ELSEVIER

PII: S0032-3861(97)10362-7

Polymer Vol. 39 No. 18, pp. 4421–4427, 1998

0032-3861/98/$19.00+0.00

Synthesis, characteristics and *in vitro* degradation of star-block copolymers consisting of L-lactide, glycolide and branched multi-arm poly(ethylene oxide)

Youxin Li and Thomas Kissel*
Department of Pharmaceutics and Biopharmacy, Philipps-University, D-35032 Marburg, Germany
(Received 21 October 1997; revised 2 December 1997)

Biodegradable star-block copolymers containing a hydrophilic 4- or 8-arm branched poly(ethylene oxide) (PEO) central unit and hydrophobic poly(L-lactide) (PLLA) or poly(L-lactide–*co*–glycolide) (PLLG) were synthesized by solution polymerization in toluene catalysed by aluminium triethylene. Using g.p.c. and laser light scattering analysis, a significant increase in the molecular weights could be demonstrated, corresponding to the ratio of the monomers relative to the multi-arm poly(ethylene oxide) in the feed. Polydispersities were of same order as those of the parent PEOs. N.m.r. spectra show that all hydroxy end groups of multi-arm PEO are esterified after copolymerization, which supports the star architecture of the products.

The star-block copolymers have physico-chemical properties differing from their starting materials: branched PEO and PLLG or PLLA. The glass transition temperature, T_g, and the crystallinity of star-block PLLA–PEO or PLLG–PEO were significantly reduced in comparison to the respective linear polymers, demonstrating the influence of the steric architecture of the star-block copolymers on the thermal properties.

The *in vitro* degradation properties of star-block PLLG–PEO copolymers differ from those of linear ABA triblock copolymers of PLLG and PEO due to the steric architecture and shorter PLLG block. While mass loss and molecular weight decay are of comparable order in the initial phase, loss of branched PEO seems to be reduced. These novel biodegradable polyether–polyesters may have potential for parenteral protein delivery systems.

(Keywords: poly(L-lactide); polyglycolide; star-block copolymer)

INTRODUCTION

Parenteral depot systems on the basis of biodegradable polymers are the subject of intensive research efforts, especially for the prolonged delivery of peptides and proteins[1]. These drug candidates present a formidable challenge for continuous release profiles both *in vitro* and *in vivo*, due to interactions between the drug substances and the polymeric matrix materials[2]. The control of the release profiles of proteins from parenteral depot systems of poly(lactide–*co*–glycolide) [PLG] is anything but straightforward. The release patterns observed are frequently interpreted in terms of at least biphasic processes[3,4]. Since PLG is thought to degrade by random scission of ester bonds in the bulk of the polymeric matrix[5], the drug remaining in the system is exposed to an environment of acidic pH increasing as a function of time. This acidic environment, combined with elevated temperatures and hydrophobic surfaces, may provide conditions in which proteins are unlikely to survive for a long time.

Recently, linear ABA triblock copolymers have been prepared from poly(ethylene oxide) (PEO) and biodegradable polyester[6–17]. The copolymerization of PEO and lactide or lactide/glycolide is now regarded as a suitable method to obtain new polymeric materials with novel physical, chemical and biological properties adaptable to specific uses[6–14].

We have recently described the synthesis of ABA triblock copolymers containing PEO and PLLA or PLLG, which are obtained by bulk copolymerization in the presence of aluminium triisopropoxide[13]. The systematic investigation of the release of hydrophilic macromolecules from biodegradable parenteral delivery systems based on the ABA triblock copolymers has demonstrated their potential as protein delivery systems[14,15]. Here, we report a novel type of new star-block copolymers from multi-arm PEO and L-lactide or L-lactide/glycolide. We are interested in the effects of the molecular architecture on the degradation properties of the polymer.

