European Polymer Journal 90 (2017) 344–353

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Functional polylactide via ring-opening copolymerisation with allyl, benzyl and propargyl glycidyl ethers

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ARTICLE INFO

Keywords:
Ring-opening copolymerisation
Epoxide
Polymer-conjugate
Fluorescent probe
Azide-alkyne cycloaddition
Polymeric nanoparticles

ABSTRACT

A versatile and simple strategy is presented to synthesize reactive polylactide derivatives and their block copolymers with polyethylene glycol. Commercially available glycidyl ethers with an allyl, benzyl or propargyl functional group were copolymerised with D,L-lactide. Tin(II)-2-ethylhexanoate-catalysis produced polymers with up to 4.6, 5.9 and 2.3 allyl, benzyl or propargyl groups per chain, respectively. In contrast, less than one reactive group per chain was obtained with the organocatalyst 1,5,7-triazabicyclo[4.4.0]dec-5-ene. By increasing the polymerisation feed ratio in glycidyl ether polymers with a higher number of reactive groups per chain were obtained, however a decrease in molar mass was observed. An azidocoumarin was conjugated to the propargylated polymers *via* copper-catalysed azide-alkyne cycloaddition. These dye-labelled polymers produced nanospheres with fluorescent properties and diameters in the 100-nm size-range, as characterised by asymmetric flow field flow fractionation hyphenated with fluorescence, static and dynamic light scattering detection. The functionalised polymers were obtained at gram-scale in one step from commercially available reagents; therefore providing a robust and easy to implement approach for the production of multifunctional nanomaterials.

1. Introduction

Polylactide is widely used in the field of biomaterials and drug delivery due to its exceptional biocompatibility and bioresorbability. The hydrophobic character of this polymer makes it suitable for the preparation of polymeric nanocarriers capable of encapsulating poorly soluble lipophilic drugs in aqueous suspensions. In such applications, where molar masses above 10,000 g mol^{-1} favour *in vitro* [1] and *in vivo* stability of the nanoparticles, PLA is prepared via ring-opening polymerisation (ROP). In order to allow the conjugation of drugs, biological ligands or medical imaging probes great efforts have been made to introduce functional groups along the polymer chain. The most successful approach consists in the copolymerisation of lactide with functional derivatives of lactide or glycolide, as recently reviewed in [2]. The similar reactivities of the co-monomers allow high levels of incorporation of the functional co-monomers. However, the applicability of this method is limited by the difficulty in synthesizing the functional cyclic esters, since the alpha-hydroxy acid precursors require tedious multi-step synthesis and purification. In addition, a “copolymerisation” approach is particularly appealing because it allows the preparation of block copolymers of functional PLA with polyethylene glycol (PEG), the amphiphilic block copolymer of choice for the preparation of long-

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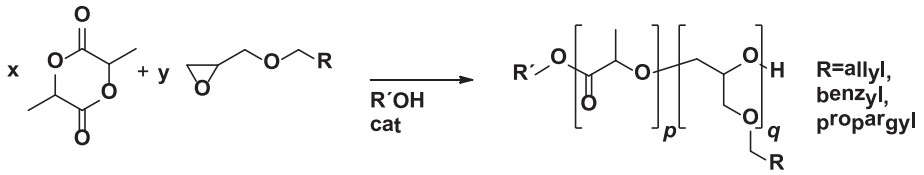
http://dx.doi.org/10.1016/j.eurpolymj.2017.03.028
Received 25 January 2017; Received in revised form 10 March 2017; Accepted 11 March 2017
Available online 14 March 2017



Scheme 1. Introduction of allyl, benzyl or propargyl functional group along a polylactide chain via copolymerisation of D,L-lactide with glycidyl ethers.

circulating nanocarriers [3]. Consequently, more readily-available functional co-monomers have been sought after for copolymerisation with lactide.

Within the library of compounds susceptible to undergo ring-opening (co)polymerisation, glycidyl ethers may be obtained from commercial sources with a variety of functional groups. In addition, conjugation chemistries with these monomers are already described for their homo- and copolymers [4]. In particular, allyl glycidyl ether (AGE) and propargyl glycidyl ether (PGE) incorporated in a polymer allow its direct modification under "click" chemistry conditions, such as thiol-ene for AGE [5] and thiol-yne and the 1,3-dipolar cycloaddition with organic azides for PGE [6]. The benzyl ether group in benzyl glycidyl ether (BGE) is a precursor to the hydroxyl group, readily obtained via catalytic hydrogenation, and for which derivatisation and coupling strategies are available.

Copolymerisation of lactide with glycidyl ethers is an attractive strategy towards functionalised polylactide derivatives for the ease of implementation. Nonetheless, limitations have already been identified, which are a low degree of incorporation of the epoxide in the copolymer, even at high epoxide feed ratio, and a detrimental impact of the initial epoxide feed ratio on the resulting polymer molar mass and molar mass distribution. In the present study we investigate the potential and limits of this approach in terms of co-monomer incorporation for three reactive glycidyl ethers, namely AGE, BGE and PGE (Scheme 1), and the impact on the copolymer molar mass and molar mass distribution. The influence of the ROP catalyst was studied by comparing the copolymer composition, monomer conversion and polymerisation times under $SnOct_2$ or TBD catalysis.

The present synthetic strategy was applied to the preparation of a macromolecular fluorescent probe, whereby an azide-functional coumarin was conjugated to alkyne-functional PLA or PEG-*b*-PLA. The conjugates self-assembled into fluorescent nanospheres, which narrow size distribution was assessed upon fractionation under asymmetric flow field flow fractionation (AF4) with fluorescence, multi-angle light scattering (MALS) and dynamic light scattering (DLS) characterisation.

