Particle Size and Distribution of Biodegradable Poly-D,L-lactide-*co*-Poly(ethylene glycol) Block Polymer Nanoparticles Prepared by Nanoprecipitation

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ABSTRACT: A biodegradable block copolymer, poly-D,L-lactide (PLA)-*co*-poly(ethylene glycol) (PEG), was prepared by the ring-opening polymerization of lactide with stannous caprylate [$Sn(Oct_2)$] as a catalyst; then, the PLA–PEG copolymer was made into nanoparticles by nanoprecipitation under different conditions. The average molecular weight and structure of PLA–PEG were detected by ^{1}H-NMR and gel permeation chromatography. The sizes and distributions of the nanoparticles were investigated with a laser particle-size analyzer. The morphologies of the nanoparticles were examined by transmission electron microscopy. The effects of the solvent–nonsolvent system, operation conditions, and dosage of span-80 on the sizes and distributions of the nanoparticles are discussed. The results show that acetone–water was a suitable solvent–nonsolvent system and the volume ratio of the nonsolvent phase to the solvent phase (O/W) (v/v), the concentration of PLA–PEG in the solvent phase, and the dosage of span-80 had important effects on the particle sizes and distributions. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 1884–1890, 2005

Key words: biodegradable; block copolymers; nanoparticles; particle size distribution

INTRODUCTION

Poly-D,L-lactide (PLA) and its copolymer PLA–poly(ethylene glycol) (PEG) are excellent biodegradable polymers that are known both for their biocompatibility and their resorbability through natural pathways.[1] As important synthetic biodegradable materials, PLA and its copolymers have wide applications in the biotechnology field, including as drug-delivery nanoparticles. Drugs can be protected and released at a controlled rate by their incorporation into biodegradable materials; this can improve the efficiency of the drugs and minimize negative drug effects. The degradation rate of the PLA–PEG copolymer and the release rate of drugs can be manipulated by the variation of the ratio of PLA to PEG because PLA is more hydrophobic and PEG is more hydrophilic.[2] At the same time, drug particles with different sizes can be selectively accumulated in different target organs or tissues, so particle size and distribution play an important role in the preparation of nanoparticles for drug targeting.

In recent years, many studies have been done on drug-delivery nanoparticles. Several methods for the preparation of nanoparticles have been developed, including nanoprecipitation[3] (also called *phase separation*), solvent evaporation,[4] multiple emulsion,[5] spray drying,[6] polymerization,[7] and supercritical fluid technology.[8,9]

There have also been some works published[10–15] on microparticles and nanoparticles based on PLA for drug delivery and targeting, especially during last 20 years. The preparation of particles, the degradation process and degradation mechanism of carrier materials, and the drug-release behavior and release mechanisms *in vivo* and *in vitro* of drug encapsulated particles have been studied; these are important characteristics of drug-entrapped particles.

The aim of this study was to attain stable PLA–PEG nanoparticles, find relations between all kinds of preparation conditions and the sizes of the particles, and select the most suitable condition for the preparation of PLA–PEG nanoparticles by nanoprecipitation.

EXPERIMENTAL

Materials

PEG with a number-average molecular weight of 4000 g/mol (PEG-4000) was used after it was dried *in vacuo*

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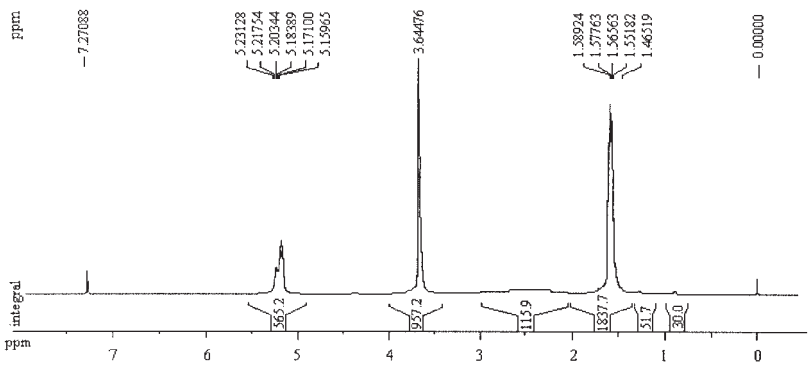


Figure 1 ^{1}H-NMR spectrum of the PLA–PEG copolymer.

at 80°C for over 10 h. D,L-Lactide (LA) was used after further purification. PEG-4000 and stannous caprylate [$Sn(Oct)_2$] were purchased from Shanghai Chemical Industry (Shanghai, China). All other reagents and solvents were analytical reagent grade.