EXPERIMENTAL

Materials

L-lactide (S-grade) and glycolide (S-grade) (Boehringer Ingelheim, Germany) were purified by recrystallization from ethyl acetate and kept over P_4O_{10} *in vacuo* prior to use. The melting points were 97°C (L-lactide) and 82°C (glycolide). Multi-arm poly(ethylene oxide) with 4 and 8 arms (Shearwater, USA) was purified in toluene and dried over P_4O_{10} *in vacuo* for 48 h prior to use. Aluminium triethylene (15% in hexane, Aldrich, Germany) was used without further purification. Ethyl acetate and toluene were

* To whom correspondence should be addressed

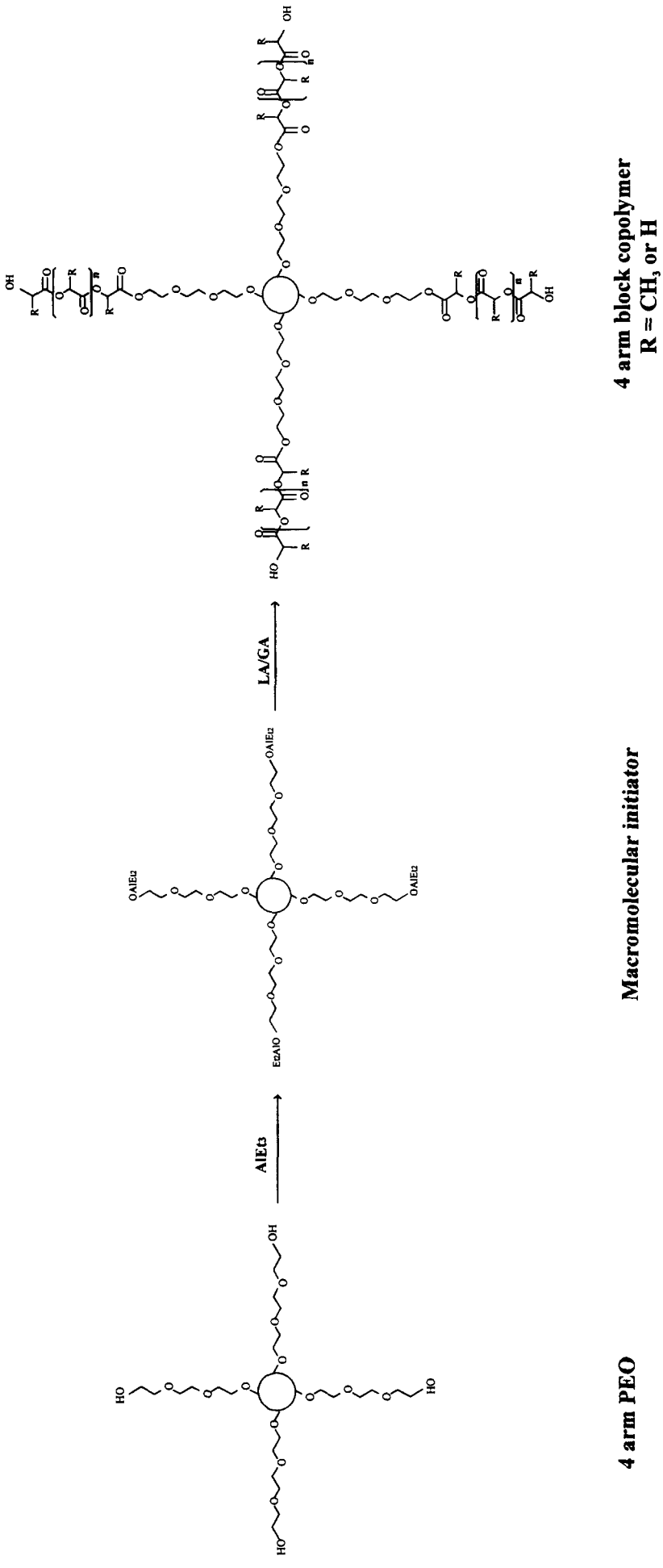

Figure 1 Schematic outline of the synthesis of the star-block copolymer containing PLLG and branched PEO

purified by refluxing and redistillation in presence of CaH_2 (ethyl acetate) and sodium (toluene).

Synthesis and characteristics of the star-block copolymers

2.0 g of the multi-arm poly(ethylene oxide) with 4 or 8 arms was dissolved in 20 ml of dried toluene in a rigorously dried 100 ml nitrogen round bottom flask under nitrogen (99.999%) purging. An equivalent amount of 15% $Al(Et)_3$ (PEO hydroxyl/Al = 1) in hexane was injected into the flask. The reaction was carried out at room temperature for half an hour under stirring. Then 20 ml of a solution containing 4.1 g of L-lactide and 1.0 g of glycolide in toluene were injected into the flask using a glass syringe, and the polymerization was initiated by immersing the flask into a preheated oil bath (70°C) under magnetic stirring. The system was kept under a nitrogen atomsphere. After 72–96 h the reaction mixture was cooled, and 50 ml of dichloromethane containing 5% of ethanol was added. The solution was extracted with 2 M HCl, washed neutral by water and precipitated into 600 ml of ethanol. The isolated polymer was dried at 40°C under vacuum for 48 h.

