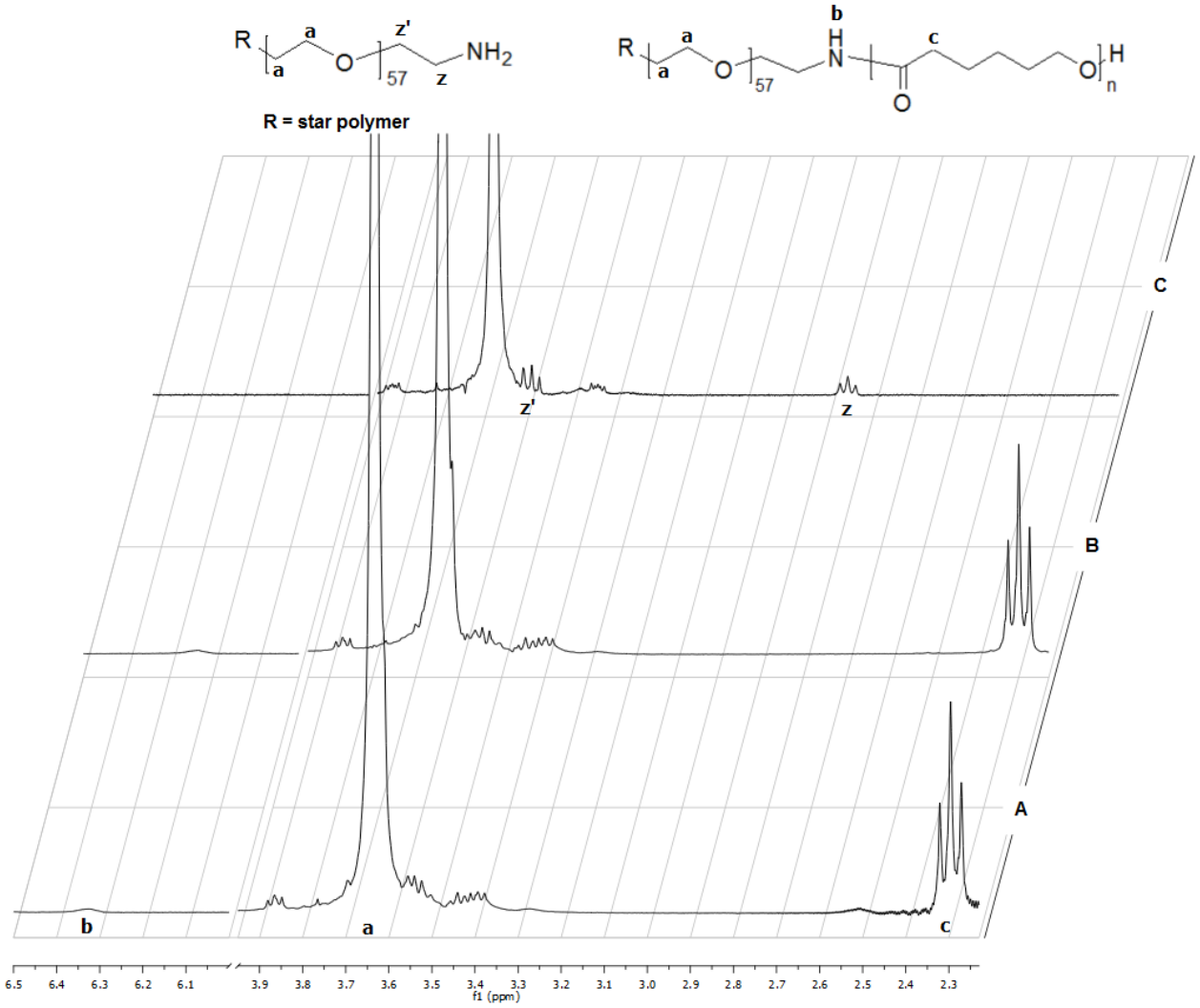

Figure 8. ^1H NMR spectra of PEG_{20K}-(NHCO)-$(PCL_9)_8$ (A), a PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogel after 5 days at 37 °C in $NaHCO_3/Na_2CO_3$ buffer (pH 10) (B) and PEG_{20K}-$(NH_2)_8$ (C). Solvent: $CDCl_3$.