Preparation of the PLA–PEG copolymer

To obtain the desired PLA–PEG copolymer, LA and PEG-4000 [4 : 1 (w/w)] were placed into a one-necked flask with a predetermined amount of $Sn(Oct)_2$. Nitrogen was directed through the reaction flask for 30 min by a syringe. Then, a high vacuum was applied for l5 min. The flask was sealed and put into a 160–170°C oil bath for 7 h to react. The resulting product was dissolved in ethyl acetate, precipitated in *n*-heptane, and then washed in distilled water; it was then dried in a vacuum oven at 50°C for 4–5 days.

Preparation of the PLA–PEG nanoparticles

Effect of solvent and nonsolvent

To prepare the nanoparticles, the PLA–PEG copolymer solution was injected drop by drop through a syringe into the nonsolvent phase. After coagulation, solvents were evaporated naturally for a period of more than 2 days. The solvent–nonsolvent systems used were acetone–water, dichloromethane–ethanol, dichloromethane–*n*-heptane, chloroform–ethanol, ethyl acetate–*n*-heptane, ethyl acetate–water, ethyl acetate–ethanol, and ethyl acetate–methanol. The effect of the volume ratio of the nonsolvent phase to the solvent phase [O/W (v/v)] and the concentration of PLA–PEG in the solvent phase were also studied.

Effect of operation conditions

In the process of preparation, the effects of some operation factors, including operation method, agitation rate, syringe size, and the temperature of the solvent and nonsolvent phases, were also studied.

Effect of the dosage of span-80

Different dosages of span-80 were dispersed in the nonsolvent by a magnetic force mixer for 10 min. Then, the PLA–PEG solution was dropped through a syringe into a span-80 dispersed water bath without agitation. The solvents were evaporated naturally.

Analysis methods

^{1}H-NMR

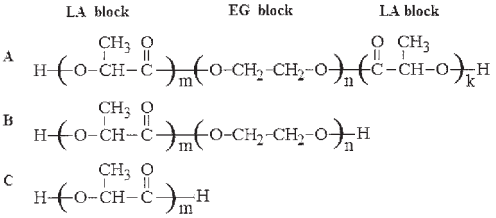
PLA–PEG copolymer was dissolved in $CDCl_3$, and ^{1}H-NMR spectra were taken with trimethylsilane as an internal reference standard with a Bruker DMX500 spectrometer (Rheinstetten, Germany).

Gel permeation chromatography (GPC)

The average molecular weight and distribution of the PLA–PEG copolymer were determined by GPC (Waters). Samples were dissolved in analytical-reagent-grade tetrahydrofuran. The number-average and weight-average molecular weights of the polymers were determined by universal calibration obtained from a polystyrene reference.

Laser particle-size analysis

The particle sizes and distributions of all nanoparticles were determined with a LS230 laser particle-size analyzer (Coulter).



Scheme 1 Three kinds of polymer structures likely to exist in the prepared polymer.

TABLE I
Different Kinds of Solvent/Nonsolvent Systems

Solvent/nonsolvent system	Experimentation phenomenon	Nanoparticles obtained?	Solubility parameter
Acetone/water	Sky-blue stable solution	Yes	19.62/49.10
Dichloromethane/ethanol	Milk-white stable solution	Yes	20.21/26.05
Dichloromethane/*n*-heptane	Film lay at the bottom of Bunsen beaker	No	20.21/15.20
Chloroform/*n*-heptane	Film lay at the bottom of Bunsen beaker	No	18.89/15.20
Chloroform/ethanol	Milk-white stable solution	Yes	18.89/26.05
Ethyl acetate/*n*-heptane	Film lay at the bottom of Bunsen beaker	No	18.34/15.20
Ethyl acetate/water	Film lay at the bottom of Bunsen beaker	No	18.34/49.1
Ethyl acetate/ethanol	Milk-white stable solution	Yes	18.34/26.05
Ethyl acetate/methanol	Film lay at the bottom of Bunsen beaker	No	18.34/29.29

Transmission electron microscopy (TEM)

A Cu halftone was immerged in the nanoparticle–water system and then dyed with 4 wt % phosphotungstic acid. The morphology of the prepared nanoparticles was examined with a H-600 transmission electron microscope (Hitachi, Tokyo).

