Protein Encapsulation in Biodegradable Amphiphilic Microspheres. I. Polymer Synthesis and Characterization and Microsphere Elaboration

PHILIPPE BOUILLOT, ALAIN PETIT, EDITH DELLACHERIE

Laboratoire de Chimie-Physique Macromoléculaire, URA CNRS 494, ENSIC-INPL, BP 451, 54001 Nancy Cedex, France

ABSTRACT: Polymerization of lactide on monomethoxypolyoxyethylene (MPOE), using stannous octoate as a catalyst, was carried out in bulk and in solvent. Polymerization in a solvent permits one to work at a lower temperature and thus to prevent transesterification reactions. The copolymers synthesized in solvent exhibited a lower polydispersity and a polylactic acid (PLA) block longer and closer to the expected one. Therefore, this procedure was used to synthesize a series of diblock copolymers MPOE−D,L-PLA, keeping the PLA chain constant (45,000 g/mol), the MPOE block increasing from 2000 to 5000, 10,000, 15,000, and 20,000 g/mol. The longer the MPOE chain, the higher the water uptake in the MPOE−PLA films and the lower the glass transition temperature of the copolymers. The synthesized copolymers were used to prepare microspheres by the double-emulsion method. The PLA microspheres possess a smooth surface, whereas those made from copolymers have a rough surface with irregularity increasing with the molecular weight of MPOE. The size of these microspheres depends on the amphiphilic nature of the copolymers, their hydrophilicity, and their intrinsic viscosity in the organic solvent. © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 68: 1695−1702, 1998

Key words: diblock copolymer; polymerization in solvent; polydispersity; water uptake; microsphere

INTRODUCTION

Polyesters and copolyesters such as polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), and polycaprolactone (PCL) are used for medical applications because of their nonimmunogenic, nontoxic, and biodegradable properties.[1] In the past 20 years, there has been a growing interest in the synthesis and use of block copolymers consisting of PLA, PLGA, or PCL blocks associated with one or more hydrophilic chains. Indeed, this type of material should allow one to cover a broad range of mechanical and physicochemical properties (such as water absorption, polymer degradation, and polymer−drug and polymer−living body interactions.) which are of particular interest in the manufacture of drug-delivery systems.

Polyoxyethylene (POE) is nontoxic and suitable for internal use in the human body[2] and, therefore, it is often chosen to form the hydrophilic block. Indeed, implants of triblock PLGA−POE−PLGA copolymers and PLGA homopolymers are equally well tolerated *in vivo*.[3] Similarly, PLA−POE−PLA and PLA implants exhibited different *in vivo* degradation kinetics, but an overall comparable biological response,[4] suggesting that the triblock copolymers might also find applications as biodegradable biomaterials. Moreover, a variety of medical applications of block copolymers have been reported, such as micelles,[5,6]

POE-coated "stealth" nanospheres,[7–9] and microspheres.[4,10,11] The synthesis of the triblock PLA–POE–PLA copolymer is described according to two principal routes:

- First, an anionic polymerization which consists of a ring-opening polymerization of lactide[12] on the POE alcoolate. However, this procedure leads to relatively low yields of conversion and shorter PLA chains than expected, because the anionic chain ends cause transesterification and back biting.[13]
- A second procedure is the pseudoanionic polymerization in bulk of lactide on the hydroxyl end group of POE using a catalyst.[14] Although the yields thus obtained are high, the high temperature generally used for the melt polymerization (140–200°C) often leads to transesterification reactions, to shorter PLA chains than expected, and to a broadening of the molecular weight distribution.[15]

The synthesis of triblock PLA–POE–PLA copolymers in a solvent gave a better control of the molecular weight together with a lower polydispersity[16] compared with the bulk polymerization at 175°C.[17] However, the polymerization mechanism of the cyclic monomers depending on the catalyst used on POE is still not perfectly understood[18] and, often, only the polymer relative molecular weights are measured by high-performance size-exclusion chromatography (HPSEC) using polystyrene[7,8,11] or POE standards.[6–9]

In our study, we aimed at comparing the bulk and solvent polymerization of lactide on monomethoxypolyoxyethylene (MPOE). The diblock copolymers thus formed were characterized by both relative and absolute methods. The latter methods do not use standards, differing in their structure of the block copolymers and often leading to erroneous average molecular weight values. A series of diblock MPOE–D,L-PLA copolymers were synthesized, keeping constant the length of the PLA block and varying the MPOE block from 2000 to 20,000 g/mol. Finally, these materials were used to prepare microspheres and their morphology and size distribution were compared to those obtained from PLA homopolymers of equivalent molecular weight.

