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Effect of preparative variables on the properties of poly(*dl*-lactide-*co*-glycolide)–methoxypoly(ethyleneglycol) copolymers related to their application in controlled drug delivery

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Abstract

The effect of certain preparative variables, such as the composition of the feed, the reaction time and the reaction temperature, on the properties of prepared poly(*dl*-lactide-*co*-glycolide)–methoxypoly(ethyleneglycol) (PLGA–mPEG) copolymers and on the yield of the reaction was investigated. The results with regard the molecular weight and yield were discussed in relation to a polymerization mechanism proposed recently (Du et al., 1995. Macromolecules 28, 2124–2132). The higher the PEG content of the feed the lower the molecular weight of the copolymer and the yield of the reaction. The breadth of the molecular weight distribution decreased initially with time, but appeared to stabilize later at low values. Both the ethylene oxide content and the lactide to glycolide molar ratio in the copolymer depended on the reaction temperature and varied with the reaction time. PLGA and mPEG appeared to be partially miscible, and copolymers containing approximately 40% mol or higher ethylene oxide exhibited crystallinity. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Copolymer properties; Poly(lactide-*co*-glycolide)–methoxypoly(ethyleneglycol) copolymers; Synthesis variables

1. Introduction

The application of block copolymers of biodegradable poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA) with poly(ethyleneglycol) (PEG) in controlled drug delivery and drug

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targeting has recently been proposed (Stolnik et al., 1994; Gref et al., 1995; Emile et al., 1996; Jeong et al., 1997). Triblock copolymers PLA–PEG–PLA and PLGA–PEG–PLGA form more hydrophilic matrices than PLA and PLGA and are considered more suitable for the controlled delivery of proteins (Youxin and Kissel, 1993). Also, diblock PLA–mPEG and PLGA–mPEG copolymers (mPEG, monomethoxypolyethyleneglycol) have been used in the preparation of nanoparticles, exhibiting prolonged residence in systemic circulation after intravenous administration and therefore having potential as targeted drug carriers (Gref et al., 1994; Bazile et al., 1995).

These copolymers are not yet commercially available. Although the synthesis of PLA–PEG–PLA triblock copolymers has been studied (Zhu et al., 1990; Deng et al., 1990; Jedlinski et al., 1993; Kricheldorf and Meier-Haack, 1993; Youxin and Kissel, 1993) and a coordinative reaction mechanism has been proposed for the polymerizations catalysed by stannous octoate (Du et al., 1995), relatively few reports appear to exist on the synthesis of PLGA–mPEG diblock copolymers (Gopherich et al., 1994; Gref et al., 1994). Moreover, none of these studies deals in a systematic way with the effect of the conditions of preparation on the properties of the prepared PLGA–mPEG copolymers.

In the present work, a systematic study of the effect of certain preparative variables, such as the composition of the feed, the polymerization time and the polymerization temperature, on the yield of the polymerization and on the properties of the resulting PLGA–mPEG copolymers was undertaken. The information provided by this study could be useful to the pharmaceutical scientist working on the development of controlled drug delivery or drug targeting systems based on PLGA–mPEG copolymers.

2. Materials and methods

2.1. Materials

Glycolide (GE) and *dl*-lactide (LE) were purchased from Boehringer Ingelheim (Germany). They were recrystallized from ethyl acetate and dried under vacuum before use. Monomethoxypoly(ethyleneglycol) (mPEG, molecular weight 2000) and stannous octoate were from Sigma (Germany). The mPEG was dried under vacuum before use. Tetrahydrofuran (THF) of HPLC grade and other organic solvents of analytical grade were obtained from Merck (Germany).

2.2. PLGA–mPEG synthesis

The method applied previously for the synthesis of poly(lactide-*co*-glycolide) (Avgoustakis and Nixon, 1991) was used with minor modifications. Briefly, lactide and glycolide at a molar ratio of 4, and the specified amount of mPEG were put in thick-walled glass tubes. The total weight of the feed was 3 g. Stannous octoate (dissolved in hexane) was added at a concentration of 0.03% by weight of the feed and the tubes were evacuated. Then the tubes were heated in an air oven for the specified time. The resulting copolymers were purified by dissolving them in chloroform and then precipitating them into excess methanol. The purified copolymers were dried under vacuum. The yield of the reaction was determined by weighing the dried copolymers.

The reproducibility of polymerization was evaluated during pre-experiments and it was found that, provided the quality of the monomers (high purity, low moisture content of lactide/glycolide) was high, the reproducibility was satisfactory (individual values of molecular weight, composition and yield varied by less than 10% of the mean).

