Polymeric Nanoparticles Based on Polylactide and Related Copolymers

Emo Chiellini, Vera L. Covolan, Lorenzo M. Orsini, Roberto Solaro*

UdR – INSTM Consortium @ Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento, 35 – 56126, Pisa, Italy

Summary: The preparation of nanoparticle suspensions was carried out by using commercial biodegradable polymers as poly(d,l-lactide), poly(d,l-lactide-*co*-glycolide) and poly(d,l-lactide-*co*-ε-caprolactone). The method of preparation was based on the controlled addition of polymer organic solution to an aqueous phase containing dispersing agents. Poly(ethylene glycol) (10, 20, and 35 kDa grade), Tween 20, and Pluronic F-127 were used as dispersing agents in the aqueous phase. Content and type of both polymeric matrix and dispersing agent resulted of paramount relevance for the attainment of monodispersed nanoparticles with average diameter of about 130 nm. The addition of a steric stabilizer allowed for nanoparticle purification and isolation while preventing their agglomeration. The best results were obtained by using 35 kDa grade poly(ethylene glycol) as dispersing agent and either mannitol or glycidyl*iso*propylidenglyceryl-β-cyclodextrin as steric stabilizer. The adopted procedure afforded biodegradable nanoparticle suspensions that could be used for the incapsulation and intravenous administration of biologically active proteins and oligopeptides.

Keywords: biocompatible polymers; biodegradable polymers; nanoparticles; polyesters; polylactides

Introduction

Increasing attention is being currently dedicated to preparation of nano-sized particles for medical and pharmaceutical uses. These applications require specific systems based on particles displaying biodegradable and biocompatible characteristics for use as drug delivery systems, including non-parenteral pulmonary, nasal, and oral routes. Systems with submicron dimensions are required for intravenous administration because of the limitations imposed by the micrmetric dimensions of capillaries (4-5 μm). Besides, features such as small size and large surface area make the particles very promising for the formulation of drug dosage forms.[1] Systems based on liposomes were widely studied as drug carrier because of their

 DOI: 10.1002/masy.200350730

reduced toxicity and ability of protecting drugs from degradation. However, these systems are affected by serious problems such as low drug payload, rapid leakage of water-soluble drugs in presence of blood components, and poor shell life.[2] Alternatively, nanoparticle systems based on biodegradable/biocompatible polymeric materials were found advantageous over liposomes because they can increase the stability of protein drugs and positively affect their potency by their targeted administration to the sites of choice.[3]

Among the biodegradable/biocompatible polymeric materials, poly(esters) based on poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers (PLGA) have been extensively investigated for biomedical applications.[4] However, the high crystallinity and low hydrophilicity of poly(l-lactide) negatively affect its degradation rate, resulting in poor compatibility with soft tissues. To overcome this drawback, alternative polymers have been synthesized by attempting to embody hydrophilic and more easily hydrolyzable segments into PLA.[5] Since the biocompatibility is one of the best characters possessed by poly(ethylene glycol) (PEG), a great deal of interest has been devoted to investigations aimed at including PEG into PLA copolymers. The presence of hydrophilic PEG segments in amphiphilic biocompatible polymeric materials may allow for the formulation of nanoparticles susceptible of escaping the RES action and hence prolonging their life span in the blood circulation with respect to PLA particles.[6]

Following our interest in the preparation of nanoparticle systems for the targeted delivery of proteic drugs,[7-13] in the present paper we report on the results of an investigation aimed at the formulation and isolation of biocompatible nanoparticles suited for the incapsulation of proteins and oligopeptides. To this goal, lactic acid homopolymers and copolymers were used in combination with PEG of various molecular weights, in order to obtain stealth biodegradable nanoparticles.

Experimental

Products

Poly(d,l-lactic acid) (PLA; Mw 15.000 - 25.000; Polyscences), poly(d,l-lactide-*co*-glycolide) 50:50 (PLGA; Mw 5.000-15.000; Aldrich) and poly(d,l-lactide-*co*-ε-caprolactone) 86:14 (PLCL; Aldrich) were used for the preparation of nanoparticle suspensions. Acetone and

ethanol were used as polymer solvents. Poly(ethylene glycol) (PEG10, MW 10,000; PEG20, MW 20,000; PEG35, MW 35,000; Polysciences), Tween 20 (TW; Fluka Chemika), Pluronic F-127 (F-127; Sigma), L-α-phosphatidylcholine (PHO; Sigma) were used as dispersing agents. Mannitol (MAN; Aldrich) and glycidyl*iso*propylidenglyceryl-β-cyclodextrin[14,15] (CD) were used as steric stabilizers.