2. Experimental

2.1. Materials

The monomer 3,6-dimethyl-1,4-dioxane-2,5-dione (D,L-lactide, Sigma, 98%) was purified by three recrystallisations from dry toluene. Benzyl alcohol, used as a polymerisation initiator, was distilled under reduced pressure (160 °C, 70 kPa). The polymerisation catalyst tin (II) 2-ethylhexanoate ($SnOct_2$, Sigma, 95%) was dried under reduced pressure (7×10^{-2} mbar) and stored under argon and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD, Sigma-Aldrich, 98%) was dried by three azeotropic distillations with toluene and stored under argon. 3-(α-Azidoacetyl)coumarin was prepared from 3-(bromoacetyl)coumarin (Aldrich, 97%) according to a literature procedure [7]. Dry dichloromethane (Aldrich, 99%) was stored over 3 Å molecular sieves and degassed under a flow of argon for 30 min prior to use in polymerisations. Allyl glycidyl ether (AGE, Aldrich, > 99%), benzyl glycidyl ether (BGE, Aldrich, 99%), glycidyl propargyl ether (PGE, Aldrich, 95%), poly(ethylene glycol) monomethyl ether ($mPEG_{5k}$, M_n 5000 g mol^{-1} and $mPEG_{2k}$, M_n 2000 g mol^{-1}), poly(ethylene glycol) (PEG_{2k}, M_n 2050 g mol^{-1}), sodium azide (Sigma, 99.5%), dry toluene (Aldrich, 99%), anhydrous Na_2SO_4, $CuSO_4$, L-ascorbic acid, sodium bicarbonate, and PA grade dichloromethane, hexane, propan-2-ol and acetone from VETEC (Brazil) were used as received.

2.2. Characterisation

^1H and ^{13}C NMR experiments were recorded at 25 °C on a Bruker AVANCE DRX400 MHz spectrometer. Copolymers with BGE were analysed in DMSO-d_6 and all other compounds in $CDCl_3$, with tetramethylsilane (TMS) as the internal reference. Chemical shifts (δ) are given in parts per million (ppm). The molar mass distribution of the polymers was characterised by gel permeation chromatography (GPC) on an Agilent Technologies 1260 Infinity unit comprising a solvent degasser, isocratic pump, an Agilent 1260 Infinity UV detector (G1314F) and a differential refractive index detector (G1362A RID at 35.0 °C) in series, a Varian PLgel 5 μm MiniMix-D 50 × 4.6 mm pre-column and two Agilent PLgel 5 μm MiniMix-D 250 × 4.6 mm columns in series at 30.0 °C with HPLC grade THF with 250 ppm BHT stabilizer as the eluent at a flow of 0.25 mL/min. The samples were prepared at a concentration of 3 mg/mL in the eluent, filtered (0.22 μm PVDF Millipore filter) and the injection volume was 20 μL. The system was calibrated using Agilent Technologies EasiVial narrow dispersion polystyrene standards (162–371,100 g mol^{-1}). Fluorescence spectra were recorded on a Shimadzu RF-5301PC spectrometer.

The polymeric nanosphere suspensions were diluted at 1:500 in 1 mM NaCl and characterised in terms of Z-average hydrodynamic diameter (D_h) by dynamic light scattering (DLS) and zeta potential by electrophoretic light scattering on a Zetasizer Nano ZS (Malvern Instrument, UK) equipped with a He-Ne Laser at 633 nm. D_h were calculated with cumulant analysis

using the Zetasizer 7.11 software (Malvern) with the Stokes-Einstein equation and values of 0.8872 cP (water viscosity) and 1.330 (water refractive index).

AF4 fractionation of the polymeric nanospheres was performed on a Postnova analytics (Landberg, Germany) AF2000 MT system equipped with two PN1130 HPLC pumps (tip and focus pumps) and AF2000 module (crossflow pump), PN5300 autosampler, PN4020 Channel oven, a separation channel fitted with a Postnova AF2000 MT Series NovaRC AQU 5 kDa cut-off regenerated cellulose membrane and a 350 μm spacer. The fractionated sample was detected using a PN3211 UV detector with absorbance at 254 nm, and a PN 3412 fluorescence detector with excitation at 310 and emission at 390 nm, the gyration diameter of the fractions were determined with a PN3621 multi-angle laser light scattering (MALS) detector with a 532 nm laser (7–164°, 21 angles) and the hydrodynamic diameter with a Zetasizer Nano ZS (Malvern Instrument, UK – as described above), in series. The carrier liquid was 10 mM NaCl in Milli-Q water filtered on a 0.1 μm PTFE membrane filter (Millipore®). The separation channel and detectors were kept at 25 °C and the detector flowrate was maintained at 0.5 mL/min. The nanosphere suspensions were diluted at 1:3 in the carrier liquid. The injection flowrate was set at 0.2 mL/min with injection time of 3 min, an injection volume of 20 μL and a transition time of 1 min. An initial crossflow of 1.5 mL/min was applied during injection, maintained for 3 min and was set to decrease exponentially to 0.05 mL/min over a period of 15 min and maintained at 0.05 mL/min for 10 min, allowing complete elution of the sample. The gyration diameters (D_g) were determined for each fraction at 3.8 s time intervals using the angular variation of the scattered light intensity at angles 20–156° (19 angles) recorded on the MALLS detector using the Postnova AF2000 software calculation for spherical shape model and D_h at 5.0 s intervals. The differential and cumulative D_g-distributions and distribution parameters D_5, D_{50} and $D_{95\%}$ were determined using the intensity of the UV signal as the value of concentration for each fraction relative to the total sample.

2.3. Polymer synthesis with $SnOct_2$ catalyst

2.3.1. Copolymers of D,L-lactide and glycidyl ethers

In a typical copolymerisation (entry 9, Table 1) D,L-lactide (1.00 g, 7.0 mmol) was weighed in an oven-dried 50-mL Schlenk flask fitted with a 3 × 10 mm magnetic stirbar. PGE (246 μL, 2.2 mmol), benzyl alcohol (38 μL of a solution in toluene at 20 mg/mL) and $SnOct_2$ (155 μL of a solution in toluene at 20 mg/mL) were added. The flask was fitted with a rubber septum. Five cycles of vacuum (1×10^{-1} mbar)/argon were applied. The flask was sealed and placed in an oil bath at 128 °C (temperature inside the flask: 120 °C) with magnetic stirring (360 rpm). The reaction was interrupted at the point where magnetic stirring was impaired due to the high viscosity of the polymerisation medium, by cooling the reaction vessel in an ice bath. The polymers were dissolved in dichloromethane (5 mL), precipitated in hexane (50 mL), re-dissolved in dichloromethane (5 mL), precipitated in propan-2-ol (50 mL) and dried under high vacuum (7×10^{-2} mbar).

