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Carbodiimide-mediated synthesis of poly(L-lactide)-based networks

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ABSTRACT

Poly(L-lactide-*co*-succinic anhydride) networks were synthesised via the carbodiimide-mediated coupling of poly(L-lactide) (PLLA) star polymers. When 4-(dimethylamino)pyridine (DMAP) alone was used as the catalyst, gelation did not occur. However, when 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS), the salt of DMAP and *p*-toluenesulfonic acid (PTSA), was the catalyst, the networks obtained had gel fractions comparable to those which were reported for networks synthesised by conventional methods. Greater gel fractions and conversion of the prepolymer terminal hydroxyl groups were observed when the hydroxyl-terminated star prepolymers reacted with succinic anhydride in a one-pot procedure than when the hydroxyl-terminated star prepolymers reacted with presynthesised succinic-terminated star prepolymers. The thermal properties of the networks, glass transition temperature (T_g), melting temperature (T_m) and crystallinity (X_c) were all strongly influenced by the average molecular weights between the crosslinks ($\overline{M_c}$). The network with the smallest $\overline{M_c}$ (1400 g/mol) was amorphous and had a T_g of 59 °C while the network with the largest $\overline{M_c}$ (7800 g/mol) was 15% crystalline and had a T_g of 56 °C.

1. Introduction

The crosslinking of degradable aliphatic polyesters is known to strongly affect the rate and mechanism of degradation (surface or bulk). Amsden et al. [1] studied both the in vivo and in vitro degradation behaviour of two photocrosslinked *star*-poly(ϵ-caprolactone-*co*-D,L-lactide) elastomers of differing crosslink densities and found that the degradation behaviour of the network with the lower crosslink density suggested a bulk erosion degradation mechanism, displaying a non-linear decrease in Young's modulus, stress at break and mass. In contrast, the degradation of the network with the greater crosslink density, displayed a linear decrease in mass and mechanical strength, which is characteristic of a surface erosion mechanism. The crosslink density of biodegradable networks also affects their mechanical properties, including strength and elastic modulus, as discussed in a recent review [2]. Depending on the ultimate application and projected function of such polymers, the ability to control their degradation behaviour will have important consequences. This is especially true when used in biomedical applications.

There are several approaches that have been developed for the crosslinking of degradable aliphatic polyesters, including peroxide treatment [3] and the addition of a crosslinking agent possessing multiple polymerisable groups into the monomer solution [3–5]. However, if the aim is to produce networks with well-defined $\overline{M_c}$ then the best approach is to crosslink well-defined degradable aliphatic linear or star polyesters through the reaction of terminal end groups with an appropriate multifunctional crosslinking reagent. A number of procedures have been reported that allow degradable aliphatic polyester networks to be synthesised in this way. The most popular involves preparing vinyl-functionalised prepolymers through the reaction of the hydroxyl polymer end groups with acryloyl chloride or another vinyl-containing acid chloride [1,6–14] or with vinyl-containing anhydrides, such as maleic anhydride and methacrylic anhydride [15,16]. The functionalised prepolymers are then cured thermally or with high energy light such as UV to create the network structure. Di- or tri-isocyanates have also been employed to facilitate the formation of crosslinked structures by coupling hydroxyl-terminated star or

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linear prepolymers [17]. However, often these approaches have significant drawbacks. The potential degradation of the polyester during functionalisation [18,19], as well as the presence of residual polyacrylate chains [10] that are released once the polymer system has degraded, are all inherent disadvantages.

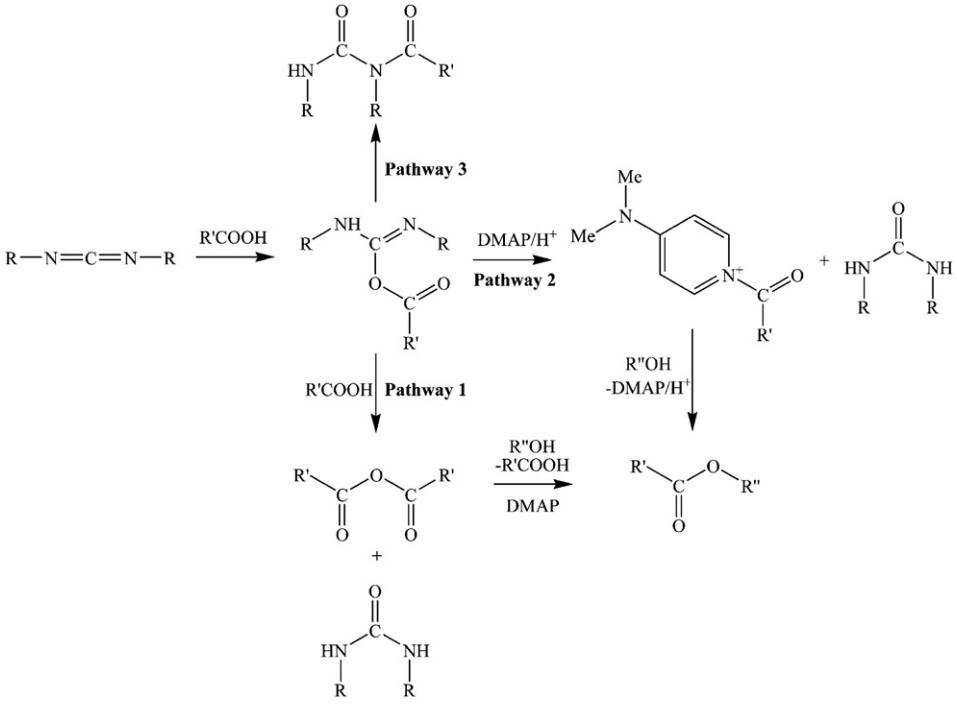
We report here the synthesis of poly(L-lactide)-based networks by carbodiimide-mediated coupling of hydroxyl-terminated star prepolymers with succinic anhydride. Unlike the procedures previously described, this reaction can be performed under mild conditions with a range of commercially available chain extending anhydrides/diacids and should not be expected to release toxic species upon degradation. Furthermore, the chirality of the prepolymer would be expected to be preserved in the network structure [20,21].