The conversion of monomer was determined gravimetrically in combination with ^{1}H-n.m.r. spectroscopy, and the composition of the products was measured by ^{1}H-n.m.r. spectroscopy. The molecular weight and polydispersity was measured by g.p.c. combined with a refractive index or laser light scattering detector[13].

Preparation of films

Films were cast from 5% dichloromethane solution (w/v) on Teflon-coated plates. Residual solvents were removed *in vacuo* at room temperature for 2 days until constant weight was obtained, and subsequently cut into 50 × 5 mm^2 specimens. The film thickness was found to be 200 ± 20 μm.

In vitro *degradation*

Films of the polymers immersed in 20 ml of 0.2 M phosphate buffer saline (pH 7.2) were stirred in a rotating metal block thermostat (Rotatherm, Liebisch, Germany) at 15 rpm and 37°C. At preset intervals the samples were recovered and frozen at − 20°C for 2 h, then freeze-dried *in vacuo* (0.4 mbar) at − 20°C for 48 h followed by secondary drying at room temperature for 48 h. Molecular weights (g.p.c.), mass loss (gravimetry) and composition (n.m.r.) were analysed.

Analytical methods

All n.m.r. spectra were obtained from $CDCl_3$ solution containing TMS as reference at 25°C on a JNMR–FX 500 (Jeol) spectrometer. D.s.c. measurement was carried out using a differential scanning calorimeter (Perkin Elmer DSC 7) in sealed aluminium pans under a nitrogen atmosphere, Thermograms covering a range of −50 to 200°C were recorded at a heating and cooling rate of 20°C min^{-1}.

Light scattering measurement in combination with g.p.c. was carried out on a miniDraw light scattering instrument (Wyatt Technology) with an SDV linear column (300 × 8 mm, 10 μm) and a K5 cell, using THF as eluant with a flow rate of 0.700 ml min^{-1}; the wavelength of the laser light source was 690 nm and scattering angles were 45.6, 90.0 and 134.4°. Weight-averaged molecular weights and averaged mean-square radii were calculated from light-scattering data using the method of Zimm[19]. Calculations were performed using the ASTRA software supplied by Wyatt Technology.

RESULTS AND DISCUSSION

Synthesis and characteristics

The polymerization of lactide in presence of hydroxy functionality containing compound and aluminium triethylene to synthesize linear homo or block copolymers has been reported by several investigators[20–22]. According to the hypothetical reaction scheme outlined in *Figure 1*, the hydroxyl terminal groups of the branched PEO were first transformed to an Al–O–PEO bond and then lactide or glycolide was inserted into the 'Al–O' bond of the initiator, followed by the selective acyl–oxygen cleavage of the monomer. Compared to linear PEO, the polymerization of lactide/glycolide in presence of multi-arm PEO does not present any significant differences. A higher conversion of the monomer can be attained by an extended reaction time. The molecular weight determined by g.p.c. in combination with a laser light scattering detector shows good correlation with theoretical values (*Table 1* and *Figure 2*). The significant smaller hydrodynamic radius (R_w) of the star-block copolymers relative to that of the linear ABA triblock copolymer indicates the steric architectures of star-block copolymers (*Table 1*)[23].