Table 1
Influence of the polymerisation conditions on the copolymer composition.

Entry	Glycidyl ether co-monomer	mol% GE in feed	Reaction time (min)	Isolated yield (%)[a]	M_n (g/mol) (Đ)[b]	mol% GE in polymer[c]	Number of GE per chain[d]
	Catalyst: $SnOct_2$						
1	–	0	34	85	31,500 (1.3)	NA	NA
2	AGE	6	52	71	25,600 (1.3)	0.4	0.7
3	AGE	24	99	61	18,500 (1.6)	1.7	2.2
4	AGE	46	192	59	6900 (1.5)	8.7	4.6
5	BGE	6	63	45	24,400 (1.2)	1.5	2.6
6	BGE	24	264	58	8000 (1.4)	5.9	3.5
7	BGE	46	267	99	6800 (1.6)	11.1	5.9
8	PGE	6	32	68	26,300 (1.2)	0.5	0.8
9	PGE	24	180	74	7600 (1.3)	3.2	1.7
10	PGE	46	270	57	4900 (1.2)	6.9	2.3
	Catalyst: TBD						
11	–	0	10	55	8600 (1.3)	NA	NA
12	AGE	24	10	ND	8600 (1.9)	0.2	0.1
13	AGE	24	1440	ND	7800 (2.1)	0.5	0.3
14	BGE	46	10	ND	5900 (1.3)	1	0.3
15	BGE	46	1440	ND	5500 (1.3)	ND	ND
16	PGE	46	10	ND	3700 (1.3)	2.8	0.7
17	PGE	46	1440	ND	3860 (1.3)	3.4	0.9

Experimental conditions: $SnOct_2$ catalyst: lactide:initiator:catalyst = 1000:1:1.1, T = 120 °C, bulk; TBD catalyst: lactide:initiator:catalyst = 88:1:1.1, T = 20 °C, in dichloromethane (1 M lactide). GE glycidyl ether; AGE allyl glycidyl ether; BGE benzyl glycidyl ether; PGE propargyl glycidyl ether; TBD 1,5,7-triazabicyclo[4.4.0]dec-5-ene.

[a] Gravimetric.
[b] By GPC.
[c] By NMR.
[d] Calculated as the product of the degree of polymerisation by gel permeation chromatography and mol% GE in the polymer.

2.3.2. Block copolymers of D,L-lactide and glycidyl ethers with PEG

In a typical copolymerisation (entry 26, Table 3) $mPEG_{5k}$ (0.15 g, 3×10^{-2} mmol) and D,L-lactide (1.50 g, 10.4 mmol) were weighed in an oven-dried 50-mL round-bottom flask fitted with a 3 × 10 mm magnetic stirbar. BGE (111 μL, 0.7 mmol) and $SnOct_2$ (155 μL of a solution in toluene at 20 mg/mL) were added. The mixture was dried by two consecutive azeotropic distillations with 5 mL toluene at 40 °C under reduced pressure on a rotary evaporator. The flask was fitted with a vacuum adaptor and five cycles of vacuum (1×10^{-1} mbar)/argon were applied. Polymerisation and polymer purification were carried out as described above.

2.4. Polymer synthesis with TBD catalyst

In a typical copolymerisation (entries 12 and 13, Table 1) D,L-lactide (0.672 g, 4.7 mmol) was weighed in an oven-dried 50-mL round-bottom flask cooled under an argon atmosphere. AGE (160 μL, 1.5 mmol) and dry dichloromethane (4.6 mL) were added. The flask was fitted with a magnetic stirbar and a rubber septum and the solution was flushed with argon via a needle for 10 min. To this solution 2.50 mL of a solution of TBD and benzyl alcohol (31 μL TBD and 20 μL benzyl alcohol in 8 mL dichloromethane, flushed with argon for 10 min) were added. The polymerisation was allowed to proceed at 20 °C for 10 min and 2 mL of the solution were withdrawn via a dry syringe and quenched with benzoic acid (0.2 mL of a 0.2 M solution in dichloromethane). The remaining solution was quenched with the same benzoic acid solution after 24 h. Dichloromethane was partially evaporated and the polymer was recovered by two successive cycles of solubilisation in dichloromethane and precipitation from propan-2-ol.

All polymers were dried under high vacuum (7×10^{-2} mbar) prior to analysis. The ratios of glycidyl ether to lactide in the copolymers were determined by integrating the signal of the methine protons of the lactide monomer unit at 5.2 ppm (2 × 1H) and the signal of the allyl, benzyl and progargyl group of the glycidyl ether co-monomer at 6.9 ppm (1H), 7.3 ppm (5H) and 2.4 ppm (1H), respectively. The number of GE per chain was calculated as the product of the degree of polymerisation (M_n by GPC divided by 144 – the molar mass of one lactide unit) by the mol percent of GE in the copolymer. Lactide conversions were determined from the isolated gravimetric yield corrected by ^{1}H-NMR data for the presence of residual monomer or solvent and proportion of lactide in the copolymer.

2.5. Conjugation of 3-(α-azidoacetyl)coumarin to alkyne functional polymers

The reaction was carried out on polymers 8–10 in Table 1. In a typical reaction P(LA-*co*-GE), entry 8 in Table 1, 400 mg, approx. 2.8×10^{-5} mmol alkyne) and 3-(α-azidoacetyl)coumarin (18 mg, 7.7×10^{-5} mmol) were dissolved in acetone (3 mL). 1 mL of a freshly prepared solution of $CuSO_4$ and sodium ascorbate (8.4×10^{-6} mmol each, 0.3 eq with respect to alkyne) was added under magnetic stirring. The reaction was allowed to proceed for 4 h at 56 °C under magnetic stirring. Acetone was evaporated under rotary evaporation. Dichloromethane (30 mL) was added and the insoluble fraction was discarded. The organic phase was washed with brine (3 × 10 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was evaporated under rotary evaporation. The polymer was solubilized in dichloromethane, precipitated from hexane followed by two cycles of solubilisation in dichloromethane and precipitation from propan-2-ol to remove unreacted coumarin.