These data indicate that the PEG_{20K}-(OCO)-$(PCL_9)_8$ star block copolymer degrades through preferential hydrolysis of linking ester groups between the PEG and PCL blocks, as observed for 8-armed PEG-PLA star block copolymers.[8] The disappearance of PCL arms on the block copolymers leads to decreased

hydrophobic interactions and disruption of the physically crosslinked network, resulting in a relatively fast disintegration of the PEG_{20K}-(OCO)-$(PCL_9)_8$ hydrogel. In contrast, the stable amide linking unit in PEG_{20K}-(NHCO)-$(PCL_9)_8$ only allows degradation to take place via hydrolysis of ester groups in the PCL chains, resulting in hydrogels which are considerably more stable.

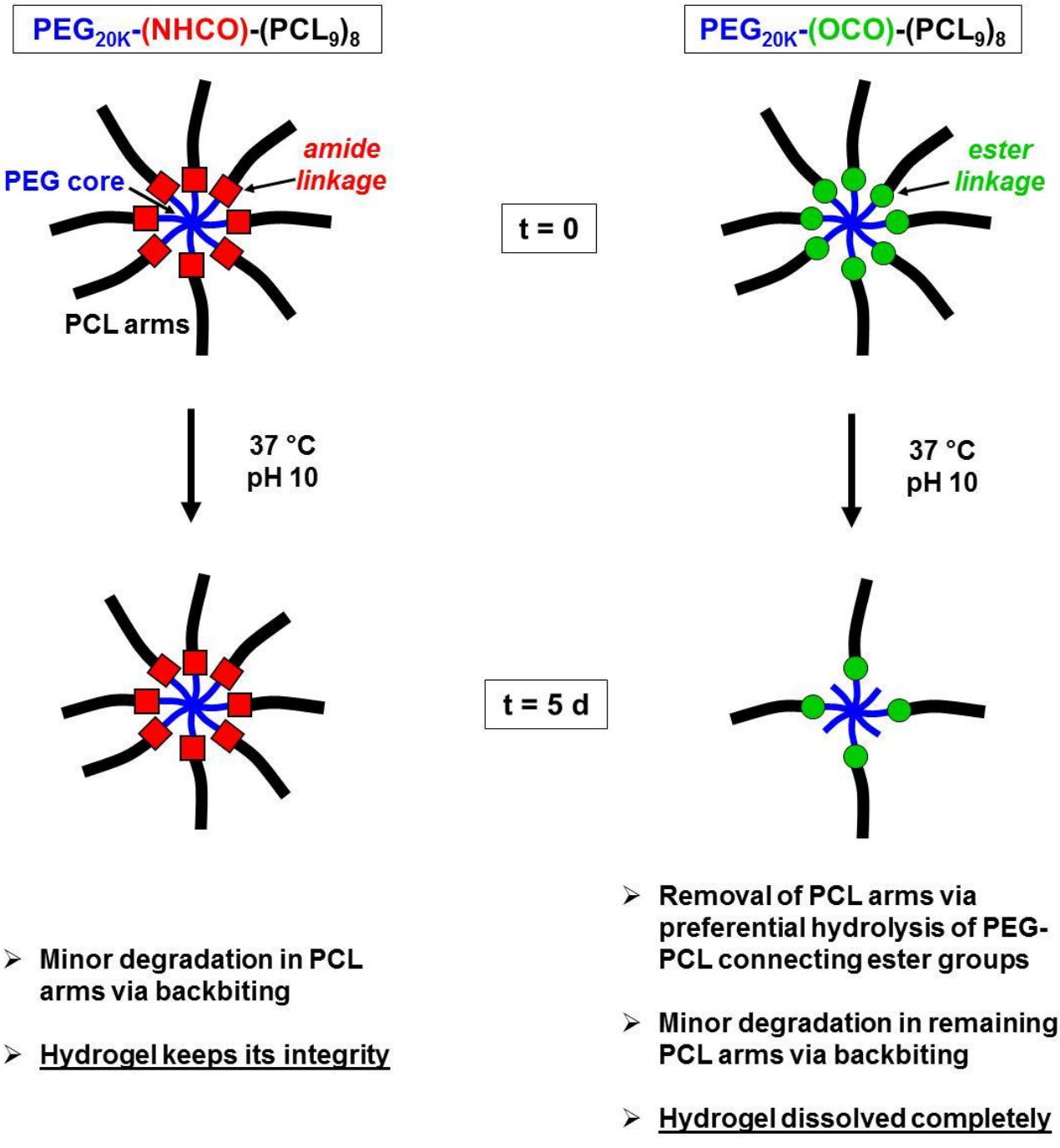

Figure 9. Schematic representation of the degradation of PEG_{20K}-(OCO)-$(PCL_9)_8$ and PEG_{20K}-(NHCO)-$(PCL_9)_8$.

These ^{1}H NMR experiments clearly show that the degradation mechanism of the star block copolymers depends on the linking group between the PEG and hydrophobic PCL. Together with previously published work on PEG-PLA block copolymers[8] these data suggest the general applicability of the proposed degradation mechanisms for block copolymers in which PEG and polyester blocks are coupled via ester or amide groups.

Oscillatory rheology measurements on a 20 w/v % PEG20K-(OCO)-$(PCL_9)_8$ hydrogel sample revealed that the storage modulus G' decreased from 8.9 kPa at the start of the degradation experiments to 3.8 kPa after 24 h at 37 °C in $NaHCO_3/Na_2CO_3$ buffer (pH 10) due to the loss of physical crosslinks, corresponding to a decrease in G' of 57 %. In contrast, G' of a 20 w/v % PEG20K-(NHCO)-$(PCL_9)_8$ hydrogel sample decreased from 12.1 to 7.7 kPa (corresponding to a decrease of 36 %) under the same conditions, confirming the higher stability against hydrolytic degradation of the PEG20K-(NHCO)-$(PCL_9)_8$ hydrogels.