The synthesis variables studied were:

1. Feed composition: feeds containing different amounts of mPEG, corresponding to 25%, 43%, 61% and 77% mol ethylene oxide (EO) in the reactant mixture, were studied.
2. Polymerization time: reaction periods ranging from 0.5 to 6 h were normally applied.
3. Polymerization temperature: based on previous experience with poly(lactide-*co*-glycolide) (Avgoustakis and Nixon, 1991), a relatively

high (190°C) and a relatively low (130°C) temperature were studied.

Poly(lactide-*co*-glycolide) (PLGA) was also prepared for comparison reasons using the same method as for the PLGA–mPEG and under the following conditions: 2 h polymerization at 190°C, lactide to glycolide molar ratio 4, stannous octoate 0.03% by weight of feed. The weight average molecular weight (M_w) of this PLGA was 7×10^4 and the composition was 79.2% mol LE and 20.8% mol GE.

2.3. PLGA–mPEG characterization

The identity and purity (with respect residual monomers) of the copolymers were examined by IR and ^{1}H-NMR spectroscopy. The IR spectra were taken in a Perkin Elmer 298 spectrophotometer over the range 400–600 cm^{-1}. KBr discs, containing 1% (w/w) of the compound being examined, were used with air as reference. The copolymers were dissolved in $CDCl_3$ and their ^{1}H-NMR spectra were recorded with a Bruker AC 250 (Germany) spectrometer.

The composition of the copolymers was determined from the integrals of the peaks in the ^{1}H-NMR spectra assigned to the lactic backbone proton $(\text{–O–CH}^*(\text{CH}_3)\text{–CO–})_x$ at 5.15 ppm, to the glycolic protons $(\text{–O–CH}_2^*\text{–CO–})_y$ at 4.78 ppm, and to the ethylene oxide protons $(\text{–O–CH}_2^*\text{–CH}_2^*\text{–})_z$ at 3.60 ppm (Fig. 1).

The M_w and the molecular weight distribution (MWD) of the copolymers were determined by gel permeation chromatography. A Waters (USA) apparatus was used in which a Waters pump 610 was connected to a Waters 410 refractometer. The following conditions were adopted: the column was Styragel HR5E (Waters), the mobile phase was THF (HPLC grade), and the flow rate was 0.8 ml/min. The sample was dissolved in THF, filtered, and then 20 μl were injected. The standard curve was prepared using a series of Shodex SM-105 (Waters) polystyrene standards. The breadth of the MWD was described in terms of the polydispersity index $PI = M_w/M_n$, where M_w is the weight average molecular weight and M_n the number average molecular weight.

The morphology of the copolymers was examined by differential scanning calorimetry (DSC) and by powder X-ray diffraction. The thermograms were obtained with a Perkin Elmer DSC 7 instrument at a heating rate of 10°C/min. The X-ray diffraction patterns were obtained using a Philips XRD PW 1840/30 apparatus.

The hydrophilicity of the copolymers was evaluated by the contact angle formed by triple-distilled water droplets on the surface of copolymer films. The films were prepared by casting 10% (w/v) copolymer solutions in dichloromethane on glass plates and allowing the solvent to evaporate. The contact angles were measured as described previously (Agathopoulos and Nikolopoulos,

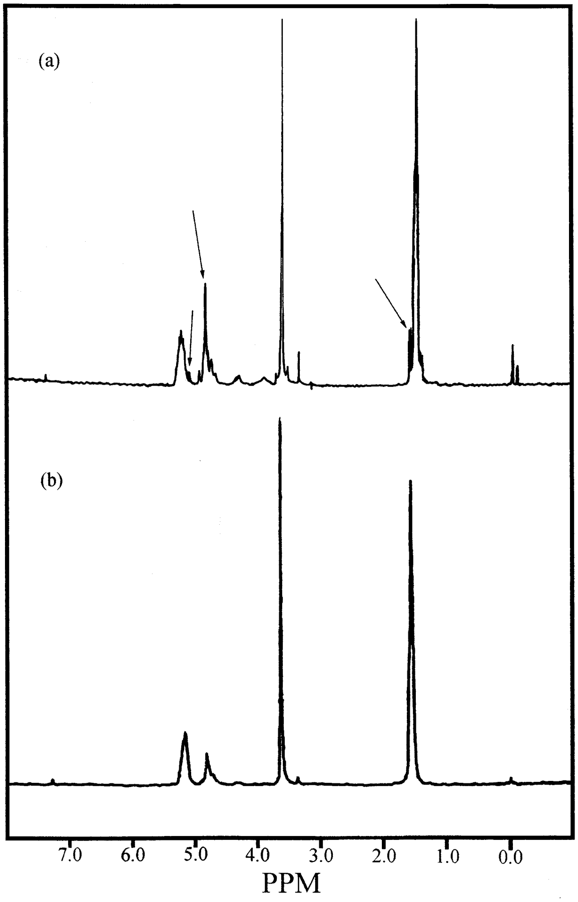

Fig. 1. ^{1}H-NMR spectra of PLGA–mPEG in $CDCl_3$: (a) non-purified (arrows indicate peaks attributed to residual LE/GE monomers); (b) purified.