Although carbodiimide-mediated coupling has not previously been reported for the synthesis of degradable aliphatic polyester networks, there have been several reports on the use of this technique for synthesising multiblock polymers from biodegradable aliphatic polyester-based prepolymers using 4-(dimethylamino) pyridine (DMAP) as the catalyst (Pathway 1 in Scheme 1) [22–29]. In these studies, the $\overline{DP_n}$ values of the resulting polymers do not appear to exceed 12. In contrast, Moore and Stupp [20] reported good yields and $\overline{DP_n}$ values greater than 50 when they used 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS), the salt of a DMAP and *p*-toluenesulfonic acid (PTSA), as a catalyst for the polycondensation of aliphatic and aryl carboxylic acids and phenols mediated by *N*,*N*′-diisopropylcarbodiimide (DIC) (Pathway 2 in Scheme 1). The addition of PTSA to the reaction mixture, not only increased the conversion but was found to suppress the formation of an unreactive *N*-acylurea side product (Pathway 3 in Scheme 1). This product was observed when only DMAP was used as the catalyst. McKie and Lepeniotis [30] have since successfully used the DPTS catalyst system, as well as the salt of DMAP and triflic acid, to polymerise (RS)-3,3,3-trifluorolactic acid using DIC. More recently, Takizawa et al. [21,31] exploited the high yield of the DPTS-catalysed carbodiimide-mediated coupling reaction in the synthesis of molecularly defined ϵ-caprolactone and L-lactic acid oligomers via a controlled stepwise coupling of protected oligomers. *N*-(3-Dimethylaminopropyl)-*N*′-ethyl carbodiimide hydrochloride (EDC) was the coupling agent of choice for the synthesis of oligomers with 32 or more lactic acid units, due to difficulties in removing the adducts formed when dicyclohexyl carbodiimide (DCC) was used [21]. Importantly, the stereoconfigurations of the lactide units do not appear to have been altered after the carbodiimide-mediated coupling reaction. Several research groups have used this catalyst system to synthesise a range of star [32,33], and dumb-bell shaped dendritic [34] block copolymers, as well as thermoplastic elastomers [35], where at least one component was a biodegradable aliphatic polyester.

2. Experimental section

2.1. Materials

Calcium hydride (99.99%), L-lactide (98%), pentaerythritol (≥99%), succinic anhydride (SA) (99+%), 4-di(methylamino)pyridine (DMAP) (99%), *p*-toluenesulfonic acid (PTSA) (98%), 1,4-dioxane (99+%), *N*-(3-dimethylaminopropyl)-*N*′-ethyl carbodiimide hydrochloride (EDC) (≥98.0%), dicyclohexyl carbodiimide (DCC) (99%) and *d*-chloroform (99.8 atom% D) were all purchased from Sigma–Aldrich. L-Lactide was recrystallised from toluene and then sublimed under vacuum, and used within 2 weeks of purification. Pentaerythritol was purified by sublimation under vacuum. Calcium hydride, purified L-lactide and purified pentaerythritol were all stored under an argon atmosphere in a drybox. 1,4-Dioxane was dried over sodium wire. EDC and DCC were stored under argon at 4 °C. Triethanolamine (TEA) and solvents (toluene, chloroform, dichloromethane, dichloroethane, diethyl ether and *n*-hexane) were all purchased from Ajax Finechem, Australia, as A.R. reagents. Dichloromethane (DCM) was supplied by Australian Chemical Refiners, Australia. Dichloroethane was dried over anhydrous magnesium sulfate. Acrylease™, a glass silanisation product supplied by Stratagene, USA, was used according to the manufacturer's guidelines.



Scheme 1. Reaction pathways for carbodiimide-mediated condensation [20].

Table 1
Hydroxyl-terminated Star PLLA prepolymers used to synthesise the networks.

Sample code	L-lactide (mmol)	Pentaerythritol (mmol)	Calcium hydride (mmol)	Reaction time (min)	Theoretical $\overline{M}_n$[a]	$\overline{M}_n$[b]	No. of polymeric arms[b]	PDI[c]
POH–1A	64.66	5.00	10.00	50	2000	2300	3.8	1.17
POH–2A	67.80	1.67	3.33	600	5900	6000	3.9	1.03
POH–3A	68.44	1.00	2.00	960	9700	9600	3.8	1.02
POH–1B	64.66	5.00	10.00	50	2000	2300	3.8	1.18
POH–2B	67.80	1.67	3.33	600	5900	6100	4.0	1.03
POH–3B	68.44	1.00	2.00	960	9700	9700	3.8	1.03

[a] Calculated from the feed ratio of L-lactide to pentaerythritol assuming complete conversion of monomer to polymer.
[b] Calculated from ^{1}H NMR spectra using Eq. (3).
[c] Calculated from GPC traces.

2.2. Syntheses

2.2.1. Hydroxyl-terminated star PLLA

Star PLLA oligomers were synthesised using pentaerythritol and calcium hydride according to our previously reported method [36]. Briefly, predetermined quantities of reagents were placed in dry glass tubular flasks in a dry box under an argon atmosphere. The tubes were flame-sealed under vacuum and fully immersed in preheated oil baths at 100 °C. After cooling and opening the tubes, the polymer mixtures were stirred in chloroform until the polymer had dissolved and gas formation had ceased. The solutions were then filtered and the solvent was removed by rotary evaporation. ^{1}H NMR was used to ascertain the absence of L-lactide. The formulations used are given in Table 1.

2.2.2. Carboxylic acid-functionalised star PLLA

Carboxylic acid-functionalised star PLLA polymers were synthesised using a method adapted from that used by Zalipsky et al. [37]. Approximately 5 g of star PLLA and a 10-fold excess of succinic anhydride were dissolved in dry 1.4-dioxane. DMAP and TEA were added to give a mole ratio of 1:1:0.25 DMAP:TEA:star PLLA and the solution was stirred at room temperature for 24 h. A white powder was obtained after rotary evaporation of the solution. The crude product was purified by repeated dissolution in dichloromethane and precipitation from a 1:1 mixture of diethyl ether and *n*-hexane. ^{1}H NMR was used to determine the purity of the product.