EXPERIMENTAL

D,L-PLA ($\overline{M_w}$ = 49,500 g/mol, $\overline{M_n}$ = 37800 g/mol, I = 1.31) was purchased from Phusis (France).

D,L-Lactide was supplied by Lancaster (England) and was recrystallized twice from anhydrous toluene. Stannous octoate [tin(II) bis-2-ethylhexanoate] (Johnson and Matthey, Germany) was used as received. MPOE with number-average molecular weights of 2000 and 5000 g/mol (MPOE2K and 5K) came from Aldrich (Germany). MPOE10K, -15K, and -20K were purchased from Shearwater Polymers Inc. (U.S.A.). Before polymerization, MPOE polymers were thoroughly dried over molecular sieves (4 Å) at 80°C a under vacuum (10 mmHg). Xylene (Aldrich, Germany), the polymerization solvent, was dried over calcium hydride and distilled before use. All other reagents were analytical grade.

Purification and Characterization of the Commercial Polymer MPOE

Commercial MPOE may contain up to 10% POE of high molecular weight.[19] The presence of this impurity affects the final properties of the synthesized copolymers. Therefore, this bifunctional compound was removed by fractional precipitation from ether. The efficacy of the purification was proved by end-group analysis of MPOE after reaction of the hydroxyl function with trichloroacetylisocyanate.[19]

The number-average molecular weight of MPOE polymers was determined by high-performance size-exclusion chromatography (HPSEC), using a TSK 3000 PW column (7.5 mm × 30 cm Touzard et Matignon, France) after calibration with POE standards with a mixture of water–acetonitrile (90 : 10% v) at a flow rate of 0.7 mL/min as the eluent.

Polymerizations

Bulk polymerization was carried out in a dried polymerization tube connected to a vacuum line. D,L-Lactide and equimolar amounts of MPOE and stannous octoate were mixed in the tube which was purged thrice with nitrogen and sealed under vacuum (5×10^{-2} torr). The reaction was carried out at 150°C for 2 h without stirring. The resulting polymer was dissolved in dichloromethane, precipitated in cold ethanol, and dried under vacuum.

Polymerization in the solvent (xylene) was performed in a stirred reactor equipped with a condenser. The reagents were dried and degassed overnight and xylene was added under a nitrogen flow using a cannulae. The polymerization reaction was conducted at 100°C for 2 h under stirring.

Then, the reaction mixture was cooled and poured into cold ethanol or cold ether to precipitate the polymer.

Copolymer Characterizations

The number-average molecular weight, the weight-average molecular weight, and the polydispersity index (I) were evaluated using a size-exclusion chromatograph equipped with a refractometer and connected to a multiangle light-scattering detector (laser photometer Dawn DSP, Wyatt Technology, USA) (MALLS). Two Ultrastyragel columns (7.8 mm $\times$ 30 cm Waters, USA) were used in series with THF as the eluent at a flow rate of 1 mL/min.

^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra of the copolymers in CDCl$_3$ were recorded on a Bruker AM 200 spectrometer using tetramethylsilane as the internal standard. Differential scanning calorimetry (DSC) measurements were conducted with a Perkin–Elmer DSC-92 Setaram thermal analyzer in aluminum pans, under a helium flow, and at a heating and cooling rate of 10°C/min.