2.6. Preparation of fluorescent nanospheres

The fluorescent polymer nanospheres were obtained by nanoprecipitation [8]. Briefly, for the one-component nanospheres 20 mg of coumarin-labelled block copolymer (obtained by reaction of azidocoumarin with PEG-*b*-P(LA-*co*-PGE) entry 27 in Table 3) were dissolved in 2 mL acetone. The solution was poured slowly in milliQ water (4 mL) under magnetic stirring (360 rpm) at 20 °C. Magnetic stirring was continued for 10 min. Acetone and part of the water were evaporated under reduced pressure at 25 °C to a volume of 2 mL, thus obtaining a suspension of nanospheres at a concentration of 10 mg of polymer per mL. The two-component nanospheres were obtained from a mixture of 10 mg of coumarin-labelled copolymer (obtained from P(LA-*co*-PGE) entry 8 in Table 1) and 10 mg of PEG-*b*-PLA block copolymer (M_n 17 kDa, prepared from $mPEG_{5k}$). The suspensions were characterised in terms of particle hydrodynamic diameter, zeta potential and luminescent properties in batch and by AF4.

3. Results and discussion

3.1. Incorporation of the glycidyl ether functional group along the polymer chain

PLA derivatives with allyl, benzyl and propargyl side-groups and their block copolymers with PEG were obtained in high yield (up to 99% lactide conversion) in bulk at 120 °C with $SnOct_2$ as the catalyst (Tables 1 and 2). Incorporation of the glycidyl ether co-monomer was confirmed via ^{1}H-NMR spectroscopy of the isolated polymer (Fig. 1 and supplementary material). The degree of co-monomer incorporation was calculated from the integration of the functional group of the glycidyl ether (signal “a” in all spectra on Fig. 1). Consequently, the values indicated in Tables 1 and 2 correspond to the amounts of functional groups per chain available for subsequent derivatisation of the copolymers. Additional 2D homonuclear and heteronuclear correlation NMR spectroscopy experiments were carried out on the copolymer prepared with the highest composition of PGE (46 mol% PGE in the feed, entry 10 in Table 1, NMR spectra in supplementary material). Thereby the signal corresponding to the terminal alkyne group along the copolymer chain was unambiguously assigned (δ [^{1}H] = 2.47 ppm, 1H, C≡C–***H***, δ [^{13}C] = 75 ppm, 1H, C≡***C***–H).

The chemical shift for the methine protons of the GE incorporated in the copolymer backbone, close to 5.2 ppm, indicates its

Table 2
Influence of the catalyst and initiator concentrations on the polymer composition and molar mass distribution.

Entry	Glycidyl ether co-monomer	LA:Ini:cat molar equivalents	mol% GE in feed	Isolated yield (%)[a]	M_n (g/mol) (Đ)[b]	mol% GE in polymer[c]	Number of GE per chain[d]
18	–	50:1:1.1	0	98	8750 (1.5)	NA	NA
19	AGE	50:1:1.1	6	95	7700 (1.5)	0.4	0.2
20	BGE	50:1:1.1	7	99	9300 (1.5)	2.6	1.7
21	PGE	50:1:1.1	6	94	7200 (1.5)	0.7	0.3
22	–	1000:1:11	0	94	29,900 (1.3)	NA	NA
23	PGE	1000:1:11	6	99	23,500 (1.4)	0.5	0.8
24	PGE	1000:1:11	24	100	11,600 (1.7)	1.5	1.2

LA: D,L-lactide; Ini: benzyl alcohol; cat: $SnOct_2$; Reaction conditions: bulk, 120 °C; Reaction time: 25 min. GE glycidyl ether; AGE allyl glycidyl ether; BGE benzyl glycidyl ether; PGE propargyl glycidyl ether.

[a] Gravimetric.

[b] By GPC.

[c] By NMR.

[d] Calculated as the product of the degree of polymerisation by gel permeation chromatography and mol% GE in the polymer.