Further evidence for the molecular architectures of the star-block copolymers is also provided by the analysis of the ^{1}H-n.m.r. spectra. *Figure 3* depicts the 500 MHz ^{1}H-n.m.r. spectra of the 4-arm star-block copolymer PLLA–*b*–PEO and unmodified 4-arm PEO. The chemical shifts of CH_3 and CH of the polylactide block main chain are found at 1.55 and 5.10 ppm, respectively, and the methylene of the PEO

Table 1 Synthesis and properties of star-block and linear copolymers consisting of LLA, GA and multi-arm and linear PEO

Star PEO	M_n (l.s.)	LA:GA:PEO in feed (g:g:g)	Conversion (%), LA + GA	LA:GA:EO (n.m.r.) (wt%)	M_n (l.s.)	R_w (l.s.) (nm)	M_w/M_n (l.s.)	M_n^a (theoretical)	T_g (°C)	T_m (°C)
4-arm	5920	3.20:0:0.40	44	78:0:22	24 700	28.7	1.26	26 900	45	165
4-arm	5920	2.10:0:0.25	26	69:0:31	23 000	28.4	1.31	19 100	42	163
4-arm	5920	2.37:0.62:0.70	60	54:18:28	21 700	—	1.55	21 100	34	—
4-arm	5920	2.40:0.30:0.70	60	60:10:30	22 100	—	1.47	19 700	37	143
4-arm	5920	2.20:0.25:0.70	53	57:8:35	19 800	—	1.52	16 900	38	149
8-arm	7100	2.00:0:0.20	62	86:0:14	42 300	11.7	1.45	51 100	35	154
8-arm	7100	14.5:0:2.00	55	86:0:20	28 500	—	1.67	35 500	33	154
8-arm	7100	2.50:0.50:0.80	62	55:15:30	21 200	—	1.73	23 700	24	—
linear	6000	17.5:5.0:8.0	72	50:17:33	19 800	34.3	18.6	18 000	42	—

[a] M_n (theoretical) = M_{nPEO} × 100/PEO wt%

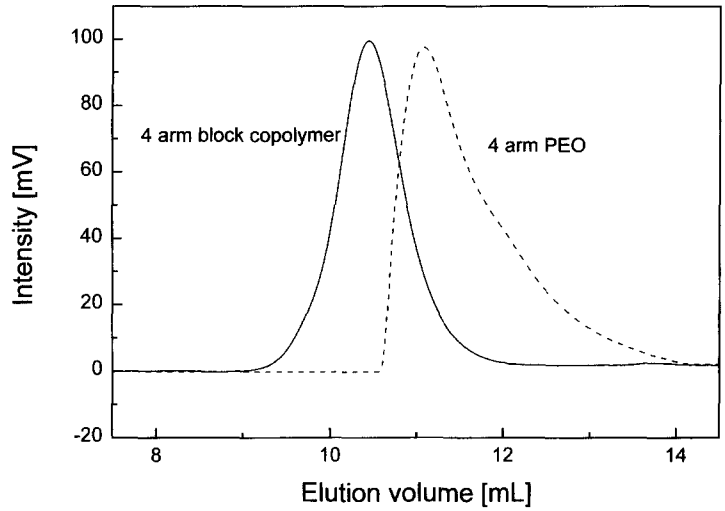

Figure 2 Size exclusion chromatograms of unreacted 4-arm PEO and the corresponding 4-arm PLLA–*b*–PEO block copolymer