Preparation of nanoparticle suspensions

The nanoparticle suspensions were obtained by mixing a polymer solution (organic phase) with a dispersing agent solution (aqueous phase). Experiments were performed according to a common procedure. Data relevant to individual runs are summarized in Tables 1-5, whereas a typical experiment is described in detail by following.

A solution of 50 mg PLA and 40 mg L-α-phosphatidylcholine in 5 ml acetone and 3 ml ethanol and a solution of 50 mg PEG10, 58 mg Tween 20, and 192 mg Pluronic F-127 in 16 ml distilled water were separately prepared. The organic solution was then added at 16 ml/h by using a motor driven syringe to the aqueous solution kept under mild magnetic stirring, at room temperature.

Dimensional and morphological characterization of suspensions

The average particle size and the particle size distribution of the suspensions were performed by an LS 230 Particle Size Analyzer (Beckman Coulter). Morphological features of nanoparticles were investigated by a JEOL JSM - T300 Scanning Electron Microscope (SEM).

Results and Discussion

Preparation of nanoparticle suspensions was carried out under diverse operating conditions by using PLA (Table 1), PLGA and PLCL (Table 2), and three kinds of lactide polymers that are known to have different biodegradation rates.[6] Initially, experiments were aimed at the selection of the best dispersing agent combinations. Tests were performed by employing PEG having molecular weight of 10 (PEG10), 20 (PEG20), and 35 kDa (PEG35), combined with

either Tween 20 or Pluronic F-127 in the aqueous phase, whereas the polymer solution contained L-α-phosphatidylcholine.

The reported preparations resulted in milky suspensions, containing particles with narrow size distribution and average diameter of about 130 nm (Table 1). However, in some cases, polymodal particle size distributions with average particle size varying from 69 to 111 μm were obtained. Apparently, the obtainment of homogeneous suspensions is inhibited when either Pluronic F-127 alone or a combination of Tween 20 with PEG10 and PEG20 are used. On the other hand, the use of PEG35 regularly resulted in nanoparticle suspensions with monomodal size distribution.

Table 1. Preparation of PLA-nanoparticle suspensions by using different dispersing agent combinations. [a)]

	Formulate compositions (mg)												
PHO	40	40	40	40	40	40	40	40	40	40	-	-	-
PEG10	50	-	-	50	-	-	50	-	-	-	-	-	-
PEG20	-	50	-	-	50	-	-	50	-	-	-	50	-
PEG35	-	-	50	-	-	50	-	-	50	-	-	-	-
TW	58	58	58	-	-	-	58	58	58	58	58	-	-
F-127	-	-	-	192	192	192	192	192	192	-	-	-	192
D [b)]	B	B	M	M	M	M	B	M	M	M	M	M	B
Φ [c)]	85 [d)]	69 [d)]	132	129	132	130	111 [d)]	131	130	132	129	128	72 [d)]

a) Organic phase: 50 mg PLA in 8 ml acetone; aqueous phase: PEG, TW, and F-127 in 16 ml distilled water. When phosphatidylcholine (PHO) was used, the polyester was dissolved in 5 ml acetone and 3 ml ethanol.
b) D = Particle size distribution: M = monomodal distribution; B = bimodal distribution. c) Average particle diameter, in nm if not otherwise stated. d) In μm.

Preparations with PLGA and PLCL copolymers resulted in suspensions with broad particle size distributions, differing widely from the previous PLA-suspensions (Table 2). In both cases, only the compositions employing PEG20 favored the formation of nanoparticles with narrow size distribution. These data indicate that modest changes in the chemical structure of the lactide polymer largely affect the interactions with tested dispersing agents, resulting in polymodal size distribution of particles.

The reported preliminary results indicated that the PEG35-Tween 20 combination was appropriate to produce stable suspensions containing a monomodal distribution of PLA-nanoparticles. On the other hand, PEG resulted to be the best dispersing agent for the preparation of PLGA and PLCL nanoparticles. Therefore, PEG35 and Tween 20 were used

independently in a series of preparations of PLA-nanoparticle suspensions and the influence of their concentration on particle size was investigated.