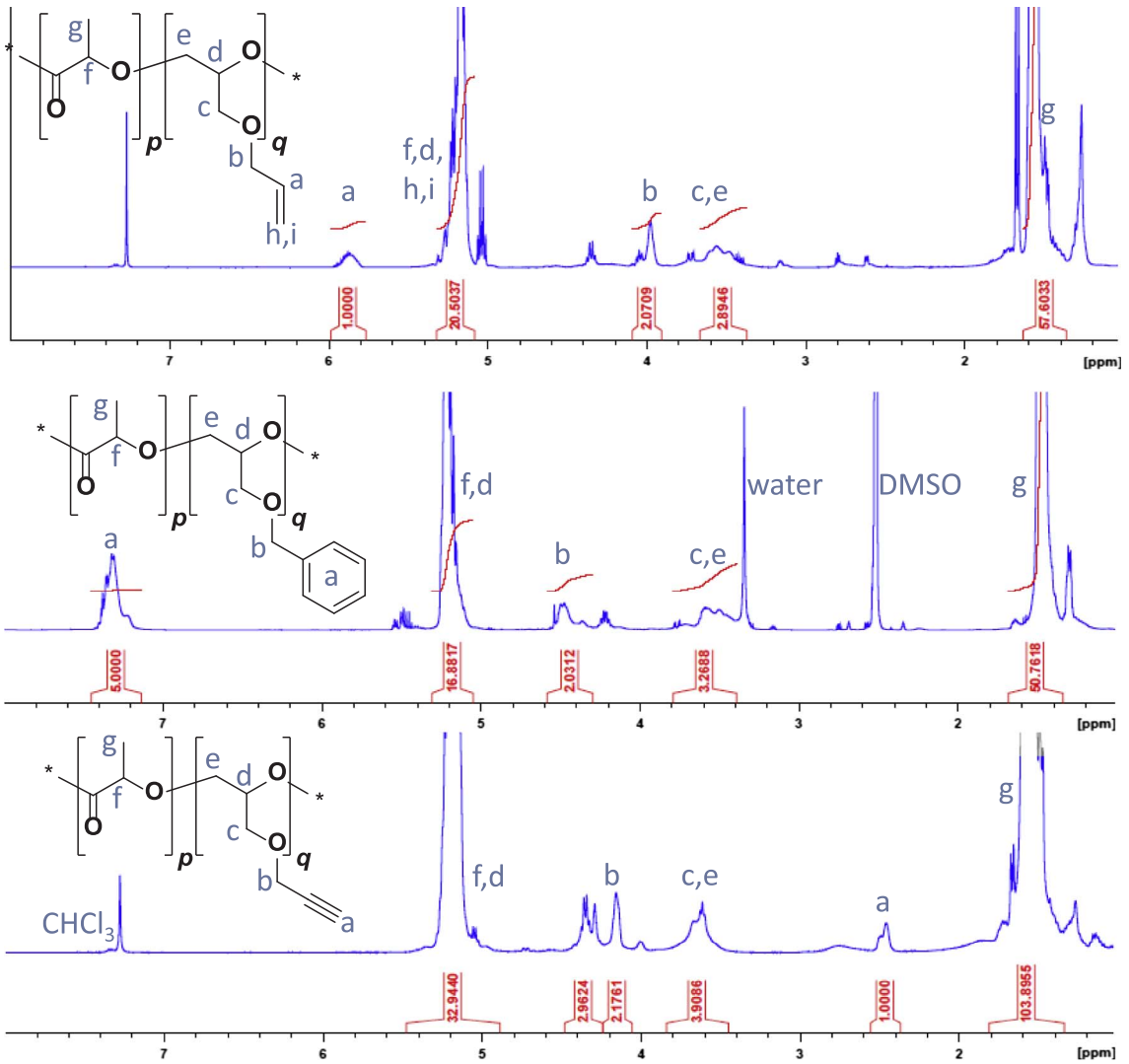


Fig. 1. ^1H NMR spectra of poly(lactide-*co*-glycidyl ethers) obtained by $SnOct_2$-catalysed copolymerisation of D,L-lactide with 46 mol% glycidyl ether in the feed. Integration of signal "a" was used to determine the mol% of glycidyl ether in the polymer.

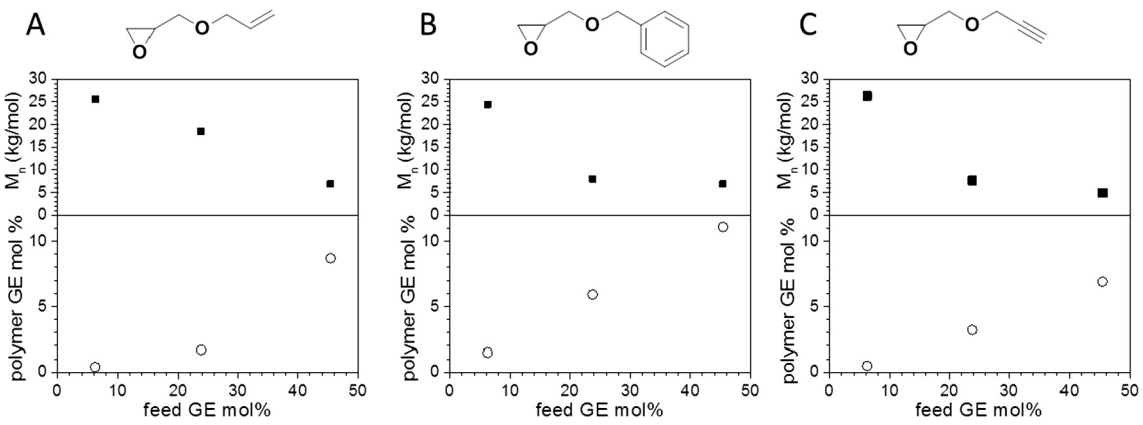


Fig. 2. Evolution of copolymer molar mass (■) and degree of functional group incorporation (○) as a function of the feed composition in the $SnOct_2$ catalysed bulk copolymerisation of lactide with allyl (A), benzyl (B) and propargyl (C) glycidyl ethers (GE) at 120 °C (n = 1 for each data point).

position adjacent to an electron-withdrawing moiety, such as the ester group of a lactide unit. Such a downfield chemical shift not only indicates that the GE co-monomer was mostly incorporated between lactide monomer units, as opposed to forming block-like GE segments, but also that the nucleophilic attack on the oxirane ring occurred on the least substituted carbon, as represented on the polymer chemical structures in Fig. 1. Previous literature reports indicate that the broad quartet at 4.3 ppm in $CDCl_3$ and 4.1 ppm in DMSO-d_6 are due to the methine proton signal of a terminal lactic acid unit at the polymeric hydroxyl chain-end [9] and could overlap with the methine signal of the lactide adjacent to a GE unit.

The percentage of glycidyl ether incorporated in the copolymer increased with increasing ratios of glycidyl ether in the polymerisation feed (Table 1 and Fig. 2). The ratio of incorporation also varied with the nature of the glycidyl ether functional group. A higher ratio of incorporation was obtained with BGE than with the other co-monomers. At high GE feed content the incorporation ratios followed the order: propargyl < allyl < benzyl. This result could be due to differences in reactivity of the oxirane ring, steric constraints or differences in glycidyl ether stability during ROP.

3.2. Influence of the co-monomer feed ratio on the polymer composition and molar mass distribution

A systematic decrease in molar mass was observed with increasing GE percentages in the feed and in the polymer (Fig. 2). The number of functional groups per chain was calculated based on the average molar mass of the polymer obtained by GPC and the percentage of GE by NMR (Table 1). While higher GE content in the feed affected significantly the molar mass, as many as 4.6, 5.9 and 2.3 allyl, benzyl and propargyl functional groups per chain, respectively, were incorporated in the copolymer (Table 1, entries 4, 7 and 10) with a GE feed content of 46 mol%. Such values, higher than unity, confirmed that copolymerisation did occur and that the decrease in molar mass observed with increasing GE in the feed and in the polymer were not due to systematic chain-termination upon GE incorporation, which would have resulted in a maximum of two GE units per chain.