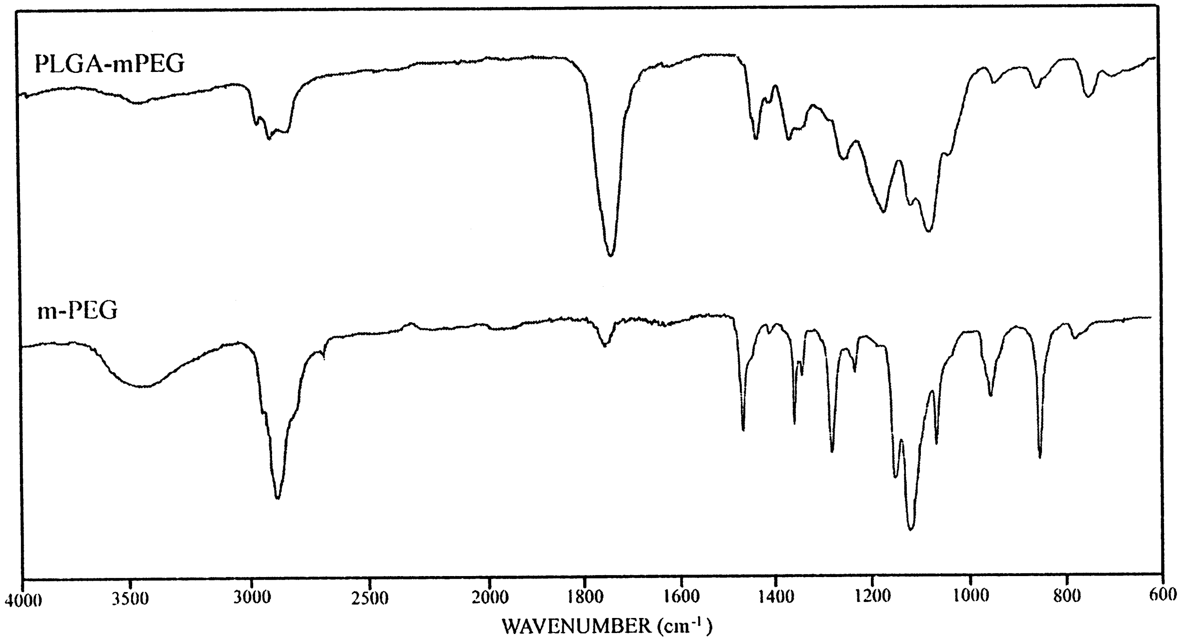

Fig. 2. IR spectra of PLGA–mPEG (26% mol EO) and mPEG.

1995). At least three measurements were made with each sample.

3. Results

3.1. Identity and purity of the synthesized copolymers

The identity and purity of the copolymers were investigated by IR and ^{1}H-NMR spectroscopy. A typical IR spectrum of a PLGA–mPEG (26% mol EO) copolymer and of pure mPEG are shown in Fig. 2. The major peaks assigned to the structure of PLGA–mPEG are: 2900–3000 cm^{-1} (C–H stretching), 1750 cm^{-1} (ester C=O stretching), and 1080 cm^{-1} (O–CH$_2$ stretching). The comparison of the IR spectrum of PLGA–mPEG with that of mPEG confirmed that the reaction between PLGA and mPEG had been effected. It is characteristic that the broad absorption band at 3500 cm^{-1} in the spectrum of mPEG, assigned to O–H stretching, was practically eliminated from the spectrum of PLGA–mPEG, indicating that the free hydroxyl groups of mPEG had reacted with the carbonyl groups of lactide/glycolide.