2.2.3. 4-(Dimethylamino)pyridinium 4-toluenesulfonate (DPTS)

The DPTS catalyst was synthesised using the method described by Moore and Stupp [20] with the exception that toluene was substituted for benzene throughout the procedure. Briefly, PTSA was dissolved in toluene and dried by azeotropic distillation. The stirred solution was cooled to 60 °C and an equimolar solution of DMAP in dry toluene was added. The resulting suspension was cooled to room temperature and the off-white solid was collected by vacuum filtration. The crude product was purified by two recrystallizations from dry dichloroethane. The product was stored in a vacuum desiccator at room temperature.

2.2.4. One-pot synthesis of PLLA-co-succinic anhydride networks (Series A)

Hydroxyl-terminated star PLLA prepolymer and succinic anhydride were mixed in a volumetric flask to give a mole ratio of 1:2 and a final combined concentration of 0.14 g/mL. To this solution, DPTS catalyst was added in 160% excess with respect to the number of hydroxyl groups. The flask was half filled with DCM to dissolve the polymer. Six molar equivalents (of polymer hydroxyl groups) of EDC were weighed under an argon atmosphere and added directly to the flask. After dissolution of EDC, the flask was filled with DCM and shaken to ensure homogeneity. A 20 mL portion of this solution was pipetted into an 8 cm diameter, 3.5 mm thick mould made from Acrylease™-coated glass sheets and a fluoropolymer O-ring. For the optimisation studies, the concentration of reagents in the reaction mixture and reaction time was varied (as detailed in the main text) and 5 mL of the solution was divided between 10 glass vials coated with Acrylease™. All moulds were sealed and allowed to react for predetermined time periods. The polymers were removed from the mould and purified in a Soxhlet extractor with DCM for 48 h. After extraction, the samples were covered with an inverted watch glass and slowly dried at 4 °C for 3 days and then in a vacuum oven at 40 °C for 2 days. Gelation time was recorded as the time when the sample no longer appeared to flow when tilted at a 90° angle for more than 20 s. All samples were stored in a vacuum desiccator.

2.2.5. Two-pot synthesis of PLLA-co-succinic anhydride networks (Series B)

Equimolar amounts of the hydroxyl-terminated star PLLA prepolymer and carboxylic acid-terminated star PLLA prepolymer were combined with a 160% excess of the DPTS in a volumetric flask to give a final combined concentration of prepolymer of 0.14 g/mL The prepolymer mixture was dissolved in a small quantity of DCM before the quantitative addition of 6 mol equivalents of EDC which was predissolved in a small volume of DCM. The flask was filled with DCM and shaken to ensure homogeneity. The solutions were pipetted into vessels and the resulting gels were treated using the same protocol described for the one-pot procedure.

2.3. Measurements

2.3.1. MALLS-GPC

MALLS-GPC analysis was carried out using a Shimadzu, Japan, system with a Wyatt DAWN EOS multiangle laser light scattering detector (690 nm, 30 mW) and a Wyatt OPTILAB DSP interferometric refractometer (690 nm). HPLC grade THF was used as the eluent with three Phenomenex phenogel columns (500, 10^4 and 10^6 Å porosity; 5 μm bead size) operated at 1 mL/min, with the column temperature set at 30 °C. Astra software (Wyatt Technology Corp., USA) was used to process the data using a known d*n*/d*c* value of 0.056 mL/g [38] to determine the molecular weight or an assumption of 100% mass recovery of the polymer where the d*n*/d*c* value was unknown.

2.3.2. ^{1}H NMR

Samples were prepared at a concentration of approximately 0.5 w/v% in $CDCl_3$. All spectra were measured using a Bruker Avance FT-NMR spectrometer (9.39 T, 400.162 MHz). Spectra were referenced to TMS using the $CHCl_3$ residual resonances at 7.26 ppm as an internal calibration.

2.3.3. Specific rotation of polymer solutions

The rotation of polarised light passing through 10.0 g/L polymer solutions were recorded on a Schmidt & Haensch Polartronic Universal Polarimeter using the 584.44 nm sodium line at 20 °C. A

5 mL quartz cell with a path length of 100 mm was used. The reported values are an average of 4 readings.

2.3.4. FT-IR-ATR

Fourier transform infrared attenuated total reflectance (FT-IR-ATR) spectra were collected using a Nicolet Nexus spectrometer equipped with a Nicolet Smart Endurance single bounce diamond ATR accessory. Each spectrum was obtained over the region 4000 cm^{-1} to 525 cm^{-1} at a resolution of 4 cm^{-1} 128 scans were co-added for each spectrum with 2 spectra collected from the surface and 2 spectra collected from the bulk for each sample. All spectra were ATR corrected with the default ATR correction in the OMNIC software (version 7.3). Grams/32 AI (version 6.00) software was used for spectral analysis.

2.3.5. Microanalysis

Microanalysis was performed on a Carlo Erba Elemental Analyser model 1106.

2.3.6. DSC

Differential scanning calorimetry (DSC) was performed using a TA Instruments DSC Q100 instrument. Approximately 5 mg of sample was sealed in an aluminium pan for each measurement. Heal/cool/heat thermograms were recorded using a heating and cooling rate of 10 °C/min. Samples were heated from −80 °C to 150 °C, cooled to −80 °C and then heated to 200 °C. T_m values reported were calculated from the first heating phase, while T_g values were calculated from the second heating phase as the mid-point of the transition. A 50 mL/min N_2 flow rate was used during the analysis.

