Thermo-Responsive Hydrogels Based on Branched Poly(L-lactide)-poly(ethylene glycol) Copolymers

Ingrid W. Velthoen,[1] *Edze J. Tijsma,*[2] *Pieter J. Dijkstra,*[1] *Jan Feijen**[1]

Summary: Branched poly(L-lactide)-poly(ethylene glycol) (PLLA-PEG) block copolymers were synthesized from trifunctional PLLA and amine functionalized methoxy poly(ethylene glycol)s. The copolymers in water formed hydrogels that showed thermo-responsive behavior. The hydrogels underwent a gel to sol transition with increasing temperature as determined with the vial tilting method and oscillatory rheology. For all copolymers, the transition temperature increased with increasing copolymer concentration. The transition temperature of corresponding branched copolymers also increased with increasing PEG molecular weight, and surprisingly decreased with increasing molecular weight of the PLLA branches. In general, the gel-sol transition is explained by disruption of micellar or aggregate interactions because of partial dehydration and shrinkage of the PEG chains. An increase in the molecular weight of the PLLA branches led to the formation of micelles and aggregates as observed with DLS at low concentrations. It is speculated that the non-uniform size distribution and possible crystallization of longer PLLA blocks may have a negative effect on the formation of micellar packing upon gelation, allowing the disruption of micellar or aggregate interactions to occur at lower temperatures. The transition temperature of the gels could be tuned closely to body temperature by varying the concentration of the solution or the molecular weight of the PEG block and the PLLA blocks, which implies that these polymers may be used as injectable systems for in-situ gel formation.

Keywords: block copolymers; gelation; gel-sol transition; hydrogels; poly(lactide)-poly(ethylene glycol)

Introduction

Thermo-responsive hydrogels have received much interest for their potential use in tissue engineering and as drug delivery systems.[1,2] Upon injection of a thermo-sensitive polymer solution into the body the temperature change can cause a transition from the fluid state, the sol, to the immobile state, the gel. This temperature response is based on the formation or disruption of physical crosslinks, such as entanglements or hydrophobic interactions. The thermo-responsive behavior of poly(ethylene oxide-poly(propylene oxide)-poly(ethylene oxide) (Pluronics or Poloxamers) was widely investigated.[3–5] With increasing temperature, Pluronics first show a sol-gel transition, followed by a gel-sol transition. The mechanism of the sol-gel transition is based on the packing of micelles in a long-range order, such as a cubic crystalline phase. The gel-sol transition is caused by dehydration of poly(ethylene oxide) (PEG) at higher temperatures which decreases the interaction between micelles and/or aggregates. A possible drawback of the Pluronic systems is that they are non-biodegradable. This prompted investigaters to design and

[1] Polymer Chemistry and Biomaterials group, Department of Science and Technology, Institute for Biomedical Technology, University of Twente, P. O. Box 217, 7500 AE Enschede, The Netherlands
E-mail: j.feijen@utwente.ac.be
[2] Medtronic Bakken Research Center, Endepolsdomein 5, 6229 GW Maastricht, The Netherlands

evaluate thermo-responsive hydrogels based on block copolymers of PEG and biodegradable polyesters, such as poly(lactide)s (PLA), poly(lactide-glycolide)s (PLGA) and poly(ε-caprolactone)s (PCL). These copolymers can be conveniently synthesized by ring opening polymerization of lactide, glycolide or ε-caprolactone, using hydroxy-PEG's as initiators. Alternative preparation methods include the coupling of diblock copolymers using a difunctional spacer, such as hexamethylene diisocyanate, and the coupling of pre-synthesized polyesters to PEG, using DCC and DMAP as coupling agents.[6,7] Thermo-responsive hydrogels based on PEG-polyester diblock copolymers, and triblock copolymers with PLLA or PLGA as the central block were investigated by the group of Kim.[6,8,9] A single gel to sol transition was observed when increasing the temperature. The gel-sol transition could be adjusted by changing the concentration of the solution and the composition of the block copolymer. In short, the gel-sol transition occurred at lower concentrations as the hydrophobicity of the polyester block increased, either by increasing the molecular weight or by changing the ratio of lactide to glycolide in the hydrophobic block. The gel to sol transition is caused by disruption of the micellar packing due to shrinkage of the PEG corona at higher temperatures. This shrinkage is caused by partial dehydration of PEG, since water is a poorer solvent for PEG at higher temperatures.[7,8] By changing the composition of the PEG-PLGA-PEG copolymers to copolymers with a PEG content close to 33 wt%, and a total molecular weight of approximately 5000 $g \cdot mol^{-1}$, triblock copolymer solutions were found to form a sol state at room temperature, and a gel at body temperature.[10] This sol-gel-sol transition behavior was comparable to the Pluronics systems. The low molecular weight inversed triblock copolymers with a central hydrophilic block were prepared by PEG-initiated ring opening polymerization. These PLGA-PEG-PLGA also exhibit sol-gel-sol transition behavior in water at concentrations between 10 and 30 wt%.[11] Ring opening polymerization using PEG as the initiator was also used to prepare high molecular weight copolymers with PLLA as the outer blocks.[12–15] These copolymers were able to form hydrogels at low temperature, and were investigated for their ability to form hydrogels via stereocomplexation with their corresponding PDLA-PEG-PDLA copolymer.[13–15] Hiemstra et al.[15] also investigated the thermo-responsive behavior of hydrogels of these PLLA-PEG-PLLA copolymers, by using the vial tilting method and oscillatory rheology. These hydrogels showed thermo-responsive gelation, and the gel-sol transition temperature increased with the copolymer concentration. A triblock PLLA-PEG-PLLA copolymer with 7.5 repeating lactide units per arm, and a PEG content of 85 wt% showed almost the same transition temperature as its corresponding eight-armed star-shaped PEG-PLLA copolymer having the same PLLA block length, but a PEG content of 74 wt%. This indicates that the gel-sol transition temperature is largely influenced by the PLLA block length. The influence of the copolymer architecture was also investigated for copolymers with PLLA as the central block. Star-shaped PLLA-PEG block copolymers with PLLA as the hydrophobic core were prepared by Park et al.[7] Hydrogels of these three-arm star-shaped copolymers showed thermo-responsive gelation, with a gel to sol transition if the temperature increased as measured with the vial tilting method. The critical gelation concentration (CGC) decreased from 20 to 10 wt% when the PLLA block length of PLLA-PEG5000 copolymers increased from 5 to 9 repeating lactide units. The transition temperature increased with increasing copolymer concentration, and increasing molecular weight of the hydrophilic or hydrophobic block. The influence of the copolymer architecture was investigated by comparing the CGC of a star-shaped copolymer with that of a triblock copolymer with the same PEG content and the same PEG molecular weight per block. It was found that the CGC of star-shaped PLLA-PEG

was about 5 wt% lower than that of the linear copolymers.