The ^{1}H-NMR spectrum can reveal the presence of residual (still unreacted at the time the reaction was terminated) lactide/glycolide in the copolymers (provided they are present in adequate quantities, e.g. when the polymerization is terminated early). Thus, in the ^{1}H-NMR spectrum of a non-purified copolymer (obtained after 1 h of polymerization at 190°C of a feed containing 25% mol EO) peaks attributed to lactide (at 1.6–1.7 ppm and 5.0–5.1 ppm) and glycolide (at 4.8 ppm) can clearly be seen (Fig. 1). These peaks did not appear in the spectra of the purified copolymers (Fig. 1), indicating the purity of the (purified) copolymers with respect to the presence of lactide/glycolide. Also, the IR spectrum of the copolymers can reveal the presence of residual mPEG, because mPEG possibly present in the copolymer would give a broad absorption band at 3500 cm^{-1}. Indeed, in the IR spectra of non-purified copolymers the absorption band at 3500 cm^{-1} was observed (spectra available on request). The ^{1}H-NMR and IR spectra (Figs. 1 and 2) showed that the synthesized copolymers were pure and genuine.

3.2. Effect of preparative variables on the yield of the reaction

The yield depended on the time and temperature of the reaction and on the composition of the feed (Fig. 3). The reaction time required to obtain collectable product depended on feed composition and temperature. With feeds rich in PEG or when a relatively low temperature was employed, a longer reaction period was required to obtain copolymer. At 190°C, a reaction period longer than 0.5 h was required to obtain copolymer from feeds containing 61% mol EO or more. At 130°C, product could only be collected after reaction periods longer than 1 h with the feed containing 43% mol EO and longer than 4 h with feeds containing 61% or 77% mol EO (Fig. 3).

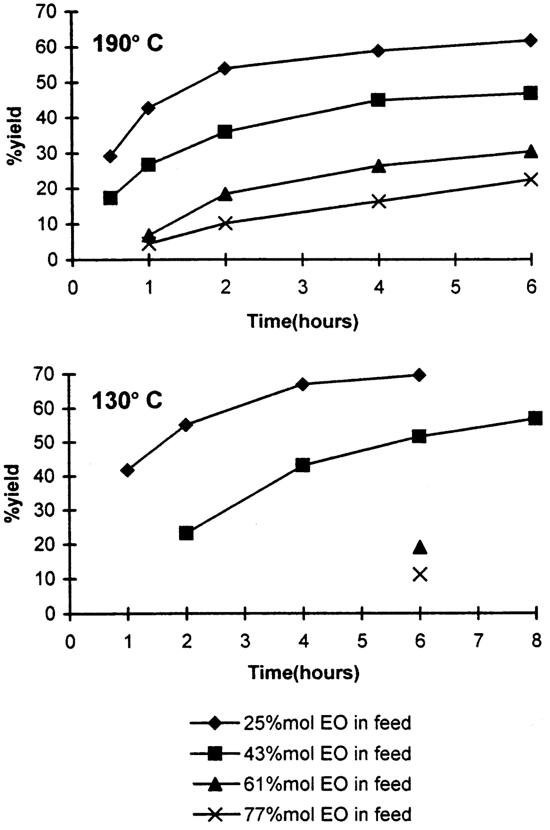

Fig. 3. Effect of the preparative variables on the yield of the reaction.

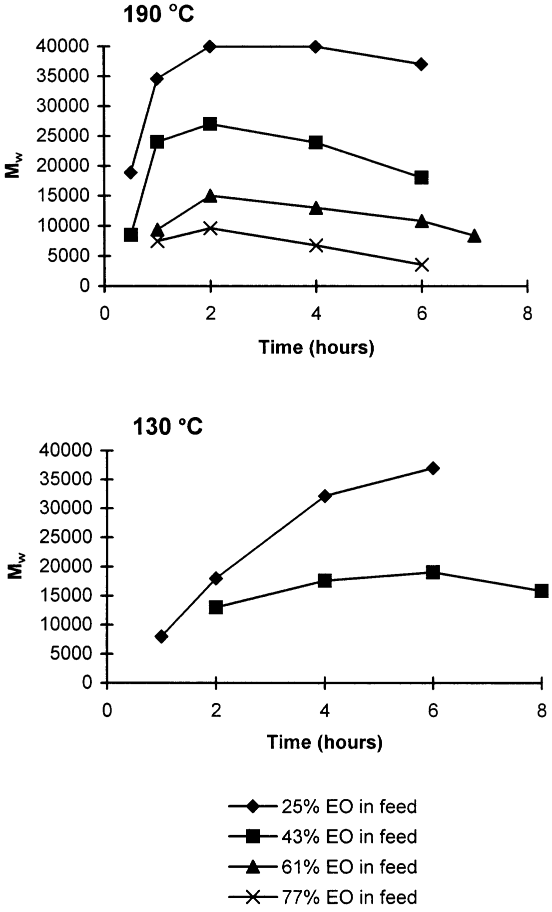

Fig. 4. Effect of the preparative variables on the weight average molecular weight (M_w) of the prepared PLGA–mPEG.