The tin content of the synthesized copolymers was determined using a plasma torch and residual tin was removed by extraction of a dichloromethane phase containing the copolymer by a 0.2M aqueous solution of diethylenetriaminopentaacetic acid (Aldrich, Germany). Polymer films (thickness $\approx$ 400 μm) were obtained by casting from a 25 wt % solution of the block copolymers in toluene. The water uptake of the dried films was measured by weighing the swollen films after various times of contact with water (37°C).

Microsphere Preparation

The microspheres were prepared by a double-emulsion technique[20] with slight modification. A water-in-oil emulsion was formed with 2 mL dichloromethane containing 400 mg of polymer and 100 μL of distilled water, by vortexing (60 s) followed by pulsed sonication (60 s, 40 W) in an ice bath. The resulting water/oil (W/O) emulsion was vortexed 30 s with 3 mL of a 1% wt PVA (Aldrich, molecular weight 13,000–23,000 g/mol, 88% hydrolyzed) solution to form a W/O/W emulsion, which was poured into 100 mL of water under stirring. The surface of the microsphere thus formed hardened in about 20 min. It was then possible to totally extract the organic solvent by using a rotary evaporator. The microspheres were
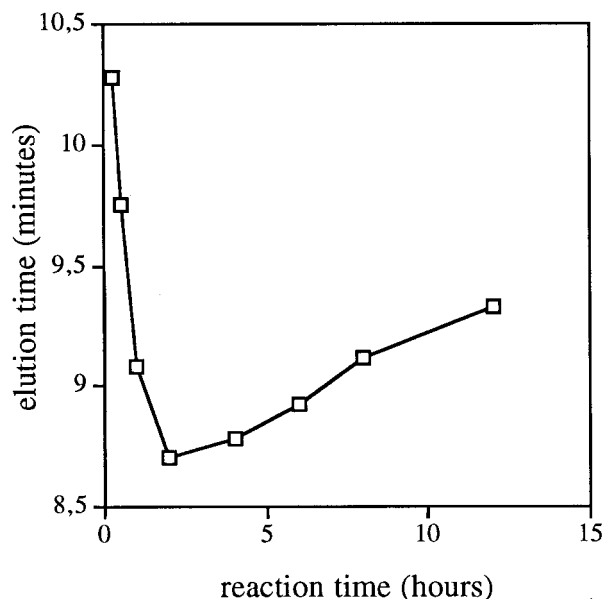
Figure 1 Evolution of the elution time of the major peak obtained during the synthesis of MPOE2K–PLA45K. Determination by HPSEC on two Lichrogel columns (PS400 and PS20, 7.8 mm $\times$ 30 cm, Merck) used in series with THF as eluent at a flow rate of 1 mL/min.

finally collected by centrifugation, washed thrice to remove the adsorbed PVA, and freeze-dried.

Microsphere Characterization

The microspheres were observed with a scanning electron microscope (JEOL JSM-T 330 A) after coating with a mixture of gold and palladium.

RESULTS AND DISCUSSION

Copolymer Synthesis

A diblock MPOE2K–PLA45K copolymer was synthesized in bulk according to the procedure described for PLA–POE–PLA[21] polymers. The same polymer was also prepared in a solvent (xylene) by modifying the solvent PLA synthesis proposed by Kricheldorf et al.[22] In both cases, the reaction course was followed by HPSEC. The peak of MPOE was shifted to the high molecular weight immediately after the reaction began. Moreover, no peak corresponding to PLA oligomers were observed on the chromatogram. Therefore, these results prove that the MPOE initiates the polymerization, which leads to only copolymer formation with no residual MPOE.