In this paper, the synthesis and characterization of branched PLLA-PEG block copolymers are described, as well as the formation of thermo-responsive hydrogels from aqueous solutions of these block copolymers.

Experimental Part

Materials

L-lactide (L-LA) was purchased from Purac (Gorinchem, the Netherlands). 2,2-Bis (hydroxymethyl)propionic acid (bis-MPA), N,N′-dicyclohexylcarbodiimide (DCC) and succinic anhydride were obtained from Acros (Geel, Belgium). Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$), N-hydroxysuccinimide (NHS), mesyl chloride, and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Aldrich (Zwijndrecht, the Netherlands). Methoxy-hydroxy poly(ethylene glycol)s with molecular weights of 2 and 5 $kg \cdot mol^{-1}$ (mPEG2000-OH and mPEG5000-OH, respectively) and aqueous ammonia (25%) were obtained from Fluka (Buchs, Switzerland). Glacial acetic acid, 4-dimethylaminopyridine (DMAP), and triethylamine (TEA) were obtained from Merck (Darmstadt, Germany). All other organic solvents were from Biosolve (Valkenswaard, the Netherlands). Dichloromethane and toluene were dried over calcium hydride (Aldrich), and sodium wire, respectively, and distilled prior to use. All other chemicals were used as received.

Synthesis

Hydrophobic PLLA macromonomers (PLLAn, with n is the number of repeating lactide units per arm) were synthesized by first ring opening polymerization of L-lactide in the presence of bis-MPA as the initiator in the melt, and subsequent reaction of the hydroxyl end groups with succinic anhydride to afford carboxylic acid end-groups (CPLLAn), which were finally activated by conversion into their NHS-esters (NHS-CPLLAn). The hydrophilic PEG blocks with amine end-groups (mPEGy-NH_2, with y is the molecular weight in $g \cdot mol^{-1}$) were synthesized from the corresponding hydroxyl functional PEGs.

PLLAn

In a typical procedure PLLA10 was prepared by adding L-lactide (25.0 g, 174 mmol) to a reaction vessel, which contained bis-MPA (1.16 g, 8.7 mmol) as the initiator and $Sn(Oct)_2$ (0.10 g, 0.25 mmol; 0.4 wt% based on L-lactide) as the catalyst. The mixture was allowed to react for 3 h at 130 °C under an argon atmosphere. The product was subsequently cooled to room temperature and dissolved in dichloromethane. To this solution, a small amount of glacial acetic acid was added, and the product was precipitated in an excess of cold diethyl ether. The product was collected by filtration, and dried in vacuo to give a white powder (Yield: 86%).

CPLLAn

The synthesis of CPLLA10 is given as a typical procedure: PLLA10 (25.0 g, 8.3 mmol), succinic anhydride (1.99 g, 19.9 mmol), DMAP (1.22 g, 10.0 mmol), and TEA (1.68 g, 16.6 mmol) were dissolved in 200 ml of dichloromethane, and stirred for 24 h under an argon atmosphere at room temperature. The solvent was partially evaporated with a rotary evaporator and the polymer was precipitated in a diethyl ether: methanol (10:1 v:v) mixture and dried in vacuo over night. The product was obtained as a white powder (Yield: 88%).

NHS-CPLLAn

The synthesis of NHS-CPLLA10 is given as a typical procedure: CPLLA10 (20 g, 6.5 mmol) was dissolved in 200 ml of dichloromethane. To the resulting solution, NHS (2.67 g, 23.2 mmol) and DCC (5.98 g, 29.0 mmol) were added. Subsequently, the reaction mixture was allowed to react for 18 h at room temperature under an argon atmosphere. The formed dicyclohexylurea (DCU) was removed after the reaction by filtration. The clear solution was concentrated by partially

evaporating the dichloromethane, and the polymer was precipitated in an excess of cold diethyl ether: methanol (10:1 v:v). The product was dried in vacuo over night to give a white powder (Yield: 88%).

mPEGy-NH_2

mPEGy-NH_2 was synthesized according to a procedure as described by Elbert and Hubbell.[16] In a typical procedure, mPEG2000-OH (25 g, 12.5 mmol) was dissolved in 700 ml of toluene and dried by the removal of 350 ml of solvent by azeotropic distillation. After the solution was cooled in an ice-bath, 25 ml of dichloromethane and TEA (5.3 ml, 37.5 mmol) were added. Subsequently, mesyl chloride (2.9 ml, 37.5 mmol) was added dropwise under stirring and allowed to react overnight. The solution was filtered and the product was precipitated in a large excess of diethyl ether. After drying, the formed mPEG2000-mesylate was reacted with 100 ml of an aqueous ammonia solution (25%) for 4 d at room temperature. Subsequently, the ammonia was allowed to evaporate and the pH of the solution was raised to 13, using 1 M NaOH. The solution was extracted with dichloromethane (100 ml) for 3 times. The dichloromethane extracts were combined and concentrated. The mPEG2000-NH_2 was isolated by precipitation in cold diethyl ether, and drying in vacuo (Yield: 78%).

PLLAn-PEGy

The synthesis of PLLA10-PEG5000 is given as a typical procedure: NHS-CPLLA10 (0.90 g, 0.22 mmol) and mPEG5000-NH_2 (3.24 g, 0.66 mmol) were dissolved in 80 ml of dichloromethane, and stirred for 24 h at room temperature under argon. The resulting solution was concentrated by partially evaporating the solvent, and precipitated in an excess of cold diethyl ether: methanol (10:1). The product was dried in vacuo and was obtained as a white powder (Yield: 95%).

Characterization

^1H (300 MHz) and ^{13}C (75.4 MHz) NMR spectra were recorded on a Varian Inova NMR spectrometer. Polymers were dissolved in $CDCl_3$ at a concentration of 0.015 $g \cdot ml^{-1}$ (^1H) or 0.2 $g \cdot ml^{-1}$ (^{13}C).

Thermal analysis of the macromonomers and the copolymers was carried out using a Pyris 1 differential scanning calorimeter, calibrated with indium and gallium. During a measurement, the polymer (5–15 mg) was cooled to −50 °C and kept at this temperature for 1 min. The sample was then heated to 200 °C, annealed for 1 min, and subsequently cooled to −50 °C. Finally, the sample was kept at −50 °C for 5 min and heated to 200 °C. The heating and cooling rate was 20 $°C \cdot min^{-1}$. Melting (T_m) and cold crystallization (T_{cc}) temperatures were obtained from the peak maxima in the second heating scan. The melt (ΔH_m) and cold crystallization (ΔH_{cc}) enthalpies were determined from the area under the curve. The glass transition temperature (T_g) was taken as the inflection point. The crystallization temperature (T_c) and enthalpy (ΔH_c) were obtained from respectively the peak maximum and the area under the curve in the cooling scan.