At both temperatures and regardless of the feed composition, the yield increased with time during the whole period studied. The rate of yield increase fell with time: the higher the proportion of PEG (% mol EO) in the feed the lower the yield of the reaction (Fig. 3).

3.3. Effect of preparative variables on the molecular weight of the copolymers

The molecular weight (M_w) of the synthesized copolymers initially increased but later began to fall with time. The molecular weight build up occurred more gradually at the lower temperature of 130°C. The higher the mPEG proportion in the feed the lower the molecular weight of the resulting copolymers (Fig. 4).

3.4. Effect of preparative variables on the MWD of the copolymers

The effect of preparative variables on the breadth of the MWD was evaluated from the PI values of the synthesized copolymers. At 190°C there was an abrupt reduction of the PI with time at the early stages, but as the reaction proceeded there appear a tendency for the PI to stabilize at values of 1.6–2.0 (Fig. 5). As can be seen in Fig. 6, bimodal MWD curves were initially obtained, but at later polymerization times the MWD curve became unimodal. At 130°C there was a drop of the PI with time too. However, this occurred more gradually than at 190°C. In the case of the feed containing 43% mol EO, the PI appeared to stabilize at about 1.8 during the last 2 h of the reaction (Fig. 5).

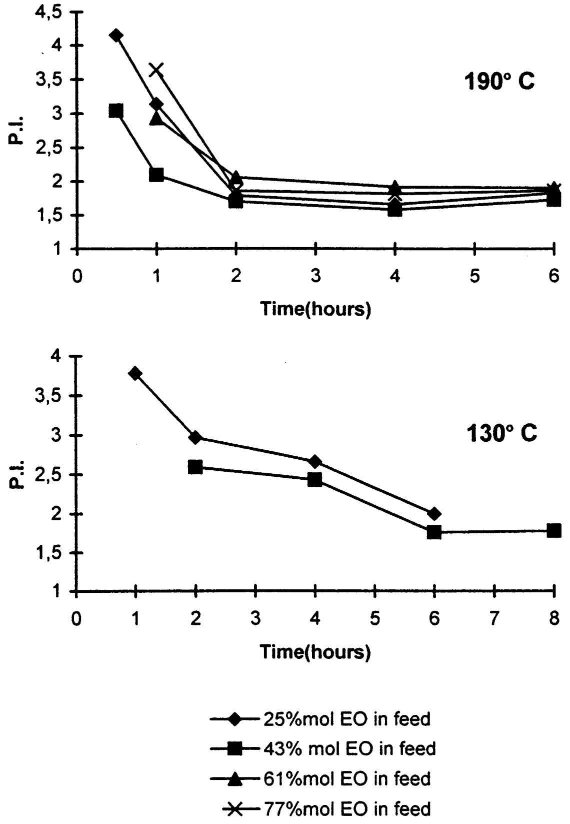

Fig. 5. Effect of the preparative variables on the molecular weight distribution (polydispersity index, PI) of the prepared PLGA–mPEG.

3.5. Effect of preparative variables on the composition of the copolymers

The conditions of synthesis significantly affected the composition of the synthesized PLGA–mPEG copolymers, i.e. the PEG content (expressed as % mol EO in the chains of the copolymer) and the LE/GE molar ratio in the copolymers (Table 1). At both temperatures, the PEG content of the copolymers initially decreased with time, but as the reaction proceeded it started to increase with time. The LE/GE molar ratio in the synthesized copolymers at the initial stages of the reaction was lower than the LE/GE ratio in the respective feeds. The LE/GE ratio in the copolymers initially increased with time and passed through a maximum before falling at late reaction times (Table 1).

3.6. Effect of preparative variables on the morphology of the copolymers

According to the thermograms obtained, the PLGA–mPEG copolymers containing 39% mol EO or higher exhibited crystallinity (Fig. 7), irrespective of the conditions of PLGA–mPEG synthesis. The observed crystallinity is probably associated with the presence of the crystalline PEG in the PLGA–mPEG copolymer chains, since the PLGA component of PLGA–mPEG of the composition used (*dl*-lactide to glycolide molar ratio 4) is amorphous (Gilding and Reed, 1979). As demonstrated by the values of the enthalpy of the transition (heat of fusion) obtained (Table 2), the degree of crystallinity increased as the EO content of PLGA–mPEG increased. The PLGA and the PLGA–mPEG containing 26% mol EO were amorphous, only exhibiting glass transition at 49 and 48°C, respectively (Fig. 7 and Table 2). The X-ray diffraction patterns (Fig. 8) confirmed the lack of crystallinity in PLGA and PLGA–mPEG containing 26% mol EO and the presence of crystallinity in the PLGA–mPEG