4. Conclusions

A series of linear and star PEG-PCL block copolymers was synthesized by the facile ring opening polymerization of ε-caprolactone initiated by hydroxyl- or amine-terminated PEG macroinitiators. In aqueous solution the block copolymers self-assembled into micelles and micellar aggregates at low concentrations. Above the CGC they formed physically crosslinked hydrogels whose thermoresponsive, mechanical and degradation behavior could be controlled via the hydrophobic block length, the total molecular weight and the PEG-PCL connecting group. Changing the ester linkage between PEG and PCL for an amide linkage, increasing the PCL block length, as well as increasing the molecular weight, generally resulted in a larger gel window, a higher gel stiffness and an enhanced *in vitro* stability. Compared to hydrogels based on PEG-PLA star block copolymers with a similar M_n and

hydrophilic/hydrophobic ratio, the PEG-PCL star block copolymer hydrogels exhibit significantly improved mechanical properties and a much higher hydrolytic stability. ^{1}H NMR analysis showed that the degradation of PEG_{20K}-(OCO)-$(PCL_9)_8$ takes place by preferential hydrolysis of the ester bond between the PEG and PCL chains, whereas the PEG_{20K}-(NHCO)-$(PCL_9)_8$ hydrogels degrade only through hydrolysis of ester bonds in the PCL block. Their robustness, their ability to be formed *in situ* and their excellent *in vitro* stability make the novel 8-armed PEG-PCL star block copolymer hydrogels promising candidates for biomedical applications such as the controlled delivery of active agents, which is the subject of current investigations in our laboratory.