The phase behavior of aqueous polymer solutions was investigated by the vial tilting method. Block copolymers were dissolved in MilliQ water (10–40 wt%) in tightly capped 5 ml vials by repeatedly heating to ~80 °C for 2 min and stirring while cooling. The block copolymer solutions were kept at 4 °C overnight prior to measurement. The temperature was increased step-wise with 2 or 4 °C and the samples were left at the measuring temperature for 10 min to equilibrate. The gel-sol transition temperature was determined by tilting the vials 90° for 1 min. If there was no flow, it was regarded as a gel state. In other cases it was regarded as a sol state.

Rheology experiments were performed on a TA instruments AR1000 rheometer with a flat plate geometry (20 mm diameter, 0.5 mm gap) in oscillating mode. Aqueous polymer solutions were prepared by dissolving the appropriate amount of polymer by repeatedly heating to ~80 °C for 2 min and stirring while cooling. The polymer solutions were then applied on the rheometer

and heated to 60 or 70 °C. To prevent evaporation of water, a solvent trap was placed over the geometry. A pre-shear was applied for 10 s, after which the polymer solution was allowed to equilibrate for 6 min. Subsequently, the polymer solution was cooled to 20 °C at 1 °C · min^{-1}, and then heated to 60 or 70 °C at 1 °C · min^{-1}. Gelation of the polymer solutions was monitored by measuring both the storage modulus G′ and the loss modulus G″ as a function of temperature. A system was considered a gel if G′ was larger than G″. The temperature at which G′ and G″ become equal is considered to be the transition temperature. A frequency ω of 1 Hz and a strain γ of 1% were applied to minimize the influence of deformation on the formation of the hydrogels. After the cooling and heating cycles, an amplitude and frequency sweep were performed at γ 0.01–10% ($\omega = 1$ Hz) and ω 0.01–10 Hz ($\gamma = 1\%$) at 20 °C, to confirm that the applied ω of 1 Hz and the γ of 1% was within the linear viscoelastic range.

The critical association concentration (CAC) of the copolymers in water at 20 °C was determined by the dye solubilization method. To 1.0 ml of aqueous copolymer solutions with concentrations ranging from 1 to 0.0001 w/v%, 10 μl of a 0.5 mM DPH solution in methanol was added. The resulting mixture was stored in the dark and equilibrated over night. UV-VIS absorption spectra of the solutions were recorded in the 300 to 500 nm range. The difference in absorption at 378 nm relative to 403 nm was plotted against the polymer concentration and the intercept of the extrapolated straight lines was defined as the CAC of the copolymer.

Dynamic light scattering experiments were performed on a Malvern Zetasizer 4000 (Malvern Corp., Malvern, UK), using a laser wavelength of 633 nm and a scattering angle of 90°. The CONTIN method was applied for data processing. The micelle or aggregate size of copolymers in water was determined as a function of temperature in the 20 to 50 °C range. The aqueous solution was allowed to equilibrate at each measuring temperature for 45 min.

Results and Discussion

Synthesis and Characterization

The synthesis of the mPEGy-NH_2's was performed according to a procedure as described by Elbert and Hubbel.[16] Hydroxy end-functionalized mPEGs were converted to their corresponding mesylates in high yield. The mesylates were quantitatively converted into amines by reaction with aqueous ammonia for 4 d at room temperature. The ^{1}H-NMR spectra of mPEGy-NH_2's revealed that only amino end-groups were present, since only a signal of the CH_2-NH_2 protons was observed at 2.86 ppm (data not shown). PLLA macromonomers bearing one carboxylic acid group and two hydroxyl groups (Figure 1) were synthesized by the $Sn(Oct)_2$ catalyzed ring opening polymerization of L-lactide using bis-MPA as the initiator. The number of repeating lactide units per arm, n, was varied from 10 to 25 by varying the monomer to initiator ratio ([M]:[I]). The integral ratio of the CH_3 protons of the monomer (1.59 ppm) to polymer (1.65 ppm) in the ^{1}H-NMR spectra of the crude samples was used to determine the conversion. In all cases, after 3 h reaction time, high monomer conversions of approximately 97% were obtained. The ^{1}H-NMR spectra of the purified PLLAn macromonomers (Figure 2A) were used to calculate the degree of polymerization (DP), and the number average molecular weight (Mn). The DP was determined from the ratio of the integrated area of the CH protons of the lactide repeating units (5.10 ppm) to the CH_3 protons of the bis-MPA moiety (1.27 ppm). The results obtained with ^{1}H-NMR (Table 1) are in good agreement with the theoretical values, based on the [M]:[I] ratio.

The hydroxyl end-groups of PLLAn were reacted with succinic anhydride to yield carboxylic acid end-groups (Figure 1). ^{1}H-NMR spectra (Figure 2B) showed the disappearance of the signals at 4.30 ppm

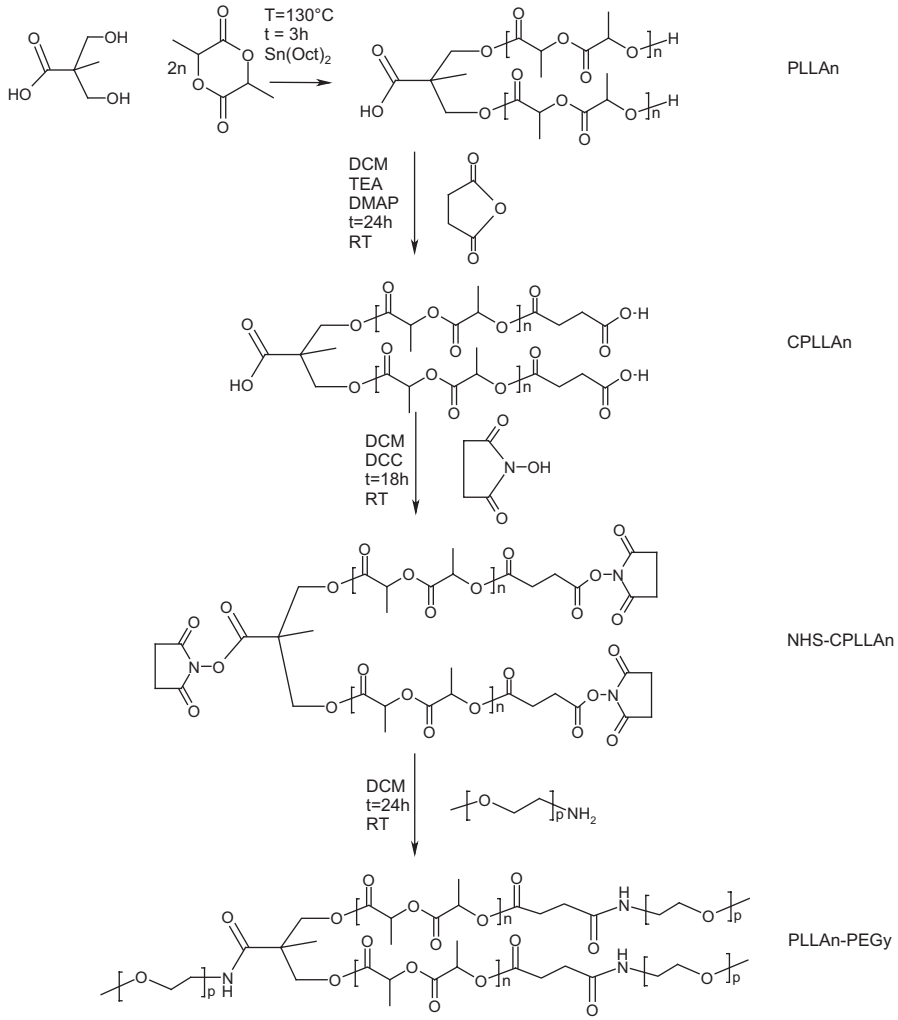

Figure 1.
Schematic synthesis route for the preparation of PLLAn-PEGy copolymers.