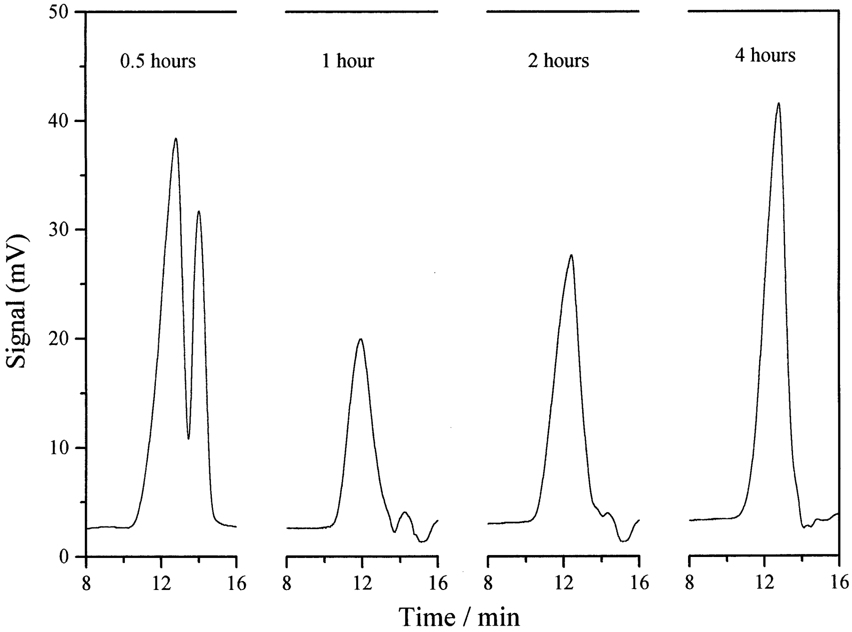

Fig. 6. Gel permeation chromatography of PLGA–mPEG at different polymerization times (feed composition: 60% mol LE, 15% mol GE and 25% mol EO; polymerization temperature 190°C).

containing 46 and 57% mol EO. The copolymer containing 39% mol EO did not exhibit peaks in the diffractogram, probably due to its low degree of crystallinity.

3.7. Effect of composition on the hydrophilicity of the copolymers

According to the contact angle values obtained (Table 3), an increase in the PEG content of the PLGA–mPEG copolymers caused an increase in the hydrophilicity of the copolymers. Contact angles ranging approximately from 31 to 74°, i.e. lower than 90°, were obtained, implying that water would wet the surface of PLGA–mPEG matrices. Also, water would penetrate into heterogeneous PLGA–mPEG matrices because $\cos\theta > 0$ when $\theta < 90°$ and, according to Eq. (1), a positive capillary pressure differential (ΔP) would exist which would draw water into the matrix:

$$\Delta P = \frac{\gamma \cos\theta}{m} \tag{1}$$

where γ is the surface tension of water, θ is the water–solid contact angle and m is the ratio of the cross-sectional area of the capillary to its perimeter (Ganderton and Selkirk, 1970).

4. Discussion

In this work, the effect of certain preparative variables on the yield of the polymerization and on the properties of the obtained PLGA–mPEG copolymers was investigated. The copolymers were characterized with regard to composition, molecular weight , MWD and morphology. The hydrophilicity of certain samples was also evaluated. These properties are expected to affect the behaviour of PLGA–mPEG during the manufacture of PLGA–mPEG-based drug delivery systems, as well as the performance of these systems in vivo.

The yield of the polymerization increased with increasing polymerization time, and the higher the PEG content of the feed the lower the yield (Fig. 3). According to the reaction mechanism pro-

Table 1
Effect of preparative variables on the composition (% mol EO and lactide to glycolide molar ratio) of the PLGA–mPEG copolymers

	% mol EO in the feed (temperature, °C)					
Reaction time (h)	25 (190)	25 (130)	43 (190)	43 (130)	61 (190)	77 (190)
0.5	23[a]/2.0[b]	–	41/3.2	–	–	–
1	15/2.7	40/1.9	23/2.9	–	46/4.0	50/2.8
2	13/3.3	34/3.0	20/4.8	35/3.9	27/4.7	45/4.6
4	13/4.0	17/3.2	21/3.8	29/4.1	30/4.4	57/4.0
6	14/3.5	14/3.6	30/3.5	27/3.6	39/4.1	79/3.9
8				31/3.6		

[a] EO (% mol) in the copolymer; –, no copolymer was obtained.