Acknowledgements

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Supporting information for:

Robust & thermosensitive poly(ethylene glycol)-poly(ε-caprolactone) star block copolymer hydrogels

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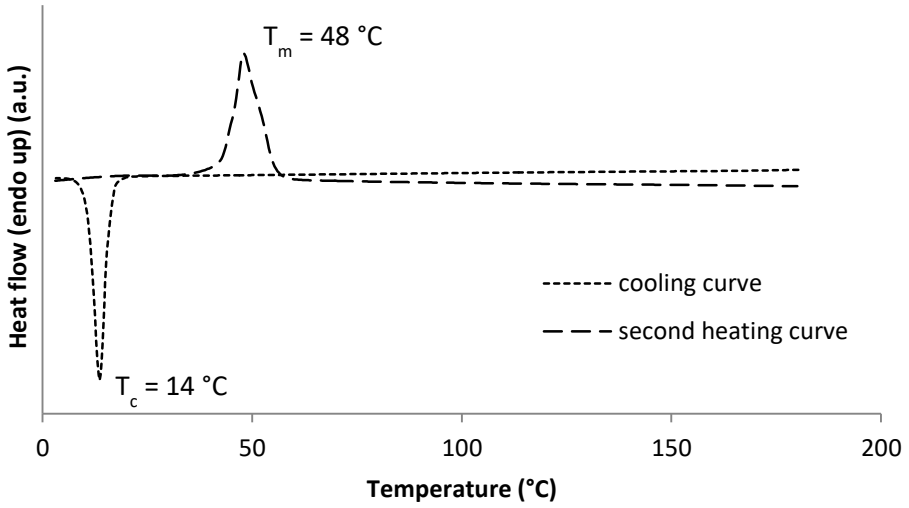

Figure S1. Second heating curve and cooling curve of the PEG_{4K}-(OCO)-$(PCL_8)_2$ block copolymer to illustrate data collection from DSC measurements.

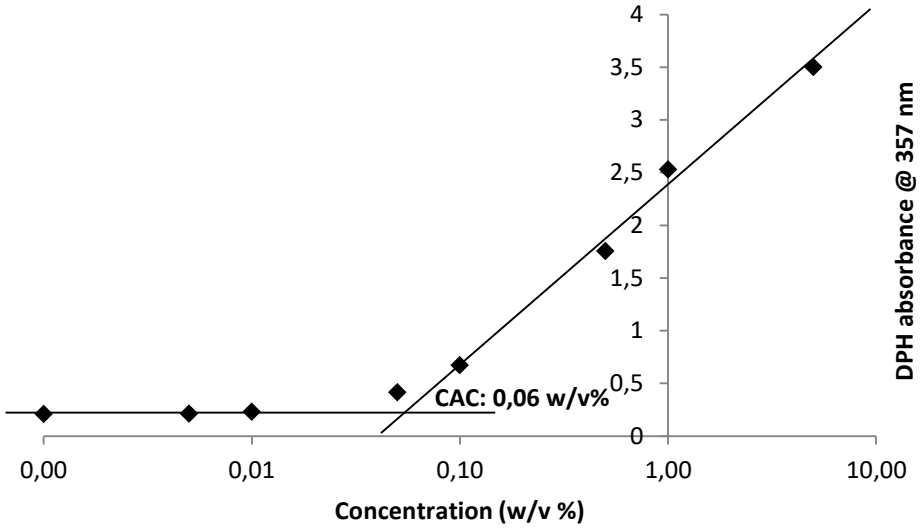

Figure S2. Representative example of the determination of the critical association concentration (CAC), in this case for the PEG_{4K}-(OCO)-$(PCL_8)_2$ block copolymer. The concentration at the intersection of the two straight lines corresponds to the CAC.