PEO
HO-CH_2CH_2-O-PEO
a b

Star-block copolymer
HO-CH(Me)-COO-PLA-$COOCH_2$-CH_2-O-PEO-
c a′ b′

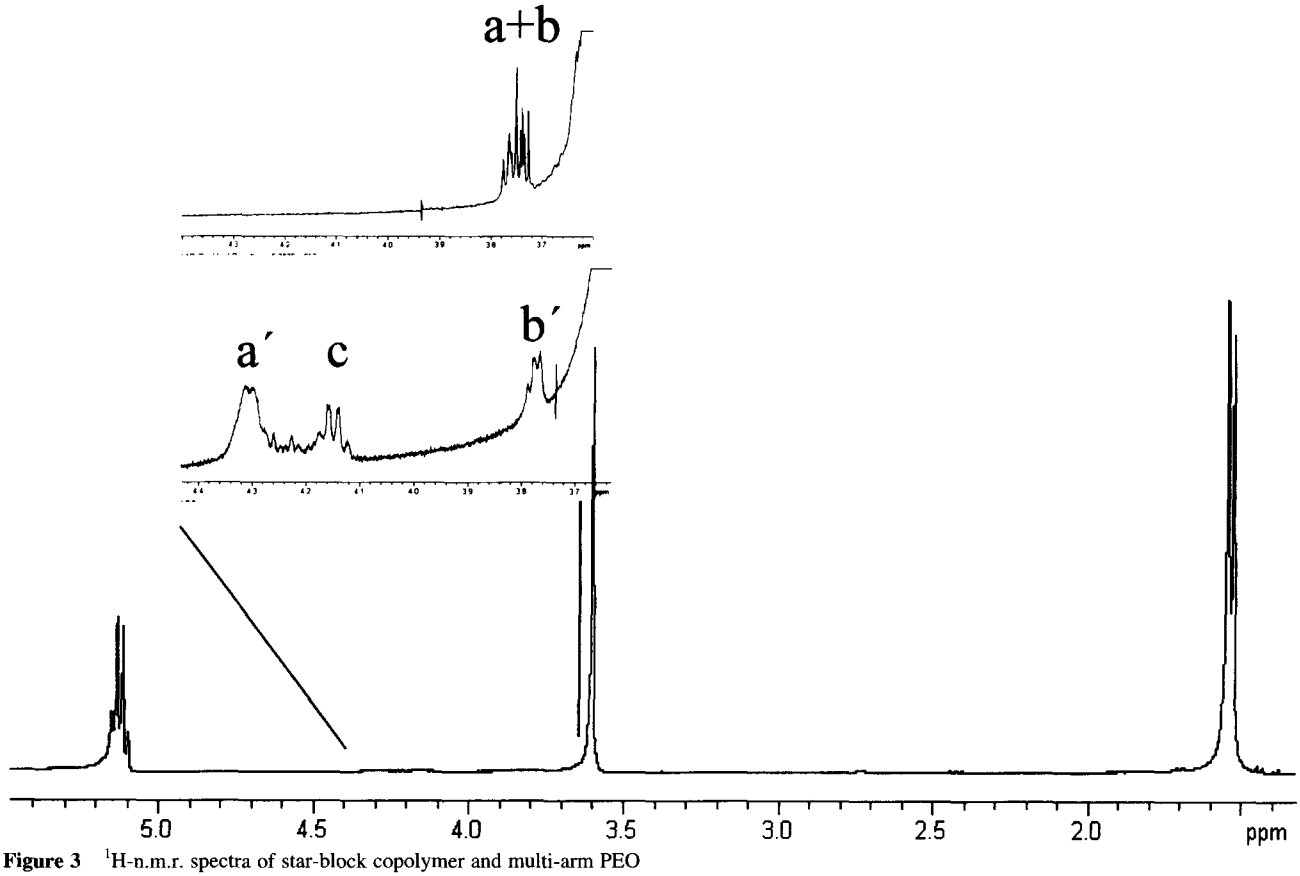

Figure 3 ^{1}H-n.m.r. spectra of star-block copolymer and multi-arm PEO

main chain resonated at 3.60 ppm. The main evidence for the star architecture is the chemical shift of the methylene groups of the terminal PEO units. Before esterification, the methylene of the hydroxylated end unit is found at 3.7–3.8 ppm (a + b). After esterification, the α-methylene protons of PLLA-connecting EO units (PLA–COO–CH_2) appear at 4.3 ppm (a′), neighboured to the methine protons of the hydroxylated lactyl end units (c). The β-methylene groups remain unchanged (b′). From this analysis it appears that all hydroxy end groups of 4-arm PEO are esterified by L-lactide and that the 4-arm PLLA–*b*–PEO is isolated as the main product. The absence of carboxyl endgroups of lactic