(c′, C*H*-OH) and 1.49 ppm (d′, CH(CH_3)-OH), belonging to the protons of the terminal lactic acid unit, and the appearance of a new peak at 2.68 ppm (e), corresponding to the CH_2 protons of the succinic half ester. Furthermore, the signal belonging to the CH_3 protons of the bis-MPA moiety shifted from 1.27 ppm to 1.21 ppm. The integral ratio of the signals a, b, and e is 3:4:4 revealing a high conversion, and is in good accordance with the values based on the macromonomer structure (Table 1).

Active esters were successfully generated by the reaction of the three carboxylic acid groups with NHS (Figure 1). ^{1}H-NMR spectra showed the appearance of a new signal originating from the CH_2 protons of the succinimide ring at 2.83 ppm (Figure 2C, peak f). The CH_2 protons of the succinic ester appeared as two triplets (e, 2.83 ppm and 2.95 ppm). Additionally, the signal corresponding to the CH_3 protons of the bis-MPA moiety (a) shifted from 1.21 ppm to 1.44 ppm, and the multiplet belonging to the CH_2 protons of the bis-

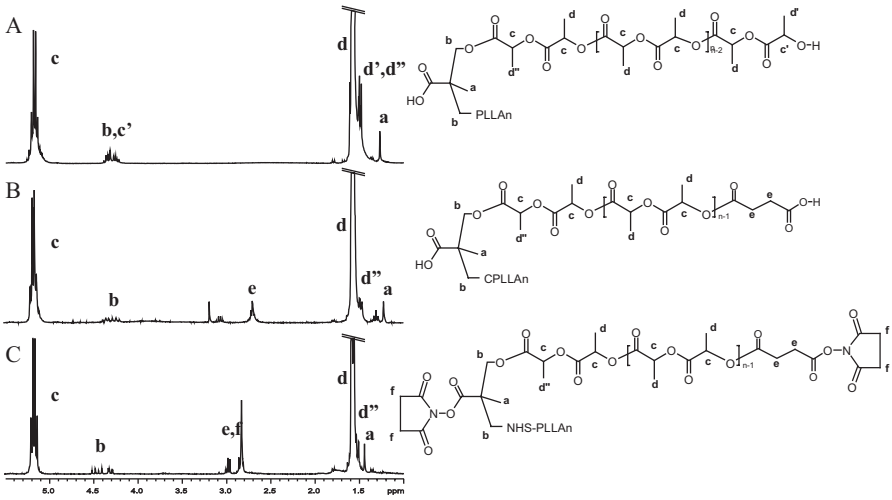

Figure 2.
^{1}H-NMR spectra of (A) PLLA10; (B) CPLLA10; and (C) NHS-CPLLA10. Solvent: $CDCl_3$.

MPA moiety shifted (b) from 4.20-4.40 to 4.25-4.55 ppm. High conversions were obtained, based on the integral ratio of the signals b, e, and f.

The ^{13}C-NMR spectral data of PLLAn, CPLLAn, and NHS-CPLLAn support the conclusions based on ^{1}H-NMR spectral data as discussed above. As a typical example, the spectrum of NHS-CPLLA20 is shown in Figure 3, and shows three major signals corresponding to the carbonyl, methine and methyl carbons (a, b, and c) of the poly(lactide) arms. The signals with low intensity correspond to the carbons of the bis-MPA (d, e and f), the succinic ester (h and i), and the succinimide moieties (j and k). The insert shows the 160-180 ppm carbonyl region of PLLA20 (A), CPLLA20 (B), and NHS-CPLLA20 (C). The signals at 176 and 172 ppm in spectrum A and B belong to carboxylic acid carbon atoms. The disappearance of these peaks, and the appearance of multiple peaks around 170 ppm (Spectrum C) confirm that all carboxylic acid groups are converted to their active NHS-esters.

Table 1.
^{1}H-NMR results and calculated molecular weight of PLLAn, CPLLAn and NHS-CPLLAn.

	Calc[a)]		^{1}H-NMR
	Mn ($g \cdot mol^{-1}$)	DP (–)	Mn ($g \cdot mol^{-1}$)
PLLA10	3020	10	2900
PLLA15	4460	15	4300
PLLA20	5900	20	6000
PLLA25	7340	25	7500
CPLLA10	3220	11	3500
CPLLA15	4660	14	4400
CPLLA20	6100	19	5900
CPLLA25	7540	25	7400
NHS-CPLLA10	3560	12	4200
NHS-CPLLA15	5000	16	5300
NHS-CPLLA20	6450	21	6700
NHS-CPLLA25	7890	28	8700

[a)] Calculated from the [M]:[I] ratio.

The coupling reaction of NHS-CPLLAn and mPEGy-NH_2 in dichloromethane at room temperature afforded PLLAn-PEGy copolymers. ^{1}H-NMR spectra showed peaks originating from the N*H* protons of the formed amide bonds at 6.32 ppm and 6.45 ppm (g and g′ in Figure 4), and signals that belong to the PEG (h, i, and j) and PLLA (a–e). Based on the complete disappearance of the signal belonging to the CH_2 protons of the succinimide ring, it is concluded that the coupling reaction was complete. Additionally, the CH_3 protons of the bis-MPA shifted from 1.44 ppm to 1.20 ppm (a), and the multiplet of