Table 2. Preparation of PLGA and PLCL nanoparticle suspensions by using different dispersing agent combinations. [a)]

	Formulate compositions (mg)									
PLGA	50	50	-	-	50	-	50	-	50	-
PLCL	-	-	50	50	-	50	-	50	-	50
PHO	-	40	-	40	-	-	-	-	40	40
PEG20	-	-	-	-	50	50	-	-	50	50
TW	58	58	58	58	-	-	-	-	58	58
F-127	-	-	-	-	-	-	192	192	192	192
Φ [b)]	P	P	P	P	M	M	P	P	P	P
F [c)]	122	158	132	134	132 [d)]	128 [d)]	164	129	154	79

a) Composition: PLGA in 8 ml acetone; PEG, TW, and F-127 in 16 ml of water. When PHO was used, the polyester was dissolved in 5 ml acetone and 3 ml ethanol. b) D = Particle size distribution: M = monomodal distribution; B = bimodal distribution. c) Average particle diameter, in μm if not otherwise stated. d) In nm.

In these experiments, the dispersing agent concentration in the aqueous phase was varied between 1.56 and 2.5 mg/ml, while maintaining constant the concentration of the other components. The preparations and related results are summarized in Table 3.

Table 3. Preparation of PLA-nanoparticles by using different PEG35 and Tween 20 concentrations.[a)]

Run	PEG35 (mg)	TW (mg)	Average diameter (nm)	Distribution [b)]
LE2/1	25	–	132	M
LE1/1	50	–	132	M
LE1/2	100	–	128	M
LE1/4	200	–	132	M
LE1/8	400	–	132	M
LT2/1	–	25	144	M
LT11/1	–	50	157	M
LT11/2	–	100	165	B
LT11/4	–	200	111	B
LT11/8	–	400	95	B

a) 50 mg PLA in 8 ml acetone, dispersing agent in 16 ml distilled water. b) M = monomodal distribution; B = bimodal distribution.

The average particle size was almost constant and close to 130 nm in all experiments carried out in the presence of PEG35. These results suggest that even the lowest dispersing agent concentration is large enough to act as steric stabilizer of nanoparticles. On the other hand, an

appreciable variation of the average particle size was observed in experiments performed by using Tween 20 as dispersing agent. Indeed, the particles size distribution changed from monomodal to bimodal by increasing the Tween 20 concentration in the aqueous phase. This behavior can be tentatively attributed to the variation of the suspension viscosity.

Purification of nanoparticles and use of steric stabilizers

Initially, centrifugation was adopted to purify and recover PLA-nanoparticles from the original suspensions. Samples of the suspensions were submitted to three cycles of centrifugation at 13,500 rpm for 20 minutes and then lyophilized. Scanning electron microscopy (SEM) analysis revealed that under the adopted conditions deformation and collapse of the nanoparticles occurred (Figure 1).

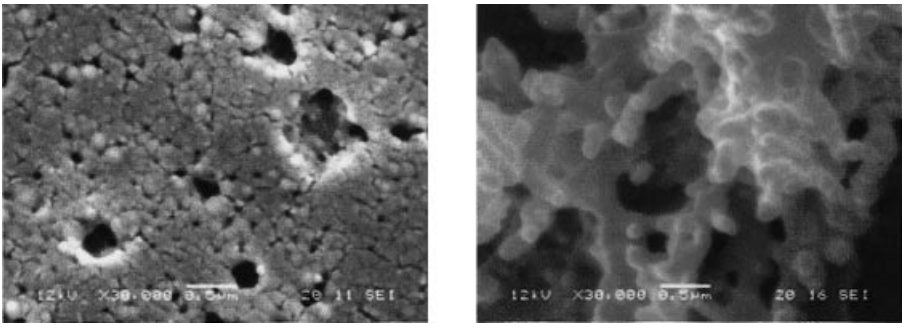

Figure 1. SEM micrographs of PLA-nanoparticles after centrifugation at 13,500 rpm.

This result clearly indicated that a less stressing isolation method was needed in order to prevent nanoparticle agglomeration. Moreover, the use of a protective agent in the suspension formulation could help overcoming this drawback. Therefore, experiments were carried out by employing steric stabilizers displaying different hydrophilicity and hindrance (Table 4), such as glycerol (GLY), propylene glycol (PG), mannitol (MAN), and glycidyl*iso*propylidenglyceryl-β-cyclodextrin (CD). The latter stabilizer was selected because of the good results obtained in the preparation of bioerodible nanoparticles for the targeted release of α-interferon.[6-12]

Table 4. Preparation of PLA-nanoparticle suspensions in the presence of different steric stabilizers.[a)]