However, the elution time of the copolymer increased after 2 h of reaction (Fig. 1). In the case

Table I   Characteristics of MPOE–PLA Diblock Copolymers Synthesized in Bulk or in Solvent

Reaction Time (h)	Type of Polymerization	$\overline{M_n}$ of Copolymers[a] (g/mol)	$\overline{M_w}$ of Copolymers[a] (g/mol)	$I = \overline{M_w}/\overline{M_n}$	Yield[b] (wt %)
2	Bulk	35,500	60,400	1.70	89
2	Solvent	43,600	57,500	1.31	94
12	Solvent	32,400	43,100	1.33	87

[a] Measured by MALLS.
[b] Amount of copolymer after purification on total amount of MPOE + lactide.

of bulk polymerization, this phenomenon is well known to be due to transesterification and back-biting[21,23] reactions. In the case of bulk PLA synthesis, transesterification reactions occur only at high temperature ($>120°C$) and lead to a large polydispersity and short chains of the copolymer,[15] whereas back-biting reactions appear after a longer time (>2 h) and decrease the yield and the length of the chains.[21]

Table I presents the values of $\overline{M_n}$, $\overline{M_w}$, the polydispersity index, and the yield, obtained for copolymers synthesized in the bulk and in solution. The same polydispersity was observed after 2 or 12 h of reaction in the solvent and it was lower than that of the copolymer synthesized in the bulk. It is likely that in solvent polymerization the decrease of $\overline{M_n}$ is caused only by back-biting reactions and not by a transesterification reaction because of the low temperature used ($100°C$). After 2 h of reaction, the yield of the bulk polymerization is lower than that in the solvent because about 10% of the lactide sublimates at the top of the tube.[24]

The copolymers obtained in the solvent have molecular weights higher than those obtained in the bulk and relatively similar to those calculated theoretically. This method was thus chosen in the following to synthesize a series of MPOE–D,L-PLA copolymers with a constant length PLA block (about 45K) and an MPOE chain with a molecular weight increasing from 2K to 5K, 10K, 15K, and 20K. The corresponding copolymers were named, respectively, MPOE2K–PLA45K, MPOE5K–PLA45K, MPOE10K–PLA45K, MPOE15K–PLA45K, and MPOE20K–PLA45K. 2K, 5K, 10K, 15K, and 20K are the molecular weights indicated by the supplier for MPOE; 45K is the expected molecular weight of the PLA block.

We determined the $\overline{M_n}$, $\overline{M_w}$, and I values by MALLS and the molar ratio of lactic acid/ethylene oxide (LA/EO) was calculated from the ^{1}H-NMR

spectra. It should be noticed that for all the synthesized copolymers the $\overline{M_w}$ calculated by an absolute method was different from that determined by HPSEC using polystyrene standards (results not shown). The latter $\overline{M_w}$ value was about 40% higher, due to the structural differences between the diblock copolymers and the standards. The characteristics of the synthesized copolymers are listed in Table II. All the copolymers synthesized have an $\overline{M_n}$ close to that expected and relatively low polydispersity. When the MPOE length increased, the yield of conversion slightly decreased. At the same time, the polydispersity index increased, probably due to the higher polydispersity of the starting MPOE itself. The information obtained by ^{1}H-NMR and light scattering is in good agreement.

The ^{1}H characteristic spectrum of MPOE–PLA copolymers classically exhibited[2,5,6] signals corresponding to polyoxyethylene (3.65 ppm) and PLA chains [1.56 ppm (methyl protons) and 5.20 ppm (methine protons)] and additional signals at 2.98 and 4.36 ppm which were assigned to the (CH_3)—$\underline{CH}$—$\underline{OH}$ end group and at 3.38 ppm corresponding to the methyl end group of MPOE. The signal at 4.28 ppm was attributed to the $\underline{CH_2}$—CH_2—O—CO connecting unit.

The ^{13}C spectra typically showed[2,25] the presence of $C=O$ and CH_3 signals of the PLA chain at 168.48 and 16.63 ppm. The peaks at 70.45 and 69.00 ppm are too close to be assigned directly. The technique of insensitive nuclei enhanced by polarization transfer (INEPT) was thus employed: The peak at 70.45 ppm appeared as a negative signal and was attributed to the MPOE chain CH_2; the peak at 69.00 ppm, as a positive signal, was assigned to the PLA chain CH.