A few examples are reported in the literature, where lactide was polymerised in the presence of epoxides in order to introduce functional groups along the polymer chain. In [10], the authors reported up to 8 mol% of incorporation of 1,2-epoxy-5-hexene with L-lactide and $Al(Et)_3{\cdot}H_2O$ as the catalyst (M_n = 16,200 g mol^{-1}, PDI = 2). Nadeau and co-workers [11] polymerised D,L-lactide in the presence of up to 30 mol% of AGE with tetraphenyltin and reported a decrease in molar mass from 19,700 to 3300 g mol^{-1} upon increasing the AGE content in the feed from 2 to 30 mol%, respectively. Recently, D,L-lactide was polymerised in the presence of propargyl 3-methylpentenoate oxide [12] and a decrease in molar mass from 5900 to 2700 g mol^{-1} was also observed upon increasing the epoxy ester co-monomer content in the feed from 5 to 30 mol%, respectively. Heiny et al. made similar observations with other epoxy ester co-monomers [13]. In most of these studies, the molar mass dispersity was higher (1.5–2.0) than the values usually reported for PLA homopolymer prepared under similar conditions with $SnOct_2$ (1.2–1.3 under our experimental conditions, Table 1, entries 1 and 11). Variations in molar mass dispersity between 1.2 and 1.6 were observed in our study (entries 1–17) but no correlation with any specific reaction parameter could be identified. Dispersity values remained significantly lower than 2, indicating that chain-transfer, whereby a chain is terminated upon GE incorporation and a new chain is initiated, is unlikely to be the main cause for the decrease in M_n with increasing GE content in the feed/copolymer. Instead, participation of the GE in chain-initiation, resulting in a higher number of chains being initiated with higher GE content in the feed would explain the decrease in M_n and limited effect on the molar mass dispersity. Longer polymerisation times were required to reach similar (or lower) lactide conversions in the presence of GE co-monomers (see entries 1, 2, 5, 8 in Table 1). Further kinetic studies would be required to identify the cause of such a decrease in lactide polymerisation rate in the presence of GE.

3.3. Influence of the catalyst on the polymer composition and molar mass distribution

$SnOct_2$ and TBD were tested as the copolymerisation catalysts. TBD, a strong organic base, is used as a ROP catalyst in lactide polymerisation [14,15] and has been employed as a catalyst for the ring-opening of epoxides with nucleophiles [16].

Table 3
Block copolymers of poly(lactide-*co*-glycidyl ethers) with polyethylene glycol.

Entry	Glycidyl ether co-monomer	Initiator	mol% GE in feed	Reaction time (min)	Lactide conversion (%)[a]	mol% GE in copolymer[b]	M_n (g/mol) (Đ)[c]
25	–	$mPEG_{5k}$	0	25	89	NA	41,700 (1.3)
26	BGE	$mPEG_{5k}$	6	25	96	1	22,000 (1.2)
27	PGE	$mPEG_{5k}$	6	25	85	1	22,600 (1.3)
28	–	PEG_{2k}	0	21	80	NA	13,300 (1.4)
29	BGE	PEG_{2k}	6	31	76	2	11,000 (1.2)
30	BGE	$mPEG_{2k}$	6	40	63	2	8200 (1.2)

Experimental conditions: $SnOct_2$ catalyst, T = 120 °C, bulk; lactide:$mPEG_{5k}$:catalyst = 330:1:1.1; lactide:PEG_{2k}:catalyst = 260:1:1.1. GE glycidyl ether; AGE allyl glycidyl ether; BGE benzyl glycidyl ether; PGE propargyl glycidyl ether.
[a] Gravimetric.
[b] By NMR.
[c] By gel permeation chromatography.

Polymers with less than one GE per chain were obtained when TBD was used as the catalyst (Table 1 entries 12–17), even at high GE contents in the feed. The reaction was left to proceed for up to 24 h in order to identify possible insertion of the epoxide via transesterification, as reported for poly(ε-caprolactone) under more strenuous conditions (using a stronger organic base and higher temperature) [17]. A slight increase in the polymer GE content was observed between the samples obtained after 10 min of reaction and 24 h (Table 1) but remained lower than one GE per chain. Other authors found that at 10 min lactide conversion was almost quantitative and longer reaction times lead to TBD-catalysed depolymerisation [18]. Limited depolymerisation could not be excluded in our case, where a slight decrease in molar mass was observed between 10 min and 24 h reaction time (entries 20 and 24).

In a pioneering study by Chen and co-workers [19], L-lactide was polymerised in the presence of ethylene oxide at 60 °C and a variety of metal catalysts. The ^1H NMR spectra of the resulting polymers indicated the presence of poly(ethylene oxide) and PLA segments, suggesting a multiblock-like nature of the copolymer. At higher reaction temperature (130 °C) the segments had shorter lengths. Pitet and co-workers [20] tuned the polymerisation temperature to obtain epoxy chain-end functional PLA using glycidol as the ROP initiator. At 80 °C in toluene with $SnOct_2$ as the catalyst, the oxirane ring was left untouched, whereas at 130 °C in bulk a branched polymer was obtained due to ring-opening of the oxirane ring of glycidol and its incorporation in the PLA chain. It is possible that higher degrees of GE incorporation were obtained in our study with $SnOct_2$ as the catalyst than with TBD because of the higher reaction temperature with $SnOct_2$ increasing the reactivity of the oxirane ring. However, previous studies demonstrated better control over lactide polymerisation and higher conversions under TBD catalysis at lower temperatures (as low as −10 °C) [18] and we were not able to obtain polymers with TBD as the catalyst at high reaction temperature (120 °C).

Altogether our data indicates that TDB was not efficient in catalysing the ring-opening copolymerisation of glycidyl ethers with lactide. Consequently, $SnOct_2$ was used in subsequent work to prepare block copolymers of functional PLA with PEG (Table 3).

3.4. Influence of the catalyst ($SnOct_2$) and initiator concentrations on the copolymer characteristics

Decreasing the ratio of monomer to initiator from 1000:1 (Table 1) to 50:1 (Table 2) with 6 mol% of GE in the feed produced shorter copolymers. With a ratio of monomer to initiator of 50:1 the polymer number average molar masses were close to the values expected, should each benzyl alcohol molecule initiate one copolymer chain (7200 g mol^{-1}at 100% conversion). At high monomer to initiator ratio (1000:1), the molar masses of the polymers deviated greatly from the expected value (close to 25 kg mol^{-1} with 6 mol % GE in the feed instead of 144 kg mol^{-1}), indicating that some chains were not initiated by benzyl alcohol. The GE copolymer content was slightly lower when the initial monomer to initiator ratio was set at 50:1 instead of 1000:1. These observations support the hypothesis that GE contributed to chain initiation.