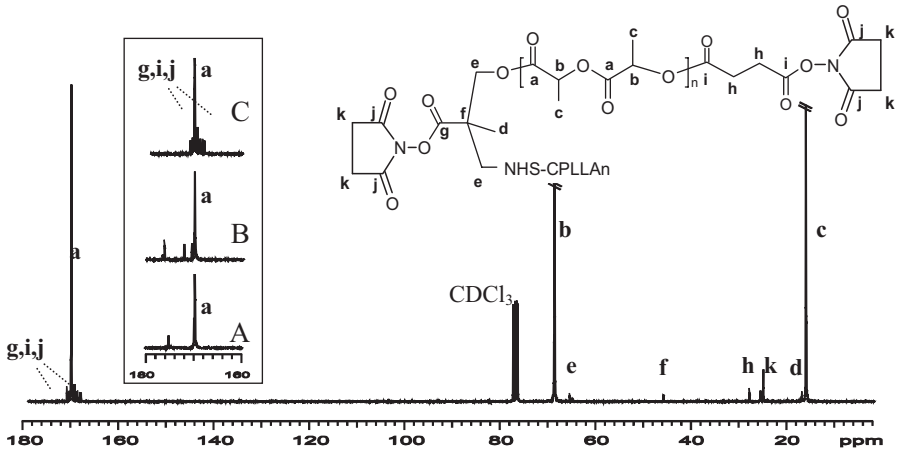

Figure 3.
^{13}C-NMR spectrum of NHS-CPLLA20. Solvent: $CDCl_3$. The insert shows the 160–180 ppm region of (A) PLLA20; (B) CPLLA20; and (C) NHS-CPLLA20.

the CH_2 protons of the bis-MPA shifted from 4.25–4.55 ppm to 4.10–4.35 ppm (b). Furthermore, the multiplets of the CH_2 protons of the succinic ester (e) shifted from 2.83 ppm and 2.95 ppm to 2.48 ppm and 2.70 ppm, respectively. The calculated and determined molecular weights based on the ^{1}H-NMR data are presented in Table 2.

The thermal properties of the macromonomers and the copolymers are listed in Tables 3 and 4, respectively. As typical

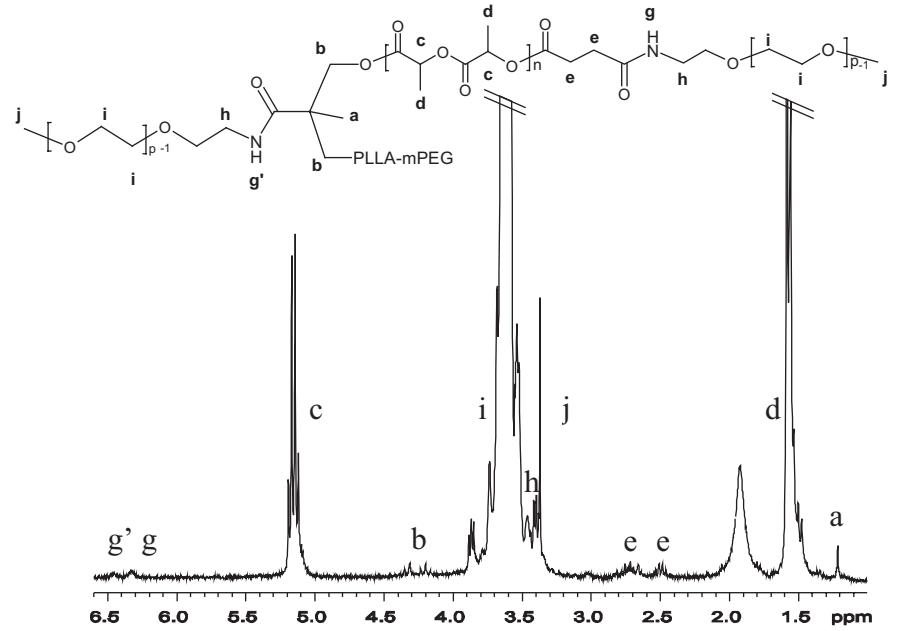

Figure 4.
^{1}H-NMR spectrum of PLLA10-PEG5000. Solvent: $CDCl_3$.

Table 2.
Degree of polymerization (DP), molecular weight and PEG content of PLLAn-PEGy copolymers.

		Theoretical		^{1}H-NMR		
y ($g \cdot mol^{-1}$)	n (–)	Mn_{tot} ($g \cdot mol^{-1}$)	PEG cont. (wt%)	DP $_{PLLA}$ (–)	Mn_{tot}[a] ($g \cdot mol^{-1}$)	PEG cont.[b] (wt%)
2000	10	9200	65	9.8	9200	62
2000	15	10650	56	15.4	10800	57
2000	20	12100	50	21.3	12500	46
2000	25	13550	44	27.1	14100	41
5000	10	18200	82	10.8	18400	80
5000	15	19650	76	14.2	19400	75
5000	20	21100	71	21.1	21400	73
5000	25	22550	67	27.1	23100	69

[a] Calculated as $Mn_{tot} = Mn\ PLLA + 3 \cdot y$.
[b] Calculated from the relative integral ratio of the peaks corresponding to the methine protons of PLLA (5.14 ppm) and the CH_2 protons of PEG (3.64 ppm).

Table 3.
Thermal properties of macromonomers NHS-CPLLAn and mPEGy-NH_2.

	T_g (°C)	T_m (°C)	ΔH_m ($J \cdot g^{-1}$)	T_c (°C)	ΔH_c ($J \cdot g^{-1}$)
NHS-CPLLA10	49	–	–	–	–
NHS-CPLLA15	53	140	2	119[a]	1
NHS-CPLLA20	54	146	6	119[a]	6
NHS-CPLLA25	54	154	13	125[a]	10
mPEG2000-NH_2	–	55	155	24	144
mPEG5000-NH_2	–	62	165	31	159

[a] Observed as the cold crystallization peak in the second heating scan.

examples, the second heating scans and the cooling scans of copolymer PLLA10-PEG2000, and the macromonomers NHS-CPLLA10 and mPEG2000-NH_2 are shown in Figure 5. A T_g was observed for all NHS-CPLLAn macromonomers, which increased with increasing molecular weight. Melting and cold crystallization were observed for these macromonomers with $n \geq 15$, and increased with molecular weight. These T_m's, T_c's and T_g's are similar to values found for the corresponding AB_2 functional PLLAn, as obtained after ring opening polymerization (Figure 1) (data not shown). The mPEGy-NH_2 macromonomers showed melting and crystallization

Table 4.
Thermal properties of the PLLAn-PEGy copolymers.