2.3.7. Swelling measurements

Pieces (7 mm × 7 mm) of polymers were weighed and placed in individual vials, each containing 50 mL of chloroform. The vials were sealed and immersed in a water bath preheated at 25 °C. After 4 days the swollen samples were removed from the vials and wiped carefully with lint-free tissue to remove residual solvent on the surface of the gels before weighing. The following modified version of the Flory–Rehner equation was used to estimate $\overline{M_c}$ from the swelling results [39]:

$$\overline{M_c} = \frac{\left(1-\frac{2}{\Phi}\right)V_1 v_{2r}^{2/3} v_{2m}^{1/3}}{\bar{v}\left[\ln(1-v_{2m}) + v_{2m} + \chi v_{2m}^2\right]} \quad (1)$$

where v_{2r} is the polymer volume fraction of the relaxed gel (i.e. after crosslinking but before swelling), calculated as $[1+(q_F-1)\rho_{polymer}/\rho_{solvent}]^{-1}$, v_{2m} is the polymer volume fraction in the swollen gel at equilibrium, calculated as $[1+(q_S-1)\rho_{polymer}/\rho_{solvent}]^{-1}$, V_1 is the molar volume of the solvent (80.01 cm^3/mol at 25 °C), $\bar{v}$ is the specific volume of dried PLLA (0.80 cm^3/g at 25 °C) [40], χ is the Flory–Huggins interaction parameter (which equals 0.1) [40], Φ is the functionality of the prepolymers (which equals 4), q_F is the ratio of mass of the relaxed gel to the mass of the dry network, q_S is the ratio of the mass of the swollen gel at equilibrium to the mass of the dry network, and $\rho_{polymer}$ and $\rho_{solvent}$ are the densities of the polymer and solvent respectively.

Vernier callipers were used to measure the networks both before and after swelling.

3. Results and discussion

3.1. Synthesis of PLLA star polymers

Table 1 gives a summary of the properties of the hydroxyl-terminated PLLA star polymers used in this study. They possess low PDI values, $\overline{M_n}$ values comparable to the theoretical $\overline{M_n}$ and a high conversion of pentaerythritol hydroxyl groups. Fig. 1 shows the ^{1}H NMR spectrum and band assignments for POH–2A [41,42]. The average number of polymeric arms per molecules and $\overline{M_n}$ values were calculated from the integrated areas of the peaks in the ^{1}H NMR spectra of each prepolymer using Eqs. (2) and (3).

$$\text{No. of arms} = \frac{d'}{d+d'} \times 4 \quad (2)$$

$$\overline{M_n} = \frac{a'+a''+a'''}{2\times a''} \times \text{no. of arms} \times 144 + 136 \quad (3)$$

3.2. Synthesis of succinic acid-terminated PLLA star polymers

Succinic acid-terminated PLLA star polymers were synthesised from prepolymers POH–1B, POH–2B and POH–3B under mild conditions with DMAP and TEA as catalysts. Table 2 shows the properties of these functionalised products. The conversion of hydroxyl groups was determined from the ^{1}H NMR spectrum. Fig. 2 shows the 4.0–4.5 ppm and 2.5–3.0 ppm regions of the ^{1}H NMR spectra of both POH–2B and PCOOH–2B. The intensity of the peaks from the terminal methine (~4.35 ppm) and terminal hydroxyl (~2.69 ppm) observed in the trace of the hydroxyl-terminated star are dramatically decreased after the esterification reaction and a new multiplet at 2.72 ppm is observed. This latter peak has been attributed to the incorporation of the succinic anhydride methylene protons into the polymer structure [43]. Although residual succinic anhydride and succinic acid also resonate around 2.70 ppm in the ^{1}H NMR spectra, sharp singlet peaks would be expected for each of these molecules.

The degree of conversion of the hydroxyl end groups to succinic acid groups was estimated from the ^{1}H NMR spectra using Eq. (4).

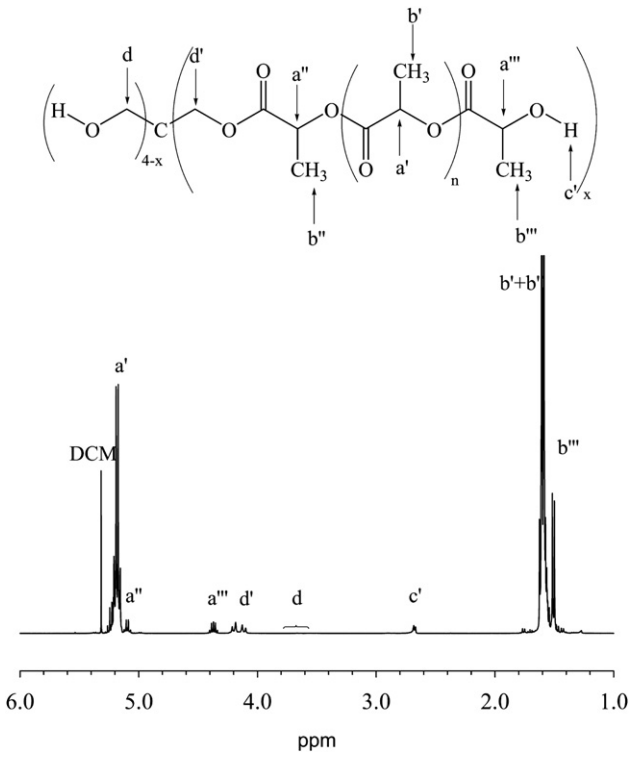


Fig. 1. ^{1}H NMR spectrum of POH–2A and peak assignment.

Table 2
Summary of succinate-terminated star PLLA synthesis.

Sample code	Hydroxyl polymer	Yield (%)	$\overline{M_n}$[a]	PDI[b]	Conversion (%)	SA/junction	$[\alpha]_D^{20}$ POH[c]	$[\alpha]_D^{20}$ PCOOH[c]
PCOOH–1B	POH–1B	43	–	–	97	1.1	−125.1 ± 0.4	−119.3 ± 0.3
PCOOH–2B	POH–2B	74	6400	1.06	~100	1.0	−143.9 ± 0.2	−147.0 ± 0.2
PCOOH–3B	POH–3B	90	9900	1.03	~100	1.0	−140.2 ± 0.1	−144.2 ± 0.2

[a] Calculated from ^{1}H NMR spectra using Eq. (6).
[b] Calculated from GPC traces.
[c] Specific rotation for the hydroxyl-terminated precursor and succinate-terminated polymer respectively.