A higher $SnOct_2$ concentration while maintaining the monomer to initiator ratio at 1000:1 (catalyst to initiator ratio 11:1, Table 2 entries 22–24, instead of 1.1:1, Table 1 entries 1, 8 and 9) produced copolymers of D,L-lactide and PGE with similar molar masses and GE contents in shorter reaction times, however, with a higher molar mass dispersity (see in particular entry 24 in Table 2). The polymerisation time of 25 min was sufficient to reach quantitative lactide conversion for all experiments in Table 2, most likely because the higher catalyst concentrations used in comparison with the polymerisations presented in Table 1 resulted in higher rates of propagation [21]. Nonetheless, the lower catalyst content was selected for the synthesis of polymers used in nanosphere preparation in order to guarantee control over the polymer molar mass distribution and minimize potential residual Sn in the formulations.

3.5. Conjugation of a fluorescent azidocoumarin to alkyne-functional polymers via copper-catalysed Huisgen-1,3-dipolar cycloaddition

An azide-functional coumarin was conjugated to alkyne-functional PLA and PEG-*b*-PLA block copolymers via copper-catalysed Huisgen-1,3-dipolar cycloaddition to demonstrate the availability and reactivity of the alkyne group on the polymer backbone (reaction scheme in Fig. 3). The GPC traces of the conjugates prepared from a polymer containing approximately 1% alkyne functional groups showed strong UV absorption at 320 nm, confirming covalent attachment of the coumarin to the polymer (Fig. 3A).

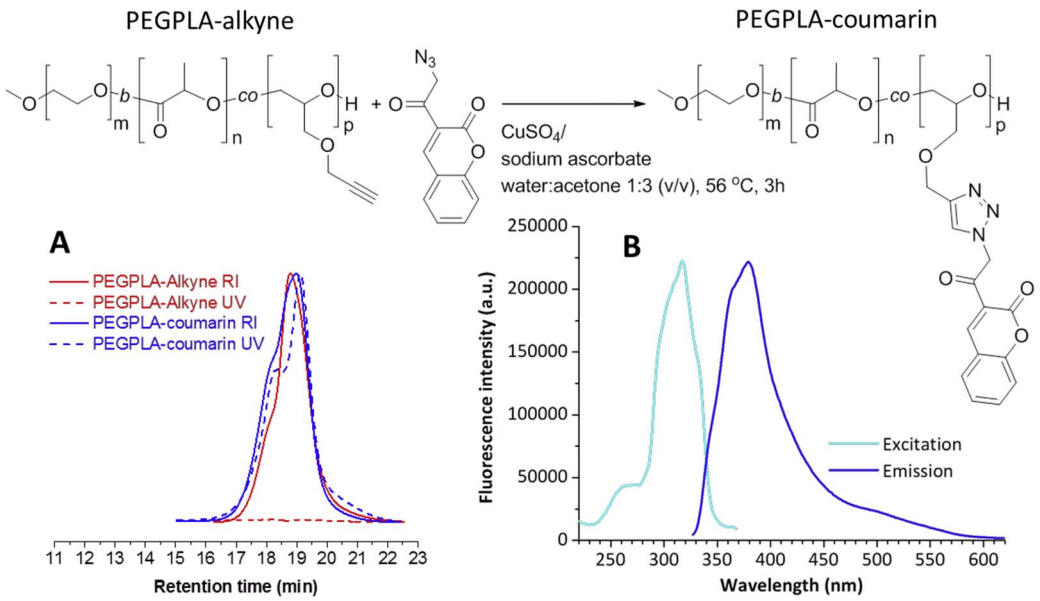


Fig. 3. Reaction scheme for the preparation of a polyethylene glycol-*block*-poly(lactide-*co*-propargyl glycidyl ether)-coumarin conjugate and characterisation of the polymer by gel permeation chromatography in THF with UV (320 nm) and refractive index (RI) detection (A) and fluorescence spectroscopy in acetonitrile (B).

The polymeric coumarin and triazole linkage were identified via NMR spectroscopy (supplementary information). The polymer showed luminescent properties in solution in acetonitrile (Fig. 3B) with a shift towards lower excitation and emission maxima upon formation of the triazole, from 450 to 319 nm and 510 to 388 nm for the excitation and emission maxima of the azido-coumarin (supplementary material) and polymer-coumarin conjugate, respectively.

Polymeric nanospheres were obtained from the PEG-*b*-PLA-coumarin conjugate (one-component nanospheres) or a mixture of PLA-coumarin conjugate and non-labelled PEG-*b*-PLA (two-component nanospheres) by the nanoprecipitation method [8]. Fractionation by AF4 with fluorescence detection revealed that the nanospheres had luminescent properties throughout their size distribution (Fig. 4). Absolute determination of the nanoparticle diameters by dynamic and static light scattering in flow mode confirmed the monodisperse character of the distribution.

The one-component nanospheres had a lower hydrodynamic diameter (66 nm) than their two-component counterparts (101 nm). In order to keep the PEG content in each formulations at 10 wt% while maintaining the PEG block length at 5 kDa, the block copolymer used in the two-component nanospheres had half the PLA content (or PLA molar mass) of that used in the one-component nanospheres. The size of self-assembled PEG_{5k}-*b*-PLA nanospheres typically increases with increasing molar mass of the PLA block [22]. Interestingly, the nanospheres composed of PEG_{5k}-*b*-PLA_{40k} only were smaller than those composed of a mixture of PLA_{22k} homopolymer and PEG_{5k}-*b*-PLA_{21k}, indicating that the polymer composition distribution had a stronger influence on the nanoparticle size than the polymer chain-length. The zeta potentials of both nanosphere formulations measured in batch were close to neutrality (Table 4). PLA-based nanospheres prepared by this method typically have negative zeta potentials (lower than −30 mV) [23], which suggests the PLA-*b*-PEG block copolymer with a PEG block of 5 kDa used in the proportion of 10 wt% with respect to the total polymer content was able to shield the negative surface charges by forming a PEG corona located at the nanoparticle interface [23,24].