[b] Lactide to glycolide molar ratio in the copolymer (4.0 ± 0.1 in the feed).

posed by Du et al. (1995) for the polymerization of lactide in the presence of PEG, an active intermediate should be formed from the reaction of lactide/glycolide, stannous octoate and mPEG in the initiation phase. The tin of this intermediate may coordinate either with the terminal OH group of the intermediate (intramolecular reaction leading to chain growth) or with the terminal OH group of another molecule, such as a mPEG molecule or another copolymer chain (intermolecular reaction leading to chain transfer growth).The higher the mPEG content of the feed the higher the probability that the active intermediate formed would react with a mPEG molecule. This would result in the formation of a relatively large number of small chains characterized by a high mPEG/PLGA ratio which probably did not precipitate in methanol with the rest of the copolymer during copolymer purification. Thus, increasing the mPEG content of the feed decreased the polymerization yield.

A similar finding was reported by Kricheldorf and Meier-Haack (1993). They used a similar purification procedure to the one used here and they observed that when LE/PEG molar ratio in the feed was lower than 200 (in this study the (LE + GE)/mPEG molar ratio in the feed was always ≤ 130) fractionation of the polymer occurred during purification and lower yields of PLA–PEG–PLA were obtained. The removal of the chains with a high mPEG/PLGA ratio during purification was probably the reason that the mPEG content of the copolymers was lower than the mPEG content of the respective feeds (Table 1).

The molecular weight of the copolymers depended on the proportion of mPEG in the feed and was affected by the polymerization time and temperature (Fig. 4). In accordance with the polymerization mechanism proposed by Du et al. (1995), the higher the mPEG proportion in the feed the lower the molecular weight of the resulting copolymers. In general, the molecular weights of the copolymers determined by GPC (Fig. 4) were in fair agreement with the molecular weights

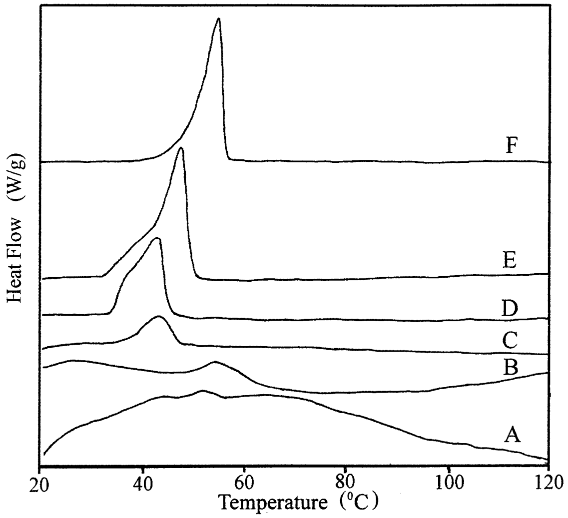

Fig. 7. DSC thermograms of PLGA, PLGA–mPEG and mPEG. (A) PLGA; (B) PLGA–mPEG (26% mol EO); (C) PLGA–mPEG (39% mol EO); (D) PLGA–mPEG (46% mol EO); (E) PLGA–mPEG (57% mol EO); (F) mPEG.

Table 2
Glass transition temperatures (T_g) and melting points (T_m) of PLGA–mPEG copolymers having different PEG (% mol EO) content

% mol EO in copolymer	T_g (°C)	T_m (°C)	ΔH^a(J/g)
0 (PLGA)	49	–[b]	–
26	48	–	–
39	–	42.8	11.9
46	–	41.1	23.6
57	–	47.6	54.5
100 (mPEG)	–	55.3	183.4

[a] ΔH, heat of fusion.

[b] –, the respective transition was not observed.

Table 3
Effect of PEG content (% mol EO) on the contact angle of PLGA–mPEG copolymers with water

% mol EO in copolymer	Contact angle (°, mean ± S.D.)
0	74 ± 4
26	65 ± 1
39	58 ± 2
57	31 ± 4

calculated on the basis of the composition of the copolymers.