		PLLA				PEG			
y ($g \cdot mol^{-1}$)	n (–)	T_m (°C)	ΔH_m ($J \cdot g^{-1}$)	T_c (°C)	ΔH_c ($J \cdot g^{-1}$)	T_m (°C)	ΔH_m ($J \cdot g^{-1}$)	T_c (°C)	ΔH_c ($J \cdot g^{-1}$)
2000	10	77	7	42	9	45	55	-8	45
2000	15	87	10	49	12	43	46	3	35
2000	20	134	24	78	20	42	43	13	42
2000	25	150	26	87	22	40	34	14	32
5000	10	–	–	–	–	57	103	16	87
5000	15	81	4	42	5	52	84	19	83
5000	20	129	12	50	2	56	93	21	90
5000	25	147	11	51	2	57	79	24	80

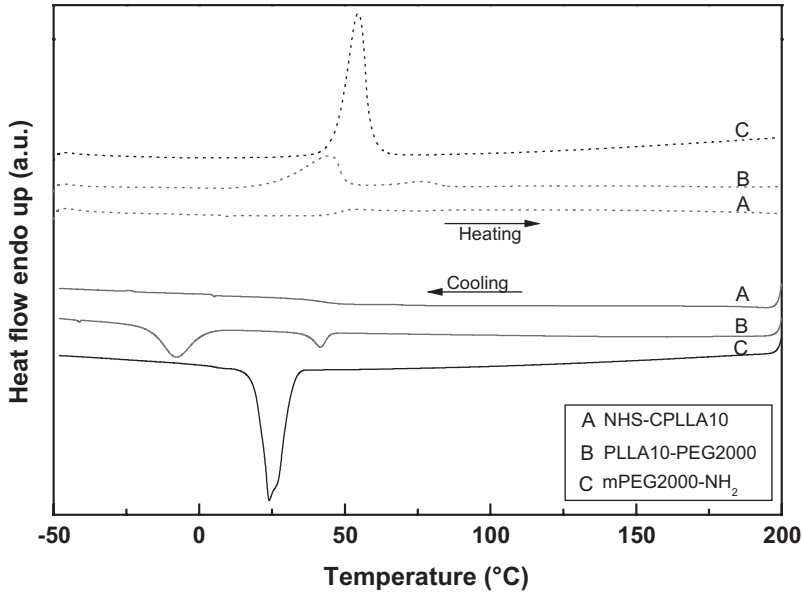

Figure 5.
Second heating scans (dotted lines) and cooling scans (solid lines) of (A) NHS-CPLLA10; (B) PLLA10-PEG2000; and (C) mPEG2000-NH_2.

temperatures that were comparable to the corresponding hydroxyl functionalized mPEG's.

In the second heating scan of the copolymers, a T_g was not observed, due to the low PLLA content in the block copolymers. A melting and crystallization peak was found for the PEG-rich phase in all cases and for the PLLA-rich phase in most cases (Table 4). The copolymer with the highest PEG content of 80 wt% (PLLA10-PEG5000) did not show crystallization of the PLLA. Interestingly, the PLLA10-PEG2000 copolymer exhibited both a T_m and T_c of the PLLA-rich phase, contrary to the corresponding NHS-CPLLA10 macromonomer (Figure 5). All other copolymers showed a T_m of the PLLA-rich phase, and of the PEG-rich phase that was lower than the T_m of the corresponding macromonomers, due to partial phase mixing.

Gel Formation

All PLLAn-PEGy copolymers were soluble in water when n was 10 or 15. The PLLA20-PEG5000 copolymer was soluble, whereas the PLLA20-PEG2000 was not, due to its too high hydrophobic content. Both PLLA25-PEG2000 and PLLA25-PEG5000 copolymers were insoluble in water. All water-soluble copolymers provided transparent solutions or gels, depending on concentration and temperature.

The temperature dependent gelation behavior of aqueous solutions of PLLAn-PEGy copolymers was studied by the vial tilting method in a temperature range of 4–70 °C. The gel-sol transition diagram in Figure 6 shows that all hydrogels turned into a mobile phase, the so-called 'sol', upon heating. It was observed that for all copolymers the gel-sol transition temperature increased with increasing concentration, and that a copolymer concentration of at least 22.5 to 27.5 wt% in water was necessary to form a hydrogel. Furthermore, the gel-sol transition diagram in Figure 6 shows that the gel-sol transition temperature is dependent on the PEG block length. For example, a 30 wt% PLLA10-PEG2000 hydrogel exhibited a gel-sol transition at 22 °C, whereas this transition was found at 56 °C for a PLLA10-PEG5000 hydrogel. Previous studies on linear and star-shaped

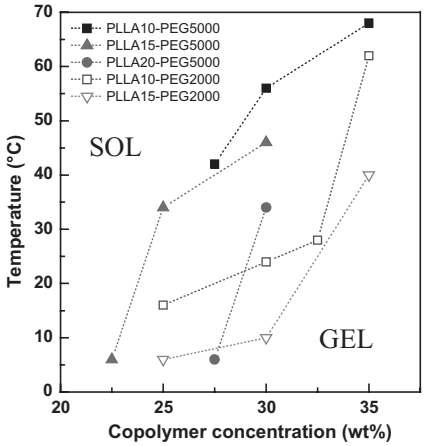

Figure 6.
Gel-sol transition diagram of PLLAn-PEGy copolymers in water.

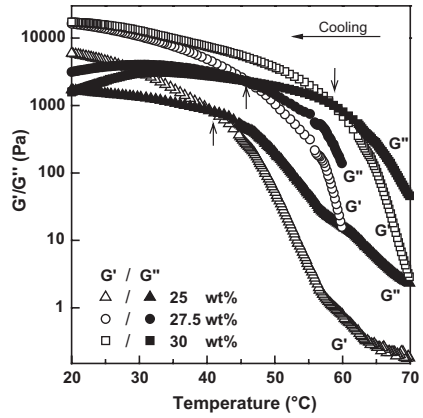

Figure 7.
The storage (G′) and loss (G″) modulus of 25, 27.5 and 30 wt% aqueous solutions of PLLA10-PEG5000, upon cooling from 70 to 20 °C. The arrows indicate the sol-gel transition temperature.

block copolymers with a central PLLA block and outer PEG blocks,[6,7] as well as block copolymers with a central multi-arm PEG block[15] give an increase in the gel-sol transition temperature with increasing hydrophobic block length. Surprisingly, the PLLAn-PEGy copolymers give a reversed behavior, a decreasing gel-sol transition temperature, with increasing PLLA block length. For example, the gel-sol transition temperatures of 30 wt% solutions of PLLAn-PEG5000 copolymer are 56, 46 and 34 °C for n is 10, 15 and 20, respectively.

The thermo-responsive behavior of the hydrogels was also studied by oscillatory rheology experiments on aqueous copolymer solutions. The storage (G′) and loss (G″) moduli were monitored as a function of temperature, when cooling the polymer solution from 70 °C to 20 °C. A system was considered a gel when G′ was larger than G″. The temperature of the cross-over point was considered as the transition temperature.[17] The storage and loss moduli of aqueous solutions of PLLA10-PEG5000 at concentrations of 25, 27.5 and 30 wt% are plotted as a function of temperature in Figure 7. At 70 °C, the G′ of all solutions was lower than the G″, and thus, the copolymer solutions were regarded as sol. While cooling, both G′ and G″ increased and showed a cross-over point, and thus, the solutions showed a transition from sol to gel. At 20 °C, all solutions formed hydrogels, with a G′ increasing from 6 to 17 kPa, with increasing copolymer concentration. Moreover, the transition temperature increased with increasing copolymer concentration, which is in accordance with the vial tilting test results. A considerable higher temperature for the cross-over point of G′ and the G″ was found for a 30 wt% PLLA10-PEG5000 solution compared to a 30 wt% PLLA10-PEG2000 solution (Figure 8A). Moreover, the storage modulus at 20 °C of the PLLA10-PEG5000 (17 kPa) was about three times higher than that of the PLLA10-PEG2000 (6.5 kPa) copolymer.