The ratio of succinate units to pentaerythritol junction units, SA/junction, calculated using Eq. (5), provided an insight into the presence of small quantities of residual succinic anhydride or acid in the final product. This ratio should equal 1 when all hydroxyl end groups have reacted to give succinate groups and all excess succinic anhydride/acid has been removed. Significant crosslinking was not assumed to have occurred based on the work of Zalipsky et al. [37], the low PDI values of the resulting polymers, and the increased ratio of succinic anhydride to polymer used in these reactions. In the cases of PCOOH–2B and PCOOH–2C, the SA/junction ratio was 1.0 and there was no evidence of the terminal lactic acid methine band, a'''. Thus all end groups were esterified and no residual succinic groups were present. However, for PCOOH–1B, residual succinic groups are present in the product. Further attempts to remove these impurities resulted in cleavage of the succinate groups from the polymer. This observation regarding the instability of the succinate terminal groups of the low molecular weight PLLA is in agreement with that reported by Sherman and Storey [44].

$$\%\,\text{Conversion} = \left(1 - \frac{a'''_{\text{succinate}}}{a'''_{\text{hydroxyl}}}\right) \times 100 \tag{4}$$

$$\text{SA/junction} = \frac{\%\,\text{conversion}}{200} \times \frac{f'}{d'} \tag{5}$$

The $\overline{M_n}$ values of the succinate-terminated polymers were calculated from their ^{1}H NMR spectra using Eqs. (2) and (6), where 100 is the molecular weight of succinic anhydride. The molecular weight of PCOOH–1B was not calculated as the excess succinic anhydride or acid present would cause the result to be biased towards a lower value. For the other two polymers, there is an increase in molecular weight observed between the pairs of hydroxyl-terminated and succinate-terminated polymers.

$$\overline{M_n} = \text{no. of arms} \times \frac{2(a' + a'' + a''')}{f'} \times 144 + 136 + \text{no. of arms} \times \text{SA/junction} \times 100 \tag{6}$$

Optical rotation measurements, corrected for the concentration of L-lactide units in each sample, showed no systematic change after the esterification reaction thus confirming that the chirality of the lactide backbone was largely unaffected by the reaction.

3.3. Coupling PLLA star polymers using procedures reported for synthesis of multiblock copolymers

Initial attempts to crosslink hydroxyl-terminated star PLLA polymers $\overline{M_n}$ = 5000–6000 g/mol with succinic anhydride (1:2 ratio PLLA to succinic anhydride) were made according to the procedures described by Huh and Bae [23] for the synthesis of PEG–PLLA multiblocks with succinic acid as a chain extender and DMAP as a catalyst. In our experiments we used DCC (as used in the original procedure) and also EDC to simplify our purification procedure. EDC was our coupling agent of choice because the urea by-product produced is soluble in DCM and can be easily removed from the networks. In contrast, the urea produced from DCC is insoluble in DCM. However, even after 14 days reaction time the solution viscosity appeared to increase only slightly, an indication that the solution did not gel. Variations in concentration of reagents and ratio of reagent to catalyst failed to result in any gelation.

3.4. Coupling PLLA star polymers with DPTS/EDC

According to Moore and Stupp the reaction pathway for the carbodiimide-mediated condensation reaction of carboxylic acids and alcohols catalysed by DMAP requires that an anhydride be formed (Pathway 1 in Scheme 1) [20]. This reaction pathway is

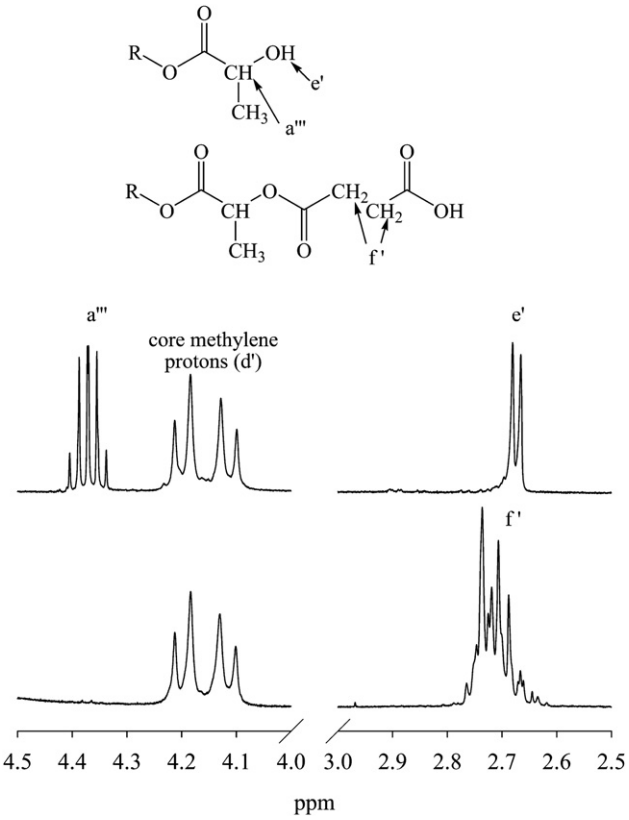


Fig. 2. 4.0–4.5 and 2.5–2.9 ppm regions of the ^{1}H NMR spectra of hydroxyl-terminated star PLLA (top) and succinate-terminated star PLLA (bottom).