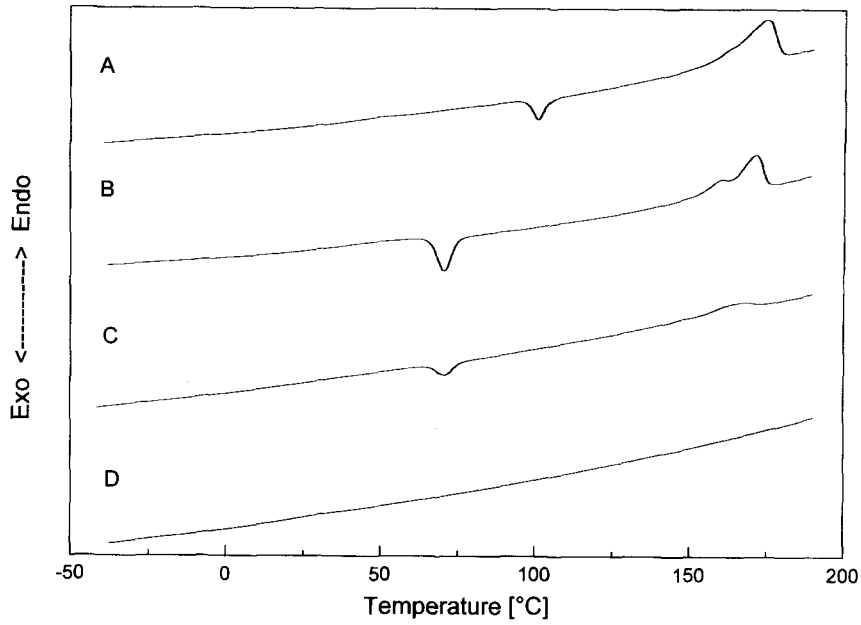

Figure 4 D.s.c. traces of linear and star-block copolymers: (A) linear triblock copolymer of LLA and PEO; (B) 4-arm block copolymer of LLA and PEO; (C) 8-arm block copolymer of LLA and PEO; (D) 4-arm block copolymer of LLA, GA and PEO

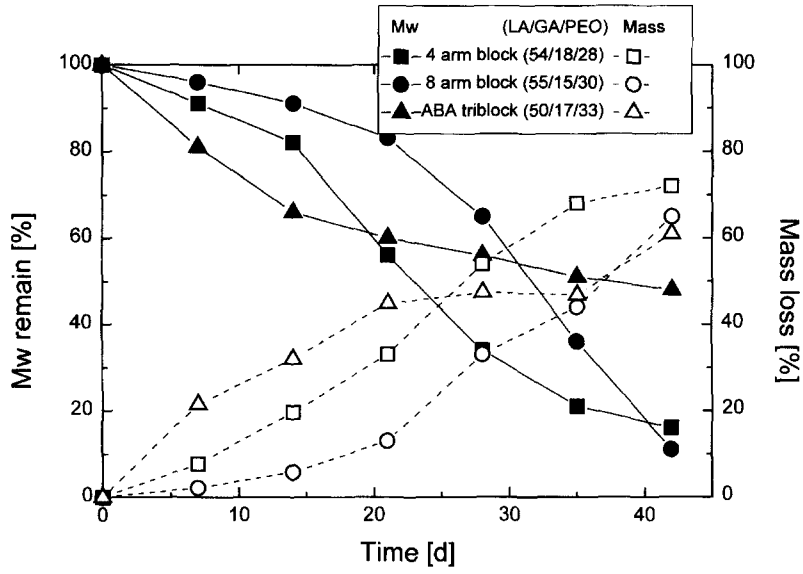

Figure 5 *In vitro* degradation of film specimens from linear and star-block copolymers in PBS, pH 7.2 at 37°C

acid and free lactic acid methine protons, which usually appear at 4.9–5.0 ppm and 4.0 ppm[16], respectively, demonstrated that homopolymerization of L-lactide did not occur to any significant extent. The ^{1}H-n.m.r. spectrum of the 8-arm block copolymer shows the same results as that of the 4-arm block copolymer.

Thermal properties of biodegradable polymers are a very important factor, influencing the formation of microparticles by various techniques relying on solvent evaporation. Drug release and biodegradation of parenteral delivery systems are also affected by the glass transition temperature. Star-block copolymers have three-dimensional branched molecular architectures and the interaction between molecules is reduced; therefore, it is not surprising that these polymers show lower glass transition temperature (T_g) and melting temperature (T_m) than their linear counterparts (*Table 1* and *Figure 4*). The T_g and T_m of the PLLA block in the 4-arm block copolymer are shifted from 67°C and 174°C of pure PLLA[13] to 42°C and 163°C, which is also lower than that of linear ABA triblock copolymers which yield 52°C and 173°C, respectively, when the MW and composition are similar (Mw~20 000 and PEO content ~30%). Furthermore, 8-arm PLLA–*b*–PEO shows a much smaller melting peak of the PLLA block and lower T_g (31°C) and T_m (152°C). These observations demonstrate that the steric architecture of the star-block copolymers reduces both the crystallinity of PLLA–*b*–PEO and T_g. A higher number of branches seems to cause less crystallinity, also lowering T_g and T_m. The lack of a melting peak T_m of PEO in the range of − 50 to 200°C suggests that the PEO blocks are in an amorphous state. The introduction of glycolide into the PLLA block leads to a random structure in this block reducing either T_g or crystallinity. When the glycolide:L-lactide ratio exceeds 1:5, no melting endotherm T_m of