Residual Tin Content

The amount of tin in the copolymers after precipitation was about 2×10^{-2} mmol/g, which repre-

Table II   Characterization of MPOE–PLA Diblock Copolymers Prepared by Polymerization in Xylene

Copolymers	$\overline{M_n}$ of MPOE[a] (g/mol)	$\overline{M_n}$[b] (g/mol)	$\overline{M_w}$[b] (g/mol)	$I = \overline{M_w}/\overline{M_n}$[b]	LA/EO Theoretical[c]	LA/OE[d]	Yield[e] (wt %)
MPOE2K–PLA45K	1700	43,600	57,500	1.30	16.4	15.9	94
MPOE5K–PLA45K	4600	46,300	58,800	1.25	6	5.6	94
MPOE10K–PLA45K	9600	50,700	71,500	1.40	2.8	2.6	90
MPOE15K–PLA45K	14,200	54,200	79,100	1.45	1.9	1.6	91
MPOE20K–PLA45K	19,400	59,800	86,100	1.45	1.4	1.2	90

[a] Determined by HPSEC.
[b] Measured by MALLS.
[c] Theoretical copolymer composition (molar ratio of units LA/EO).
[d] Molar ratio LA/EO calculated by ^{1}H-NMR.
[e] Amount of copolymer after purification (mg) on total amount of MPOE + lactide (mg).

sents about 90% of the catalyst used. Although stannous octoate is accredited by FDA, the amount of tin was reduced to 10^{-3} mmol/g after two extractions with a $0.2M$ DTPA solution. The result of this extraction shows that tin is not chemically bound to the copolymer, but may form strong complexes with it.

Polymer Swelling in Water

Films of MPOE–PLA and PLA, about 400 μm thick, were cast from toluene. The results of the water uptake (Fig. 2) indicate that the water content of the MPOE–PLA copolymers rapidly increases with the EO content. In the case of copolymers containing MPOE chains of 15K and 20K,
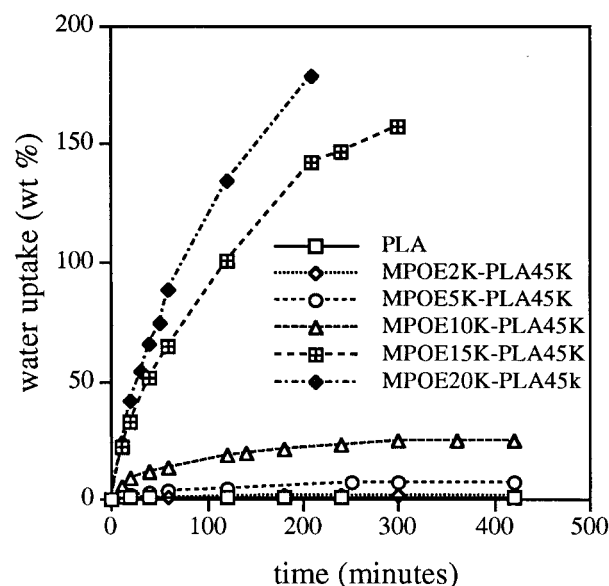
Figure 2 Water uptake of PLA and MPOE–PLA films at 37°C.

the water uptake was so important that the films were desorganized in, respectively, 4 and 3 h. Therefore, the experiment had to be stopped before reaching the absorption equilibrium. Interestingly, microspheres prepared from these copolymers highly swelled in water, but kept their structure intact (did not disintegrate in 3 days), making, thus, possible the studies of drug release and polymer degradation (results to be published). Possibly, the morphology of the films and microspheres is different. At a high EO content, the polymer films form hydrogels in water and absorb a very high amount of water, as was observed with PLA44K–POE41K–PLA44K triblock copolymers.[25] The water uptake of the PLA45K film was about the same as that of the MPOE2K–PLA45K copolymer (respectively, 1.4–1.8% wt of water), probably due to the low EO content in the latter polymer.

Thermal Behavior

The MPOE–PLA-synthesized copolymers differ in their physical properties from the PLA homopolymer: MPOE is semicrystalline, while D,L-PLA is amorphous. The thermal properties of the synthesized copolymers are listed in Table III. It is shown that, first, the glass transition temperature (T_g) decreases slightly with increase in the molecular weight of MPOE chains between 2K and 10K. Then, T_g decreases quickly between 10K and 20K. These results show that the flexibility and the mobility of the copolymer chains are related to the EO content. The higher the EO content, the higher the flexibility and the mobility of the copolymer.