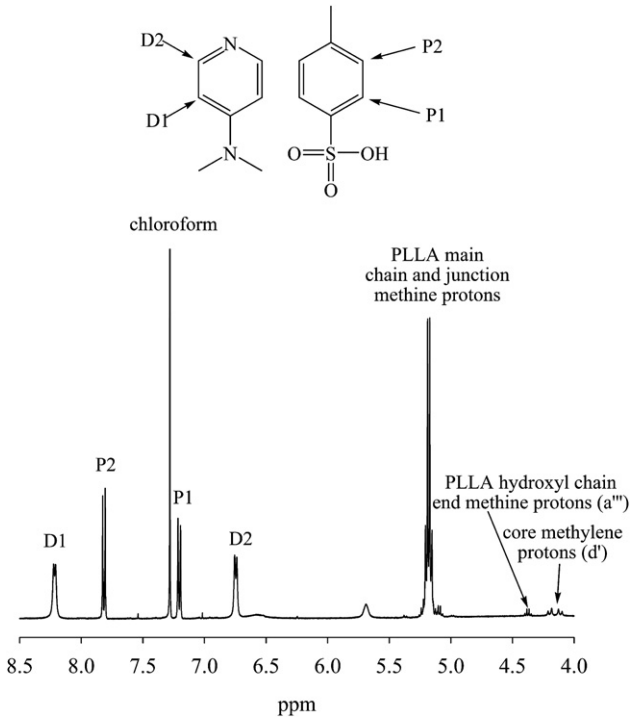


Fig. 3. Typical ^{1}H NMR spectrum of the liquid extract of a PLLA-based network.

associated with a side reaction that converts the carboxylic acid and carbodiimide into an unreactive *N*-acylurea (Pathway 3 in Scheme 1). This side reaction prevents the formation of high molecular weight products. Furthermore, it can safely be predicted that the requirement that 2 acid groups are needed to form the anhydride which then reacts with the alcohol also reduces the rate of reaction. By using DPTS as catalyst the need for an anhydride to be formed is avoided and the formation of the unreactive *N*-acylurea is dramatically reduced. In our studies with this catalyst system, gels were obtained for all star prepolymers within 13 h. In addition, elemental analysis failed to detect any nitrogen in the purified networks, thus confirming that the presence of *N*-acylurea attached to the polymer chains was very low.

Since both DMAP and PTSA are toxic, their removal from the networks needs to be considered. As neither sulphur or nitrogen were detected in the purified networks by elemental analysis, there

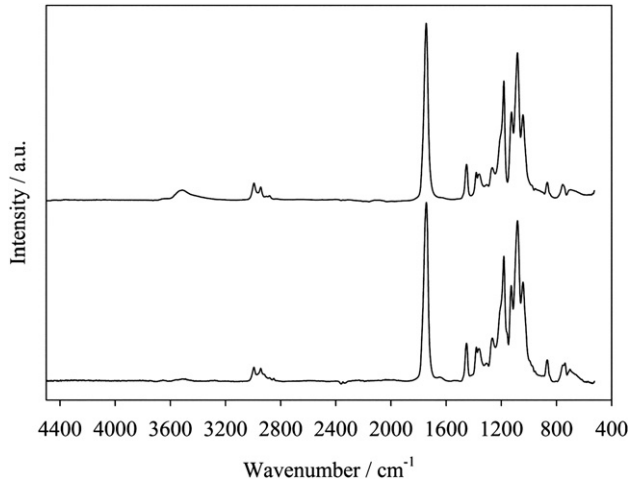


Fig. 4. FT-IR-ATR spectra of POH–1A (top) and network synthesised from this prepolymer (bottom).

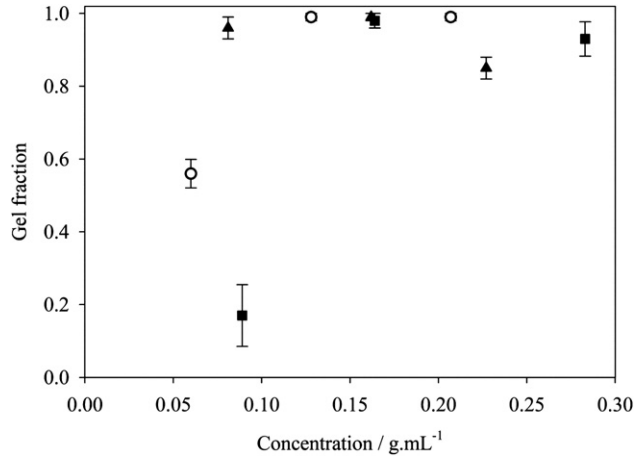


Fig. 5. Gel fraction versus concentration of precursors for networks synthesised from succinic anhydride and POH–1A (▲), POH–2A (○) and POH–3A (■).

appears to be efficient removal of the DPTS catalyst during the Soxhlet extraction step. In addition, the ^{1}H NMR spectra of the water-soluble degradation products of purified networks which were left in basic aqueous solution for several weeks, did not show any peaks that could be contributed to PTSA or DMAP, despite PTSA being water-soluble and DMAP being partially water-soluble.

As this is the first reported synthesis of gels via carbodiimide-mediated condensation reactions, we investigated the factors that control gel fraction and coupling efficiency in more depth. The main focus was on the coupling of hydroxyl-terminated star PLLA with succinic anhydride in a one-pot reaction system.

The gel fraction was determined according to Eq. (7) using the ratio of lactic acid units to PTSA and DMAP in the liquid fraction of the material which had been isolated by Soxhlet extraction. In this equation, a is the integral of all lactic acid methine resonances and x is the integral of resonances from DMAP and PTSA as shown in Fig. 3. The term, $(a/x)_{\text{feed}}$ is the theoretical initial ratio of these resonances based on the quantities of reagents used.

$$\text{gel fraction} = 1 - \left(\frac{\left(\frac{a}{x}\right)_{\text{experimental}}}{\left(\frac{a}{x}\right)_{\text{feed}}} \right) \tag{7}$$

The FT-IR-ATR spectra of POH–1A and a typical network synthesised from this prepolymer are shown in Fig. 4. The most notable difference in these spectra is the reduction of the hydroxyl stretching in the 3300–3600 cm^{-1} region. Consequently, this band was used to estimate the coupling efficiency in the gel fraction using Eq. (8). This equation describes the change in the area of the hydroxyl stretching band when normalised to the carboxylic stretching band. The $(\text{DP}_\text{n} + 4/\text{DP}_\text{n})_{\text{prepolymer}}$ term corrects for the increase in the number of ester bonds on formation of the network. In this equation, the subscript 'prepolymer' refers only to the hydroxyl-terminated prepolymer.

$$\text{Conversion of hydroxyl groups} = \frac{\left(\frac{A_{3750-3300}}{A_{1850-1550}}\right)_{\text{network}} \times \left(\frac{\text{DP}_\text{n}+4}{\text{DP}_\text{n}}\right)_{\text{prepolymer}}}{\left(\frac{A_{3750-3300}}{A_{1850-1550}}\right)_{\text{prepolymer}}} \tag{8}$$