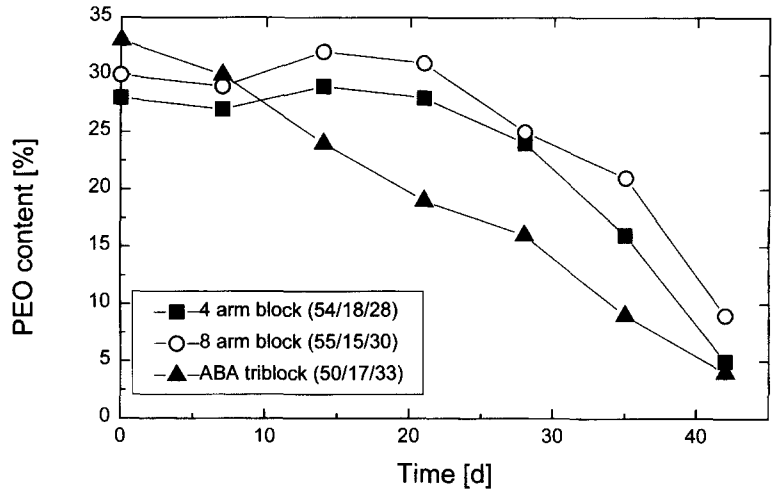

Figure 6 Composition of linear and star-block copolymers during *in vitro* degradation

the hydrophobic PLLG blocks can be found by d.s.c., suggesting that the entire polymeric matrix is in an amorphous state.

In vitro *degradation*

The degradation properties of the star-block copolymers were investigated under *in vitro* conditions (phosphate buffer saline, pH 7.2, 37°C). Previously, we reported that the linear ABA triblock copolymers of lactide, glycolide and PEO showed a degradation behaviour different from that of random copolymers of lactide and glycolide[14]. The introduction of hydrophilic PEO block led to rapid swelling in water, resulting in a faster degradation rate under both *in vitro* and *in vivo* conditions. The star-block copolymers possess similar swelling properties in water. But, compared to the fast erosion of linear ABA triblock copolymers[14] (*Figure 5*), the star-block copolymers show a slower M_w degradation and mass erosion in the first 2–3 weeks. In the case of ABA triblock copolymers, the rapid mass erosion is mainly caused by the fast cleavage of the PEO block by hydrolysis. This leads to a change of the matrix composition of the ABA triblock copolymer, decreasing the hydrophilicity of the polymeric matrix. These factors may affect the compatibility of protein and matrix. Star-block copolymers show a different degradation pattern due to star architecture. A slower mass erosion of star-block copolymers was found up to about 20 days, as shown in *Figure 5*. N.m.r. analysis of the degradation polymers demonstrated that the star-block copolymers had nearly constant PEO/PLLG ratio during the degradation up to about 20 days (*Figure 6*). This means that the slower mass erosion is due to the slower cleavage of the PEO–PLLG bond. After about 2–3 weeks, it was found that the degradation of the star-block copolymer was accelerated. In contrast, the erosion of both M_n and mass of linear ABA triblock copolymers slowed down significantly after the initial erosion period, due to preferential PEO cleavage, and at later phases of the degradation the picture is dominated by the PLLG block. Star-block copolymers PLLG–*b*–PEO possess on average a shorter chain length of PLLG block due to the star architecture in comparison to the linear block copolymer when the molecular weights are of similar magnitude. Water-soluble breakdown products, such as PLLG oligomers and monomers, can be produced after fewer hydrolytic cleavage steps, leading to a fast erosion of the matrix. This biphasic degradation property of the star-block copolymer offers a uniform environment for the encapsulated drug and a fast elimination of the polymeric matrix after exhaustion of the drug.

CONCLUSIONS

Using solution polymerization in presence of aluminium triethylene, star-block copolymers of L-lactide, glycolide and the multi-arm poly(ethylene oxide) were synthesized. These star-block copolymers were characterized by g.p.c., l.s., n.m.r. and d.s.c.. The analysis of g.p.c./l.s. and n.m.r. data is compatible with the branched architecture of star-block PLLA–*b*–PEO and PLLG–*b*–PEO.