These temperatures were determined according to the DSC thermograms of quenched

Table III Glass Transition Temperatures (T_g) of PLA and MPOE–PLA Copolymers, Melting Temperatures (T_m) of MPOE Homopolymers Used as Starting Material for the Synthesis of MPOE–PLA, and Melting Temperatures (T_m) of MPOE Crystallites in the MPOE–PLA Copolymers

Copolymers	T_g (°C)	T_m of MPOE Homopolymer (°C)	T_m of MPOE in the MPOE–PLA Copolymer (°C)
PLA45K	32		
MPOE2K–PLA45K	28	53	None
MPOE5K–PLA45K	21	58	None
MPOE10K–PLA45K	22	64	None
MPOE15K–PLA45K	−3	68	53
MPOE20K–PLA45K	−20	75	51

polymers. Only the DSC curves of the quenched copolymers, MPOE15K–PLA45K and MPOE20K–PLA45K, exhibit a recrystallization exotherm followed by an endotherm due to the fusion of the MPOE block. The melting temperatures of MPOE15K and 20K in the copolymers are lower than the melting temperatures of the MPOE homopolymers 15K and 20K. In the case of MPOE2K–PLA45K, MPOE5K–PLA45K, and MPOE10K–PLA45K, the presence of PLA inhibits the crystallization of the MPOE chains. The EO content has to be higher than 18% (wt) to lead to crystallization. However, the MPOE crystallites in the copolymers are polluted by the PLA chains, which explains the difference of melting temperatures between homo- and copolymers.
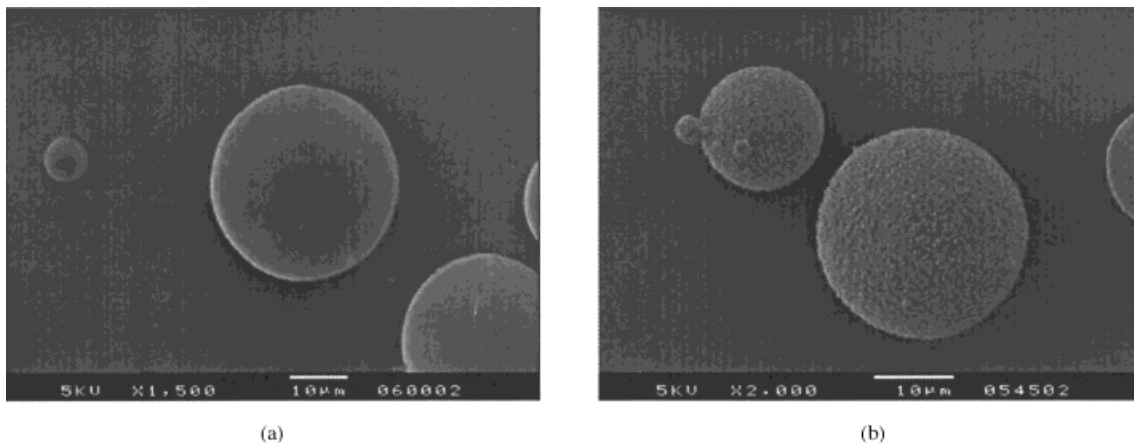
Microspheres

Morphology

Microspheres were prepared by a double-emulsion method using the series of MPOE–PLA45K-synthesized copolymers and a commercial PLA45K used as a reference. All the particles were spherical when observed by scanning electron microscopy (Fig. 3). The PLA microspheres possess a smooth surface, whereas those made from copolymers have a rough surface with irregularity increasing with the molecular weight of MPOE. Similar irregular surface structures were observed in the case of microspheres prepared with multiblock copolymers PLA–POE–PLA[10] or PLGA–POE–PLGA.[11]

Size Distribution

The volume-size distribution of the microspheres was determined before and after freeze-drying.