Figs. 5 and 6 show the effect of the concentration of network precursors on both the gel fraction and conversion of the hydroxyl groups after 48 h reaction time. The network precursor concentration was defined as the concentration of hydroxyl-terminated prepolymer and the succinic acid-terminated prepolymer or

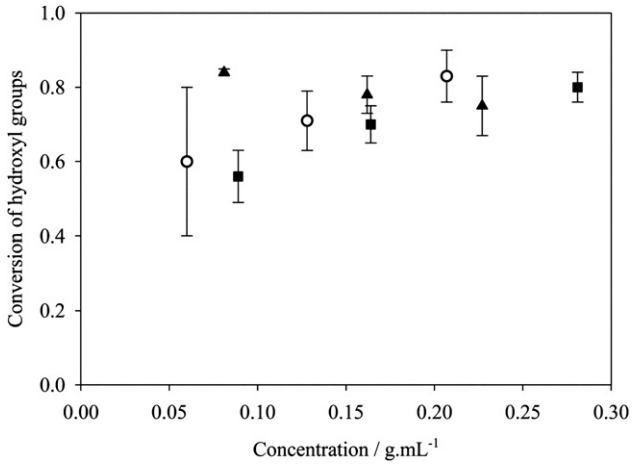


Fig. 6. Conversion of hydroxyl groups versus concentration of precursors for networks synthesised from succinic anhydride and POH–1A (▲), POH–2A (○) and POH–3A (■).

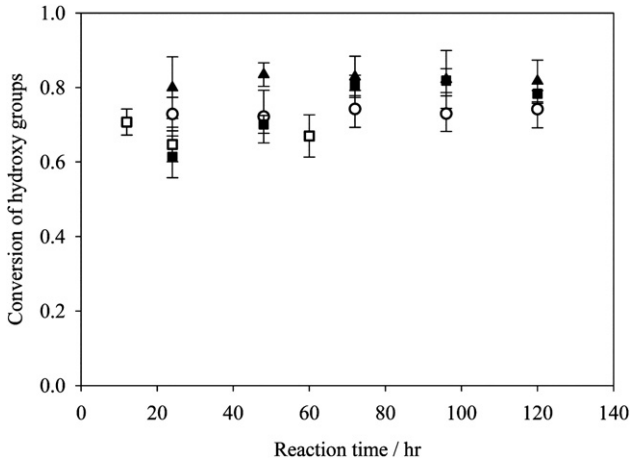


Fig. 8. Conversion of hydroxyl groups versus reaction time for networks synthesised from succinic anhydride and POH–1A (▲), POH–2A (○), POH–3A (■) and POH–3B and PCOOH–3B (□).

succinic anhydride in the DCM-based solution. There is an initial increase in gel fraction with concentration. For all three prepolymer molecular weights, a maximum gel fraction of ~0.97 is obtained for precursor concentration of 0.12–0.20 g/mL. When the precursor concentration was greater than 0.20 g/mL a decrease in the gel fraction was observed. This is possibly due to the increased initial viscosity of the reaction solution which may retard diffusion of the prepolymer and consequently favours the formation of microgel particles. The conversion of hydroxyl groups in the gel also increases with increasing precursor concentration. At ~0.13 g/mL the conversion of hydroxyl groups was ~0.7 for all networks. Based on the data presented in these two sets of experiments, it can be concluded that the optimal precursor concentration was 0.14 g/mL.

The effect of the reaction time was studied for all three prepolymer molecular weights and also for the networks created from POH–3B and PCOOH–3B. Figs. 7 and 8 show the effect of the reaction time on the gel fraction and on conversion of the hydroxyl groups. These graphs clearly demonstrate that the conversion of hydroxyl groups in the gel fraction is independent of the reaction time. However, significant differences were observed in the gel fraction plot. Firstly, the gel fraction for the network synthesised from equimolar amounts of acid and hydroxyl-terminated prepolymers had the lowest gel fraction. The significant difference for this reaction is that intrastar coupling is impossible, unlike the reaction where the network is synthesised from the hydroxyl-terminated prepolymer and succinic anhydride. However, although it was expected that this may affect the conversion of the hydroxyl groups more than the gel fraction, it cannot solely account for the lower gel fraction that is observed. The decreased time to gelation (Table 3) and greater increase in viscosity observed for this reaction strongly suggest that the diffusion of prepolymers may become limited very early in the reaction process. This is in agreement with theoretical calculations for the conversion at the gel point for the two reactions. Using a general expression for the Carothers equation, Eq. (9), and values of 2.67 and 4 for the average initial functionality, f_{avg} in the one-pot (A series) and two-pot (B series) synthesis, the conversion at the gel point were found to be 0.75 and 0.5 respectively.

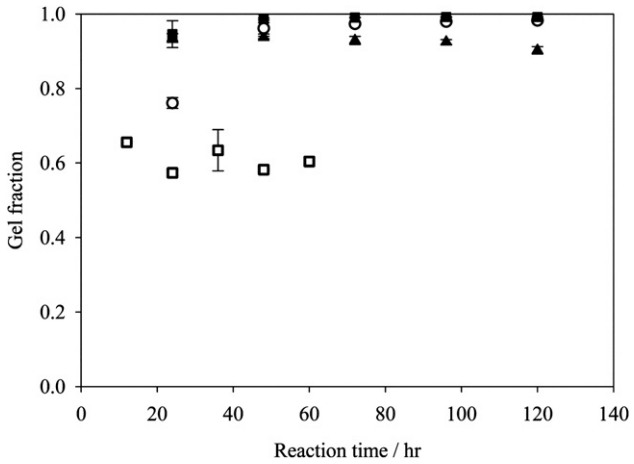


Fig. 7. Gel fraction versus reaction time for networks synthesised from succinic anhydride and POH–1A (▲), POH–2A (○), POH–3A (■) and POH–3B and PCOOH–3B (□).

$$\text{Conversion}_{\text{gel point}} = \frac{2}{f_{\text{avg}}} \tag{9}$$

For the networks synthesised from the hydroxyl-terminated prepolymers and succinic anhydride there is an increase in gel fraction during the earlier stages of the reaction (<48 h) which reaches a plateau at a value of 0.93. Based on the results obtained for these studies the optimal reaction times for each reaction were determined (Table 3). Based on the optimal reaction times measured for the networks synthesised from POH–1A, POH–2A and POH–3A, the synthesis of the networks from equimolar quantities of succinate and hydroxyl-terminated polymers, POH–1B and PCOOH–1B, and POH–2B and PCOOH–2B were assumed to have reached maximum conversion and gel fraction within the same time frame as those synthesised from POH–3B and PCOOH–3B.