Run	PEG35 (mg)	TW (mg)	MAN (mg)	GLY (mg)	PG (mg)	CD (mg)	H_2O (ml)	Φ[b)] (nm)
LM2w	50	50	100	–	–	–	16	136
LGLT1w	50	50	–	100	–	–	16	127
LPGT1w	50	50	–	–	100	–	16	129
LD2w	50	50	–	–	–	100	16	132
LGLEw	50	–	–	50	–	–	16	261
LGLT2w	–	50	–	50	–	–	16	130 [c)]
LPGEw	50	–	–	–	50	–	16	550
LPGT2w	–	50	–	–	50	–	16	191 [c)]

a) 50 mg PLA in 8 ml acetone. TW = Tween 20, MAN = mannitol, GLY = glycerol, PG = propylene glycol, CD = glycidyl*iso*propylidenglyceryl-β-cyclodextrin.
b) Avearge particle diameter, monomodal distribution if not otherwise stated.
c) Polymodal size distribution

Nanoparticle suspensions with average particle size of 130 nm and monomodal size distribution were obtained by using a combination of PEG35 and Tween 20 with glycerol (Run LGLT1w) or propylene glycol (Run LPGT1w). However, a significant increase of particle size and/or a polymodal size distribution was observed when glycerol and propylene glycol were used with either PEG35 (Runs LGLEw and LPGEw) or Tween 20 (Runs LGLT2w and LPGT2w).

Mannitol (Run LM2w) and glycidyl*iso*propylidenglyceryl-β-cyclodextrin (Run LD2w) afforded the most stable suspensions containing PLA-nanoparticles of average size around 130 nm and very narrow particle size distribution (Table 4). These preparations were considered very well suited for the assessment of the purification technique. The PLA-nanoparticle suspensions obtained in run LD2w were purified by 2 cycles of centrifugation at 8.000 rpm, whereas those obtained by using mannitol were submitted to cross-flow ultrafiltration by using 500 kDa cut-off membrane. Both samples were then lyophilized and analyzed by SEM (Figure 2).

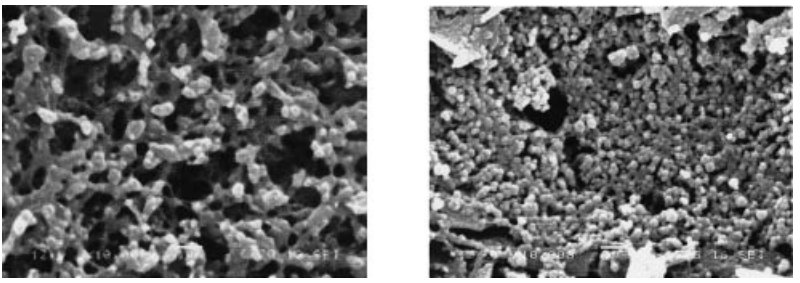

Figure 2. SEM micrographs of purified PLA-nanoparticles obtained: by using mannitol and submitted to ultrafiltration process (left); by using glycidyl*iso*propylidenglyceryl-β-cyclodextrin and submitted to centrifugation at 8.000 rpm (right).

SEM micrographs evidenced that nanoparticles prepared in the presence of steric stabilizers were more resistant to the stress of purification process and their aggregation and coalescence was inhibited. However, the recovery of the particles from the suspensions was rather limited under the adopted mild centrifugation conditions, and in some cases a significant amount of particles remained in suspension. On the other hand, the ultrafiltration process allowed for an almost quantitative retrieval of the particles that displayed a satisfactory purity degree.

Mannitol was also successfully used as steric stabilizer for the preparation of PLGA and PLCL nanoparticle suspensions (Table 5).

Table 5. Preparation of PLGA and PLCL nanoparticle suspensions by using mannitol as steric stabilizer.

Run	Copolymer		Acetone	EtOH	PEG35	MAN	H_2O	Φ [a]
	(type)	(mg)	(ml)	(ml)	(mg)	(mg)	(ml)	(nm)
LAEM1	PLGA	25	8.0	–	50	50	16	128
LAEM2	PLGA	50	8.0	–	100	100	16	126
LAEM3	PLGA	100	8.0	–	200	200	16	159
LAEM4	PLGA	200	8.0	–	400	400	16	132 [b]
LCEM1	PLCL	25	8.0	–	50	50	16	121
LCEM2	PLCL	50	8.0	–	100	100	16	132
LCEM3	PLCL	100	8.0	–	200	200	16	176 [b]
LCEM4	PLCL	100	5.0	3.0	200	200	16	132
LCEM5	PLCL	200	5.0	3.0	400	400	16	98 [b]

a) Avearge particle diameter, monomodal distribution if not otherwise stated.
b) Polymodal size distribution

The results presented in Table 5 indicate that the concentration of the organic phase has a significant influence on the resulting nanoparticle suspensions. Indeed, the average particle size increased on increasing the organic phase concentration and eventually a polymodal size distribution was attained. This effect was more pronounced in the case of the more hydrophobic PLCL. Also the composition of the organic solvent affected particle sizes and distributions.