The reduction in the interaction between the molecules due to the steric architecture of the star-block copolymer affects the thermal properties of these polymers, as demonatrated by d.s.c. Compared to linear block copolymer, T_g and T_m of the star-block copolymer are lowered, and a significant decrease in the crystallinity of the PLLA block is also observed.

In vitro degradation of the star-block copolymers shows a different behaviour compared to linear triblock copolymer. A longer retention of the PEO component due to there being more connecting bonds between PLLG and PEO blocks leads to a slower change in the physico-chemical properties of the polymeric matrix. The acceleration of the degradation after 3 weeks offers a fast elimination of the polymeric matrix after the exhaustion of the drug.

REFERENCES

1. Pitt, C. G., *Int. J. Pharm.*, 1990, **59**, 173–196.
2. Hutchinson, F. G. and Fur, B. J. A., *J. Controlled Rel.*, 1990, **13**, 279–294.
3. Sanders, L. M., Kent, T. J., McRae, J. L., Vickery, B. H., Tice, T. R. and Lewis, D. H., *J. Pharm. Sci.*, 1984, **73**, 1294–1297.
4. Shah, S. S., Cha, Y. and Pitt, C. G., *J. Controlled Rel.*, 1992, **18**, 261–270.
5. Ogawa, Y., Okada, H., Yamamoto, Y. and Shimamoto, T., *Chem. Pharm. Bull.*, 1988, **36**, 1095–1108.
6. Zhu, K. J., Xiangzhou, L. and Shilin, Y., *J. Appl. Poly. Sci.*, 1990, **39**, 1.
7. Kriecheldorf, H. R. and Meier-Haack, J., *J. Makromol. Chem.*, 1993, **194**, 715.
8. Deng, X. M., Xiong, C. D., Cheng, L. M. and Xu, R. P., *J. Polym. Sci., Part C: Polym. Lett.*, 1990, **28**, 411.

9. Jedlinski, Z., Kurcok, P., Walach, Z., Janeczek, H. and Radecka, I., *Makromol. Chem.*, 1993, **194**, 1681.
10. Hu, D. S. and Liu, H. J., *Polym. Bull.*, 1993, **30**, 669.
11. Cohn, D. and Younes, H., *J. Biomed. Mater. Res.*, 1988, **22**, 993–1009.
12. Cerrai, P., Tricoli, M., Lelli, T., Guerra, G. D., Sbarbati del Guerra, R., Casone, M. G. and Giusti, P., *J. Mater. Sci.: Mater. Med.*, 1994, **5**, 308.
13. Li, Y. X. and Kissel, T., *J. Controlled Rel.*, 1993, **27**, 247–257.
14. Li, Y. X., Volland, C. and Kissel, T., *J. Controlled Rel.*, 1994, **32**, 121–128.
15. Kissel, T., Li, Y. X., Volland, C., Görich, S. and Koneberg, R., *J. Controlled Rel.*, 1996, **329**, 315–326.
16. Li, S. M., Rashkov, I., Espartero, J. L., Manolova, N. and Vert, M., *Macromolecules*, 1996, **29**, 57–62.
17. Rashkov, I., Manolova, N., Li, S. M., Espartero, J. L. and Vert, M., *Macromolecules*, 1996, **29**, 50–56.
18. Ronneberger, B., Kao, W. Y. J., Anderson, J. M. and Kissel, T., *J. Biomed. Mat. Res.*, 1996, **30**, 31–40.
19. Zimm, B. H., *J. Chem. Phys.*, 1948, **16**, 1099.
20. Jacobs, C., Dubois, Ph., Jerome, R. and Teyssie, Ph., *Macromolecules*, 1991, **24**, 3027–3034.
21. Dubois, Ph., Jerome, R. and Teyssie, Ph., *Macromolecules*, 1991, **24**, 977–981.
22. Endo, M., Aido, T. and Inoue, S., *Macromolecules*, 1987, **20**, 2982–2988.
23. Li, Y. X., Nothangel, J. and Kissel, T., *Polymer*, 1997, **38**(25), 6197–6206.