3.5. Network properties

A summary of the networks synthesised on a 20 mL scale as thick sheets in a cylindrical mould with optimal precursor concentration (0.14 g/mL) and reaction times is given in Table 3. The much shorter gel time for the B series (two-pot reaction) is immediately apparent, as is the lower gel fraction and hydroxyl conversion for these networks. For each series, there is an increase in gel time with increasing molecular weight of the prepolymer(s). This is accompanied by a decrease in hydroxyl conversion in the gel

Table 3
Summary of gel times and properties of PLLA-based networks.

Sample	Prepolymer/s	Reaction time (h)	Gel time (h)	Gel fraction	Hydroxyl conversion in gel	$\overline{M_c}_{,theoretical}$ (g/mol)	$\overline{M_c}_{,experimental}$ (g/mol)	T_g (°C)	T_m (°C)	X_c (%)
N–1A	POH–1A	48	3.9 ±0.4	0.957 ±0.002	0.87 ±0.02	1300	1400	59	–	0
N–2A	POH–2A	48	7.5 ±0.5	0.978 ±0.008	0.87 ±0.02	3100	2500	57	–	0
N–3A	POH–3A	72	13 ±2	0.984 ±0.008	0.77 ±0.01	4900	3500	57	111.4	13
N–1B	POH–1B PCOOH–1B	24	0.20 ±0.06	0.18 ±0.02	0.87 ±0.08	1300	ND	ND	ND	ND
N–2B	POH–2B PCOOH–2B	24	0.16 ±0.06	0.81 ±0.05	0.69 ±0.03	3200	4700	56	102.4	1.5
N–3B	POH–3B PCOOH–3B	24	0.24 ±0.06	0.68 ±0.05	0.63 ±0.09	5000	7800	56	111.1	15

ND – not determined due to the low yield and poor shape retention during swelling.

fraction. These observations are consistent with the lower concentration of reactive alcohol and acid groups of the prepolymers which consequently lowers the probability of coupling. The gel fractions obtained for these networks are comparable to, and often greater than those synthesised either from star polymers fuctionalised with vinyl end groups [13,14,16] or coupled together using isocyanate linkages [17].

Swelling experiments using $CHCl_3$ were used to estimate the molecular weight between crosslinks, $\overline{M_c}$. Theoretical values were calculated according to the molecular weight of the prepolymer using Eq. (10). The experimental $\overline{M_c}$ values calculated for the Series A are less than, or equal to the theoretical values. In contrast the experimental values are significantly greater that the theoretical values for the Series B (Table 3). This latter observation is consistent with the lower conversion of hydroxyl groups in the gel fraction and the resulting increase in free volume arising from the lower gel fraction and consequential leaching of soluble reagents from the material. Because of this difference between the two series, the swelling data cannot be used to study the occurrence of intrastar coupling which can only occur in the A series.

$$\overline{M_c}_{theoretical} = \frac{\overline{M_n}_{hydroxyl\ prepolymer}}{2} + 100 \tag{10}$$

Thermal analysis of the networks showed that the T_g values of the networks generally decreased with increasing $\overline{M_c}$. This reflects the greater rigidity in the networks with small $\overline{M_c}$ values. Both T_m and % crystallinity, X_c, the latter being estimated using Eq. (11), where $\Delta H_{100\%}$ is 93 kJ/g [45], increased with increasing molecular weight of the prepolymer(s). The networks with the smallest $\overline{M_c}$ values, N–1A and N–2A, were completely amorphous, whereas network N–3B, with the largest $\overline{M_c}$ value of 7800 g/mol was 15% crystalline. This variation in crystallinity is most probably related to several factors, including the decreased chain mobility in the network structure, which is greatest when $\overline{M_c}$ is small [16], the bulkiness surrounding the pentaerythritol core and the length of the PLLA chains, and the length of the PLLA chains. Hao et al. [46] studied the crystallisation of a series of star PLLA polymers with different numbers of arms. They reported that the activation energy of nonisothermal crystallisation increased with an increasing number of arms, hence crystallizability decreased with an increasing number of polymeric arms. In our studies, the lower X_c values obtained from the A series compared to the B series, are consistent with the greater conversion of hydroxyl groups in the gel and corresponding restricted chain mobility.

$$X_c = 100 \times \frac{\Delta H_{melt}}{\Delta H_{100\%}} \tag{11}$$

4. Conclusion

PLLA networks were synthesised using a mild carbodiimide-mediated polyesterification reaction. DPTS proved to be a successful and suitable catalyst for network formation. Gel fractions for our networks were comparable to those obtained using conventional free radical and isocyanate methods. The one-pot method, where hydroxyl-terminated star prepolymers were coupled using succinic anhydride as the chain extender produced optimum results: greater gel fractions, better conversion of hydroxyl groups in the gel fraction and experimental $\overline{M_c}$ values close to theoretical values. As predicted, both T_m and X_c values increased with $\overline{M_c}$ with a concomitant decrease in conversion of hydroxyl groups in the gel fraction. This versatile synthetic strategy lends itself to being easily adapted for the synthesis of a range of different polyalcohol/amine and polycarboxylic acid networks. Unlike other, more studied, crosslinking methods, this strategy should not result in degradation of the polymer during the reaction or release toxic species after implantation. The degradation of our synthesised networks in accelerated in vitro studies has been investigated and will be reported in a future paper.

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