Conclusions

The reported results clearly demonstrate that the composition of the organic phase has a deep influence on the formation mechanism of nanoparticle suspensions. In particular, the amount and type of both the synthetic polymer and the dispersing agent are of paramount relevance in determining the particle sizes and distributions. However, fine-tuning of the experimental parameters allowed for the preparation of PLA, PLGA, and PLCL nanoparticles with 130 nm average diameter and fairly narrow monomodal size distribution.

The use of steric stabilizers was fundamental to obtain stable suspensions that could undergo purification procedures without significant particle aggregation and coalescence. Among the investigated stabilizers, mannitol and a β-cyclodextrin derivative turned out to be the msot effective in affording stable nanoparticles.

Both centrifugation and ultrafiltration allowed for nanoparticle purification and isolation, even though quantitative nanoparticle recovery was attained only by the latter technique.

The reported results allowed to select conditions suitable for the preparation of biodegradable nanoparticle suspensions with great potential for the incapsulation and targeted release of proteins and oligopeptides.

Acknowledgments

The financial support by MIUR Cofin 2000 project is gratefully acknowledged.

[1] H. Murakami, M. Kobayashi, H. Takeuchi, Y. Kawashima, *Int. J. Pharm.*, **1999**, *187*, 143.
[2] J. Kreuter, "Colloidal Drug Delivery Systems", Marcel Dekker, Inc., New York 1994, p.219.
[3] K. S. Soppimath, T. M. Aminabhavi, A.R. Kulkarni, W. E. Rudzinski, *J. Controlled Release*, **2001**, *70*, 1.
[4] H. Rossignol, M. Boustta, M. Vert, *Int. J. Biol. Macromol.*, **1999**, *25*, 255.
[5] K. Y. Cho, C. H. Kim, J. W. Lee, J. K. Park, *Macromol. Rapid. Commun.*, **1999**, *20*, 598.
[6] K.E. Uhrich, S. M. Canizzarro, R. S. Langer, K. M. Shakesheff, *Chem. Rev.*, **1999**, *99*, 3181.

[7] E. Chiellini, G. Leonardi, R. Solaro, R. Lisciani, G. Mazzanti (to Istituto Sierovaccinogeno Italiano), *Eur. Pat.* EP0509968 (1992).
[8] J. Cowdall, J. Davies, M. Roberts, A. Carlsson, R. Solaro, G. Mazzanti, E. E. Chiellini, E. Chiellini, E. Söderlind (to Istituto Sierovaccinogeno Italiano I.S.I. S.P.A., Italy), *PCT Int. Appl.* WO9902131 (1999).
[9] J. Cowdall, J. Davies, M. Roberts, A. Carlsson, R. Solaro, G. Mazzanti, E. E. Chiellini, F. Chiellini, E. Söderlind (to Istituto Sierovaccinogeno Italiano I.S.I. S.P.A., Italy) *PCT Int. Appl.* WO9902135 (1999).
[10] E. Chiellini, E. E. Chiellini, D. Giannasi, R. Solaro, E. Grillo Fernandes, in "Frontiers in Biomedical Polymer Applications", R.M.Ottenbrite, Ed., Technomics, Lancaster 1998, Vol. 1, p. 13.
[11] R. Bizzarri, E. Chiellini, E. E. Chiellini, F. Chiellini, C. Fiumi, F. Signori, R. Solaro, *Chim. Ind. (Milan)*, **2001**, *83* (10), 51.
[12] E. Chiellini, E. E. Chiellini, F. Chiellini, R. Solaro, *J. Bioact. Compat. Polym.*, **2001**, *16*, 441.
[13] R. Solaro, F. Chiellini, F. Signori, C. Fiumi, R. Bizzarri, E. Chiellini, *J. Mat. Sci. Mat. Med.*, **2002**, in press.
[14] E. Chiellini, R. Solaro, S. D'Antone, L. Bemporad (to Istituto Sierovaccinogeno Italiano), *Eur. Pat.* EP0486445 (1992).
[15] R. Solaro, S. D'Antone, L. Bemporad, E. Chiellini, *J. Bioact. Compat. Polym.*, **1993**, *8*, 236.