The rheology experiments revealed a decreasing sol to gel transition temperature with increasing PLLA block length (Figure 8B). It also has to be noted that at 20 °C, the storage modulus of gels with the same PEG chain length decreased almost two orders of magnitude when the PLLA block length was doubled.

Mechanism of the Gel-Sol Transition

The critical association concentration (CAC) of the copolymers in water was

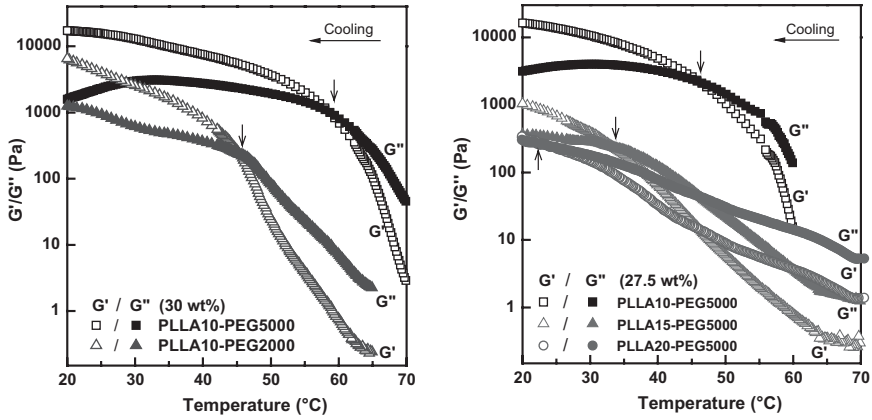

Figure 8.
The storage (G′) and loss (G″) modulus of (A) 30 wt% aqueous solutions of PLLA10-PEGy copolymers, upon cooling from 70 to 20 °C and of (B) 27.5 wt% aqueous solutions of PLLAn-PEG5000 copolymers, upon cooling from 70 to 20 °C. The arrows indicate the sol-gel transition temperature.

measured by UV using the hydrophobic dye DPH. All CAC values of the water-soluble copolymers were in between 0.02 and 0.03 w/v% (Figure 9). The CAC decreased by increasing the hydrophobic block length, or decreasing the hydrophilic block length of the copolymer. Thus, the CAC decreases if the copolymers become more hydrophobic, and therefore tend to associate more readily. The CAC of copolymers PLLA10-PEG2000 and PLLA20-PEG5000 appeared to be independent on the temperature within the 20–50 °C temperature range (data not shown).

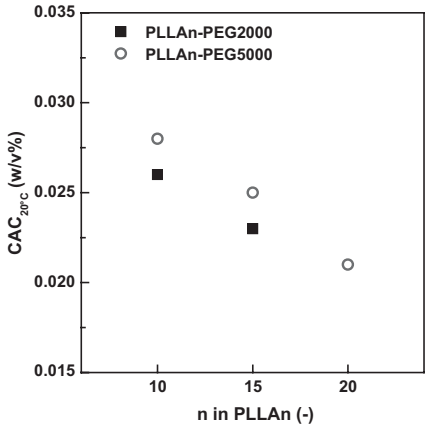

Figure 9.
Critical association concentration (w/v%) of PLLAn-PEGy copolymers in water at 20 °C.

The aggregate size and aggregate size distributions of the copolymers in aqueous solutions were determined using dynamic light scattering (DLS) at a 90° angle, and the results of PLLA10-PEG2000 and PLLA20-PEG5000 at different temperatures are presented as typical examples in Figure 10A and B. The intensity plot of PLLA10-PEG2000 at 25 °C (Figure 10A) shows that mainly micelles with an average diameter of 14 nm are present. A second distribution was observed at 50 nm, which is attributed to small micellar aggregates. It should be emphasized that the intensity of scattered light can not directly be related to the number of particles, since the intensity of light scattered by larger particles is larger than that of smaller particles.

The PLLA20-PEG5000 solution consisted mainly of small micellar-like aggregates with an average diameter of 55 nm (Figure 10B). A second distribution of larger size aggregates of ~200 nm was also found. The shift in size to micellar-like aggregates results from both the higher molecular weight of the copolymer and the length of the hydrophobic block.

Furthermore, it was observed for all aqueous copolymer solutions that the size of the micelles or smaller aggregates was decreasing with increasing temperature, which is attributed to the shrinkage of the

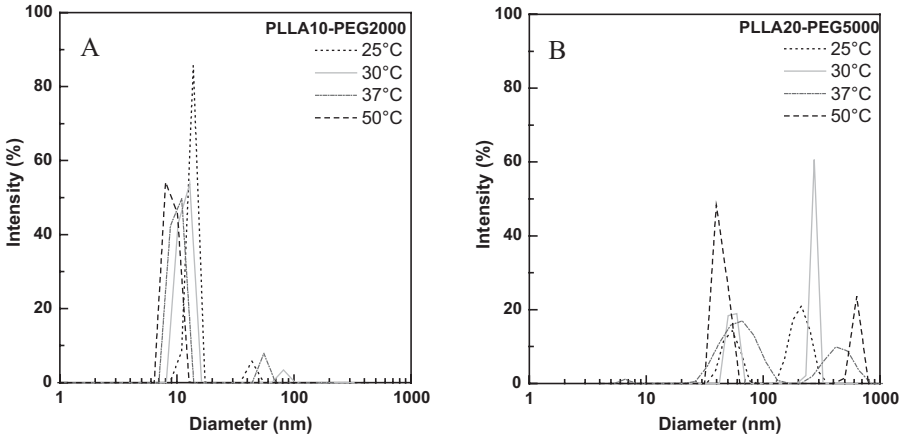

Figure 10.
Intensity plot of aqueous solutions of (A) PLLA10-PEG2000 (1 w/v%) and (B) PLLA20-PEG5000 (0.3 w/v%) at various temperatures.

PEG corona upon partial dehydration.[7] On the other hand, the size of the large aggregates became larger, due to a more favored association of the micelles at higher temperatures that are no longer shielded off by the PEG corona. In Figure 11, the average diameter of the micelles and aggregates of the copolymers in water at 25 °C are plotted as a function of the PLLA block length. It is observed, that the PLLAn-PEG2000 copolymers in water formed smaller micelles and aggregates (Figure 11A) than the corresponding PLLAn-PEG5000 copolymers (Figure 11B), which can be attributed to the smaller PEG chains. Furthermore, an increase was observed in the micelle and especially the aggregate size when increasing the PLLA block length from 10 to 15 repeating lactide units.