The molecular weight of the copolymers assumed a maximum at a time that depended mainly on the temperature of polymerization. After this time, the molecular weight began to fall with time (Fig. 4). This may be caused by the successive inclusion in the purified material of relatively small copolymer chains (produced, for example, by chain transfer reactions; see above discussion on yield) which as the polymerization proceeds become large enough to precipitate with the rest of the copolymer chains during purification. These relatively small chains are characterized by a high mPEG/PLGA ratio and when they become large enough to escape removal from the rest of the copolymer during purification they should not only decrease the molecular weight, but they should also bring about an increase in the proportion of mPEG in the purified copoly-

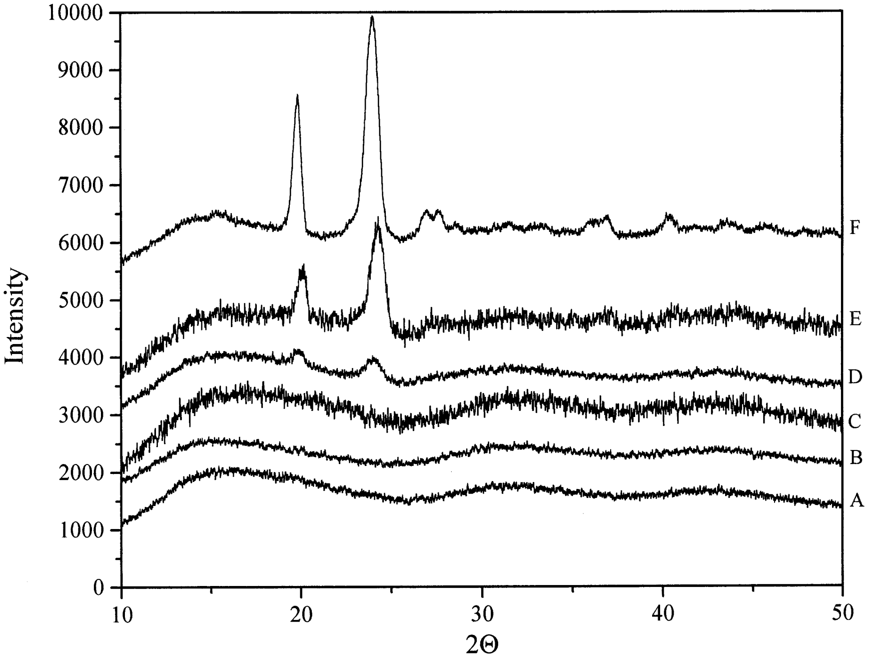

Fig. 8. X-ray powder diffractograms of PLGA, PLGA–mPEG and mPEG. (A) PLGA; (B) PLGA–mPEG (26% mol EO); (C) PLGA–mPEG (39% mol EO); (D) PLGA–mPEG (46% mol EO); (E) PLGA–mPEG (57% mol EO); (F) mPEG.

mer. Indeed, at late polymerization times, the mPEG content of the copolymers began to increase with time (Table 1).

The MWD of the copolymers was too broad at the initial stages of polymerization but later it became narrow (Fig. 6). At late polymerization times the PI appeared to stabilize at relatively low values (Fig. 5), indicating that transesterification reactions (Vanhoorne et al., 1992), which would increase the PI, did not take place to any significant extent during the polymerization periods studied here.

The composition of PLGA–mPEG is expected to affect significantly major copolymer characteristics, such as crystallinity, hydrophilicity, solubility and biodegradation. For example, as seen from the contact angle values obtained (Table 3), an increase in the EO content of PLGA–mPEG caused an increase in PLGA–mPEG hydrophilicity. The EO content of PLGA–mPEG also affected its morphology and the copolymers containing approximately 40% mol EO or higher exhibited crystallinity (Fig. 7). Nakafuku and Mitoshi (1993) working with (*l*)PLA–PEG blends and Youxin and Kissel (1993) working with (*l*)PLA–PEG–(*l*)PLA copolymers concluded, based on DSC data, that PEG and *l*–PLA were partially miscible. Our DSC data (Table 2 and Fig. 7) show that the T_{m} of mPEG segment is affected in a non-linear fashion by the molar fraction of mPEG in the copolymer, indicating a partial miscibility for mPEG and (*dl*)PLGA too.

The mPEG content of the copolymers initially fell with time as lactide and glycolide units were added in the growing copolymer chains. Later, the mPEG content of the copolymers begun to increase with time for reasons discussed already (Table 1). The variation of the LE/GE molar ratio in the synthesized copolymers with time (Table 1) may be attributed to the different reactivity of the two monomers. Glycolide, being more reactive than lactide (Gilding and Reed, 1979), would be preferentially polymerized in the initial stages, but later lactide would be incorporated in the copolymer to an increasing extent as the glycolide proportion in the reactants would have been reduced.

In conclusion, the yield of the polymerization and the properties of the PLGA–mPEG copolymers are affected by the interplay of the conditions of polymerization. Also, the purification step could interfere with the obtained results. It would appear possible to tailor the properties of the synthesized copolymers by adjusting the polymerization conditions.

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