The results are listed in Table IV. Before freeze-drying, in the case of PLA45K, MPOE2K–PLA45K, and MPOE5K–PLA45K microspheres, the higher the length of the MPOE chains, the lower the size of the microspheres. However, the microspheres made from copolymers having longer MPOE chains (10K, 15K, and 20K) present all the same mean diameter, which is slightly increased compared to that of MPOE5K–PLA45K microspheres. This observation could be explained by the effect of several parameters such as the hydrophilicity of copolymers, their amphiphilic properties, and the viscosity of the polymer solution, which have antagonistic effects. First, the MPOE–PLA copolymers have amphiphilic properties, and, thus, they lower the interfacial tension of the W/O emulsion during the microsphere preparation procedure. Therefore, the mean size of the microspheres prepared by the W/O/W emulsion procedure is smaller than for pure PLA. Second, by increasing the MPOE chain length, the viscosity of the polymer solution increases and, therefore, the size of the resulting particles tends to increase. Third, the copolymers with long MPOE blocks (10K, 15K, 20K) highly absorb water, as shown in Figure 4, leading to a matrix expansion. As a consequence, the size of the microspheres (measured after solvent removal, just before freeze-drying) might also depend on their water uptake.

The mean size of the microspheres made from PLA45K, MPOE2K–PLA45K, and MPOE5K–PLA45K is lower after freeze-drying, probably due to a compression of the matrix. The microspheres made of MPOE–PLA with longer chains (10K, 15K, and 20K) have the tendency to form aggregates after freeze-drying, probably due to

Figure 3 Photographs of microspheres made (a) from PLA45K and (b) from MPOE5K–PLA45K obtained with a scanning electron microscope.

the association of the high molecular weight MPOE chains on their surface. Thus, the size of these particles, measured immediately after freeze-drying, was greatly increased (Table IV). However, after vigorous stirring (vortex, 15 min), the microspheres eventually recovered the same mean diameter as before freeze-drying. We suppose that the hydrophilic matrices reabsorbed the water amount lost during freeze-drying, and because of the more flexible nature of the polymers, we did not observe the irreversible compression effect of freeze-drying, as in the case of PLA45K, MPOE2K–PLA45K, and MPOE5K–PLA45K.

CONCLUSIONS

Polymerization of lactide in a solvent occurs at a lower temperature than for bulk polymerization and permits one to prevent transesterification reactions. Thus, diblock MPOE–PLA copolymers can be synthesized with a better control of the length of the PLA chains and a lower polydispersity. A series of copolymers was synthesized in solvent with a constant PLA chain (about 45K) and an MPOE block of molecular weight progressively increasing from 2K to 5K, 10K, 15K, and 20K. The longer the MPOE chain, the higher the water uptake in the MPOE–PLA films and the lower the glass transition temperature of the copolymers. The synthesized copolymers were used to prepare microspheres by the double-emulsion method. The size of these microspheres depends on the amphiphilic nature of the copolymers, their hydrophilicity, and the viscosity of their solution in the organic solvent. Further articles will discuss in detail the influence of the physicochemical nature of the copolymers on the microsphere in-

**Table IV   Mean Size of Microspheres Made from PLA45K
and from Synthesized Copolymers Before and After Freeze-Drying,
Expressed as the Average of Measurements of Three Batches**

Copolymers	Mean Size Before Freeze-Drying (μm)	Mean Size After Freeze-Drying (μm)	Mean Size After Freeze-Drying and 15 min of Stirring (μm)
PLA45K	44 ± 1	40 ± 1	40 ± 1
MPOE2K–PLA45K	39 ± 1	33 ± 1	33 ± 1
MPOE5K–PLA45K	30 ± 1	26 ± 1	26 ± 1
MPOE10K–PLA45K	33 ± 1	56 ± 4	34 ± 1
MPOE15K–PLA45K	34 ± 1	52 ± 4	35 ± 1
MPOE20K–PLA45K	33 ± 1	53 ± 4	33 ± 1

Sizes measured by Coulter multisizer II.

ternal morphology and degradation and on drug release.