The gel-sol transition is proposed to be a result of breaking of the micelle packing structure, due to a decrease in effective diameter of the micelles, which is caused by partial dehydration of the PEG.[7] This

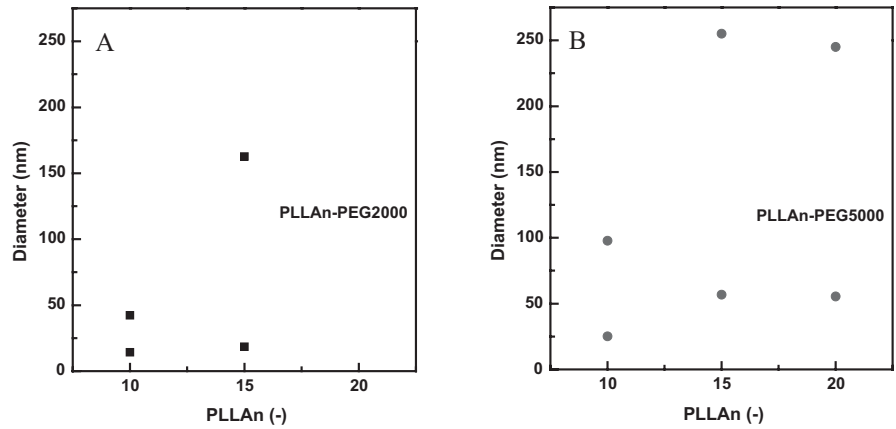

Figure 11.
Diameter of the micelles and aggregates in copolymer solutions (0.3–1 w/v%) at 25 °C plotted as a function of the PLLA block length, with n is the average number of repeating lactide units per arm for (A) PLLAn-PEG2000, and (B) PLLAn-5000.

mechanism is supported by the decrease in micellar size upon heating. Moreover, the higher molecular weight PEG chains have a higher degree of entanglement in the micellar packing, which hampers the transition to the sol phase, as observed from higher gel-sol transition temperatures. It further seems that the gel-sol transition temperature and the storage and loss moduli depend on the uniformity of micellar particles in solution. The presence of larger aggregates may have a negative influence on the packing of micelles into a gel state upon cooling, and irregular packing will result in lower storage and loss moduli than of a packing of micelles of a uniform size. This may be an explanation for the surprising decrease in transition temperature with increasing PLLA block length. Further, it can not be excluded that crystallization takes place in the aqueous state. The previously mentioned triblock and multi-arm copolymers,[6,7,15] and the three-armed PLLAn-PEGy described here show a major difference in the degree of crystallization of the hydrophobic PLLA block in the solid state. At a similar PEG content in the block copolymers, the eight-arm PEG-PLLA copolymers are non-crystalline, whereas the three-arm PLLAn-PEG5000 copolymers show crystallization of the PLLA segments (Table 4). It is speculated that the increased hydrophobic interactions upon increasing temperature may lead to crystallization of PLLA, for longer PLLA blocks, and apparently leads to lower transition temperatures of the gels. It is therefore suggested that crystallization may have a negative effect on the stability of the hydrogels as compared to the previous systems. It is further noted that no degradation is observed for all copolymers during the time period of the measurements.

Conclusion

Branched poly(L-lactide)-poly(ethylene glycol) (PLLA-PEG) copolymers were conveniently synthesized from trifunctional PLLA of controlled molecular weight and amine functionalized methoxy poly(ethylene glycol)s. These copolymers were able to form thermo-responsive hydrogels in water at high concentrations (>22.5 wt%). The gel-sol transition behavior was investigated with the vial tilting method and oscillatory rheology. The transition temperature increased with increasing copolymer concentration, or increasing PEG molecular weight, for copolymers with corresponding PLLA block lengths. The gel-sol transition is considered to be due to partial dehydration of the PEG. Surprisingly, the transition temperature decreased when the molecular weight of the hydrophobic block increased. It is speculated that the non-uniform size distribution of the micelles and aggregates, for longer PLLA blocks at low concentrations, and possible PLLA crystallization may have a negative effect on the micelle packing, resulting in lower transition temperatures, and lower storage and loss moduli. The transition temperature could be tuned closely to body temperature by varying the concentration of the solution or the molecular weight of the PEG block and the PLLA block, which makes these hydrogels of interest as injectable systems for biomedical applications.

[1] B. Jeong, S. W. Kim, Y. H. Bae, *Adv. Drug Deliv. Rev.* **2002**, *54*, 37–51.
[2] E. Ruel-Gariepy, J. C. Leroux, *Eur. J. Pharm. Biopharm.* **2004**, *58*, 409–426.
[3] G. Wanka, H. Hoffmann, W. Ulbricht, *Macromolecules* **1994**, *27*, 4145–4159.
[4] K. Mortensen, W. Brown, E. Jorgensen, *Macromolecules* **1994**, *27*, 5654–5666.
[5] B. K. Lau, Q. Q. Wang, W. Sun, L. Li, *J. Polym. Sci. Polym. Phys.* **2004**, *42*, 2014–2025.
[6] B. Jeong, Y. H. Bae, D. S. Lee, S. W. Kim, *Nature* **1997**, *388*, 860–862.
[7] S. Y. Park, B. R. Han, K. M. Na, D. K. Han, S. C. Kim, *Macromolecules* **2003**, *36*, 4115–4124.
[8] B. Jeong, D. S. Lee, J. I. Shon, Y. H. Bae, S. W. Kim, *J. Polym Sci. Polym. Chem.* **1999**, *37*, 751–760.
[9] S. W. Choi, S. Y. Choi, B. Jeong, S. W. Kim, D. S. Lee, *J. Polym. Sci. Polym. Chem.* **1999**, *37*, 2207–2218.
[10] B. Jeong, Y. H. Bae, S. W. Kim, *Macromolecules* **1999**, *32*, 7064–7069.
[11] D. S. Lee, M. S. Shim, S. W. Kim, H. Lee, I. Park, T. Y. Chang, *Macromol. Rapid Comm.* **2001**, *22*, 587–592.

[12] I. Rashkov, N. Manolova, S. M. Li, J. L. Espartero, M. Vert, *Macromolecules* **1996**, *29*, 50–56.
[13] S. M. Li, M. Vert, *Macromolecules* **2003**, *36*, 8008–8014.
[14] D. W. Grijpma, J. Feijen, *J. Control. Release* **2001**, *72*, 247–249.
[15] C. Hiemstra, Z. Y. Zhong, P. J. Dijkstra, J. Feijen, *Macromol. Symp.* **2005**, *224*, 119–131.
[16] D. L. Elbert, J. A. Hubbell, *Biomacromolecules* **2001**, *2*, 430–441.
[17] C. Y. M. Tung, P. J. Dynes, *J. Appl. Polym. Sci.* **1982**, *27*, 569–574.