These well-defined single- or two-component fluorescently labelled nanostructures could potentially serve as drug carriers and imaging agents for *in vitro* applications. Fluorescent labels suitable for *in vivo* imaging [25,26] could be conjugated to the polymers following the same synthetic strategy. Covalent attachment of the fluorescent label to the nanoparticles circumvents the issue of organic dye leakage or release from the nanoparticles and thus guarantees co-localisation of the dye with the nanoparticles in *in vivo* biodistribution studies. In addition, some of the brightest fluorescent probes have been obtained with organic nanoparticles [27] and the use of the bioresorbable polylactide is particularly attractive in the biomedical field.

4. Conclusions

Copolymers of allyl, benzyl and propargyl glycidyl ethers with D,L-lactide were obtained. Up to 8.7, 11.1 and 6.9 mol% of allyl, benzyl and propargyl functional groups, respectively, were incorporated along the polymer backbone with $SnOct_2$ as the polymerisation catalyst. Good control over the molar mass distribution was maintained. However, interference of the GE with the molar mass was observed, whereby the higher the ratio of GE co-monomer in the feed, the lower the copolymer average molar mass. TBD as the catalyst produced mostly PLA homopolymer, with less than 1 mol% of functional group in the polymer even at high feed content in GE.

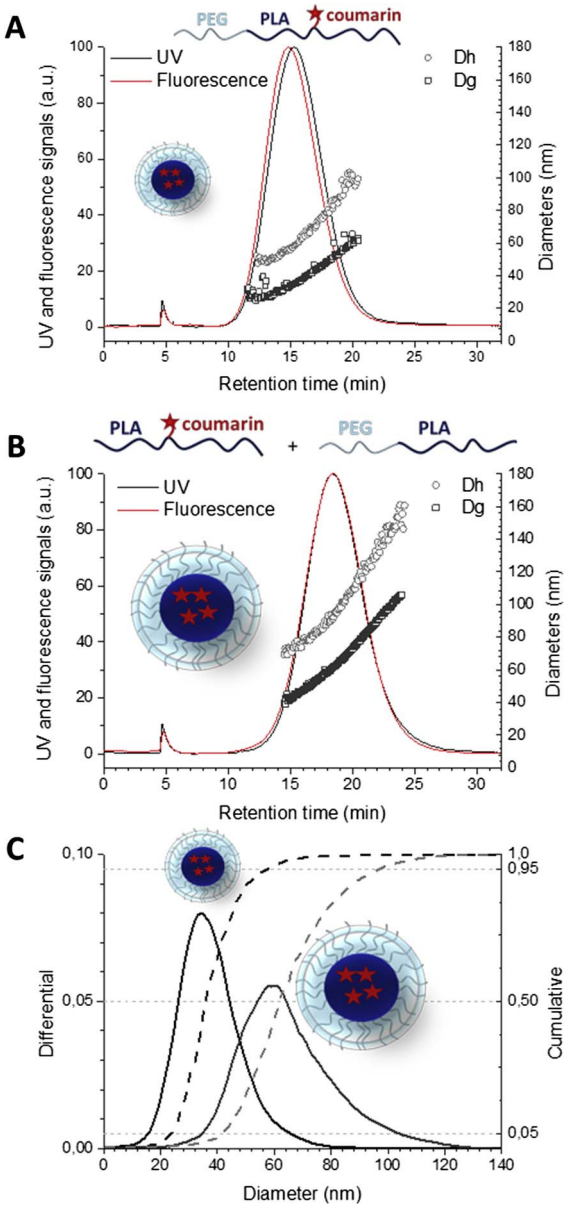


Fig. 4. Asymmetric flow field flow fractionation of nanospheres of coumarin-labelled PLA and PEG-*b*-PLA. The nanospheres were prepared with coumarin-labelled PEG_{5k}-b-PLA_{40k} block copolymer (A) or a 50:50 (w/w) mixture of coumarin-labelled PLA_{22k} and non-labelled PEG_{5k}-b-PLA_{21k} (B). Both nanospheres contained approx. 1 mol% of coumarin (with respect to lactide) conjugated to the polymer and 10 wt% of PEG with respect to the total polymer content. The hydrodynamic diameter (D_h) was determined by dynamic light scattering and the gyration diameter (D_g) by multi-angle light scattering. Graph C represents the differential and cumulative distributions of D_g corresponding to fractograms A (black lines) and B (grey lines).

Table 4
Physico-chemical characterisation of the fluorescent nanospheres.

Nanosphere composition	Batch D_h (nm) (PDI)	Batch zeta potential (mV)	Flow D_h[a] (nm)	Flow D_g[a] (nm)	$D_{5\%}$ (nm)	$D_{50\%}$ (nm)	$D_{95\%}$ (nm)
PEG_{5k}-*b*-PLA_{40k}-coumarin	66 ± 1 (0.16)	−2 ± 1	63 ± 1	40 ± 1	24	36	57
PLA_{22k}-coumarin + PEG_{5k}-*b*-PLA_{21k} (50:50 w/w)	101 ± 1 (0.09)	−7 ± 3	99 ± 1	64 ± 2	42	62	97

Values are averages of three measurements ± standard deviation.
[a] At distribution peak maximum.

The reactive polymers were obtained at gram-scale in one step from commercially available reagents and therefore our approach is robust and easy to implement. The reactivity of the alkyne functional group along the polymer chain was demonstrated on alkyne-functional PLA and PEG-*b*-PLA block copolymers, leading to the formation of a fluorescent polymer-coumarin conjugates via copper-catalysed azide-alkyne cycloaddition under mild conditions. These fluorescently labelled polymers self-assembled in water to form nanospheres that retained their luminescent properties in aqueous medium. The nanospheres had narrow size distributions with diameters below 100 nm, as determined by AF4 with hyphenated MALS and DLS detection.

Acknowledgements

This work was financially supported by CAPES, FAPEMIG, CNPq, and NANOBIOMG network, Minas Gerais, Brazil. The authors thank the NMR facility staff and Welmarie van Schalkwyk at Stellenbosch University, South Africa, Ivana Lula and Jarbas Magalhães at Laboratório de Ressonância Magnética Multi-Usuário, Universidade Federal de Minas Gerais and Patrícia Monteiro at University of Nottingham for NMR spectroscopy analyses and Bruna Postacchini at UFOP for fluorescence spectroscopy.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eurpolymj.2017.03.028.

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