REFERENCES

1. E. J. Frazza and E. E. Schmitt, *J. Biomed. Mater. Res. Symp.,* **1,** 43 (1971).

2. K. J. Zhu, L. Xiangzhou, and Y. Shilin, *J. Appl. Polym. Sci.,* **39,** 1 (1990).

3. B. Ronneberger, W. J. Kao, J. M. Anderson, and T. Kissel, *J. Biomed. Mater. Res.,* **30,** 31 (1996).

4. H. Younes, P. R. Nataf, D. Cohn, Y. J. Appelbaum, G. Pizov, and G. Uretzky, *Biomater. Artif. Cells, Artif. Organs,* **16,** 705 (1988).

5. E. Piskin, X. Kaitian, E. B. Denkbas, and Z. Küçükya-vur, *J. Biomater. Sci. Polym. Ed.,* **7,** 359 (1995).

6. X. Zhang, J. K. Jackson, and H. M. Burt, *Int. J. Pharm.,* **132,** 195 (1996).

7. D. Bazile, C. Prud'homme, M. T. Bassoulet, M. Marlard, G. Spenlehauer, and M. Veillard, *J. Pharm. Sci.,* **84,** 493 (1995).

8. R. Gref, Y. Minamitake, M. Peracchia, Y. Trubet-skoy, V. Torchilin, and R. Langer, *Science,* **263,** 1600 (1994).

9. S. Stolnik, S. E. Dunn, M. C. Garnett, M. C. Davies, A. G. A. Coombes, D. C. Taylor, M. P. Irving, S. C. Purkin, T. F. Tadros, S. S. Davies, and L. Illum, *Pharm. Res.,* **11,** 1800 (1994).

10. T. Kissel, Y. X. Li, C. Volland, S. Gôrich, and R. Koneberg, *J. Control. Release,* **39,** 315 (1996).

11. M. K. Au-Yeh, P. G. Jenkins, S. S. Davis, and A. G. A. Coombes, *J. Control. Release,* **37,** 1 (1995).

12. H. R. Kricheldorf and C. Boettcher, *Makromol. Chem. Macromol. Symp.,* **73,** 47 (1993).

13. H. R. Kricheldorf and I. Kreis, *Makromol. Chem.,* **191,** 1057 (1990).

14. L. Youxin and T. Kissel, *J. Control. Release,* **27,** 247 (1993).

15. J. W. Leenslag and A. J. Pennings, *Makromol. Chem.,* **188,** 1809 (1987).

16. E. Çelikkaya, E. B. Denkbas, and E. Piskin, *J. Appl. Polym. Sci.,* **61,** 1439 (1996).

17. W. M. Stevels, M. J. K. Ankone, P. J. Dijkstra, and J. Feijen, *Makromol. Chem. Phys.,* **196,** 3687 (1995).

18. Y. J. Du, P. J. Lemstra, A. J. Nijenhuis, H. A. M. van Aert, and C. Bastiaansen, *Macromolecules,* **28,** 2124 (1995).

19. R. de Vos and E. J. Goethals, *Polym. Bull. (Berl.),* **15,** 547 (1986).

20. M. J. Blanco Prieto, F. Delie, E. Fattal, A. Tartar, F. Puisieux, A. Gulik, and P. Couvreur, *Int. J. Pharm.,* **111,** 137 (1994).

21. H. R. Kricheldorf and J. Meier-Haack, *Makromol. Chem.,* **194,** 715 (1993).

22. H. R. Kricheldorf, C. Boettcher, and K. U. Tônnes, *Polymer,* **33,** 2817 (1992).

23. X. Zhang, U. P. Wyss, D. Pichora, and M. F. A. Goosen, *Pure Appl. Chem.,* **30,** 933 (1993).

24. D. S. G. Hu and H. J. Liu, *J. Appl. Polym. Sci.,* **51,** 473 (1994).

25. S. M. Li, I. Rashkov, J. L. Espartero, N. Manolova, and M. Vert, *Macromolecules,* **29,** 57 (1996).