RESULTS AND DISCUSSION

Composition of the PLA–PEG copolymer

The PLA–PEG copolymer was synthesized by ring-opening polymerization, and the feed ratio of LA to PEG-4000 was 4 : 1. The number-average molecular weight of the PLA–PEG copolymer obtained from GPC was 5196 g/mol, and the weight-average molecular weight was 8421 g/mol.

Figure 1 shows the ^{1}H-NMR spectrum of the PLA–PEG copolymer. The peaks at 1.56 ppm (CH_3) and 5.18 ppm (CH) were attributed to PLA blocks, and peaks at 3.64 ppm (CH_2) were characteristic of the main-chain methylene units in the PEG blocks; the area of these three peaks were 1837.7 (CH_3), 565.2 (CH), and 957.2 (CH_2). The areas of the CH and CH_2 peaks were used to calculate the PLA–PEG block weight ratio with the following equations:

$$\text{Block number ratio} = (\text{Number of PLA blocks})/(\text{Number of PEG blocks})$$
$$= (4 \times \text{Area of CH peaks})/(\text{Area of } CH_2 \text{ peaks})$$
$$= 4 \times 565.2/957.2 = 2.36$$

$$\text{Block weight ratio} = (\text{Weight of PLA blocks})/(\text{Weight of PEG blocks})$$
$$= \text{Block number ratio} \times \text{Molecular weight of LA block/Molecular weight of EG block}$$
$$= 2.36 \times 72/44 = 3.86$$

where EG is ethylene glycol. This block weight ratio (3.86) was close to the weight ratio of LA and PEG before the reaction (4.00). From these calculations, we

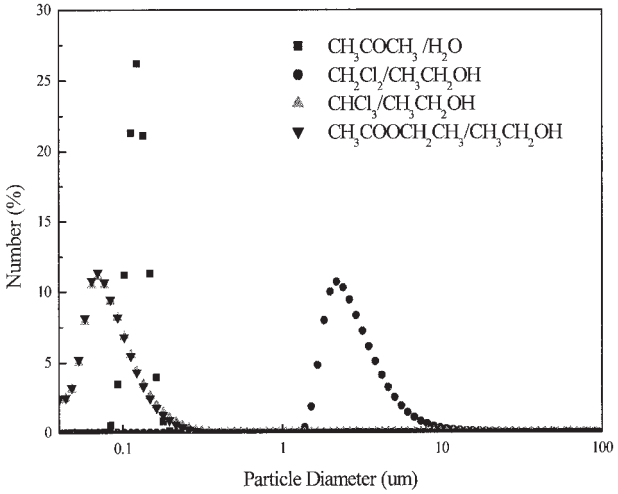


Figure 2 Effect of solvent–nonsolvent systems on the sizes and distributions of the nanoparticles.

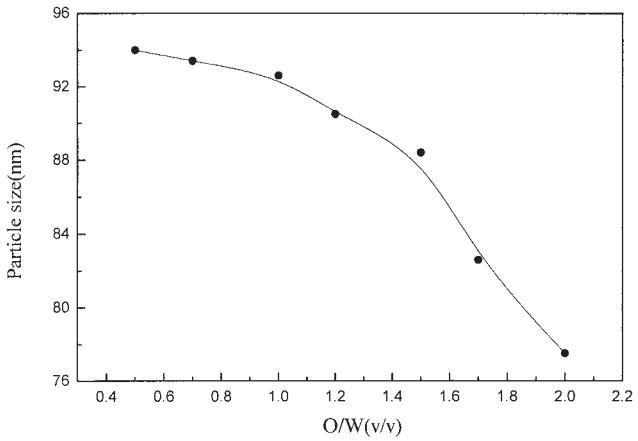


Figure 3 Effect of O/W (v/v) on the sizes of the nanoparticles.

TABLE II
Effect of the Solvent/Nonsolvent Systems on Nanoparticle Sizes

Solvent/nonsolvent system	Mean particle size (μm)	SD	Coefficient of variation (%)	Median size (%)	Analyses	
					Size	Distribution
Acetone/water	0.130	0.018	13	27	−	−
Dichloromethane/ethanol	3.343	2.235	67	10	+	+
Chloroform/ethanol	0.0942	0.088	93	11	−	+
Ethyl acetate/ethanol	0.0933	0.106	114	11	−	++

− = small; + = large.

surmised the three main kinds of structure that were likely to exist in the prepared polymers, as shown in Scheme 1.

Effects of different parameters on the particle sizes and distributions

Effect of the solvent–nonsolvent system

For the formation of nanoparticles by nanoprecipitation, the solvent–nonsolvent system is very important. We studied nine different solvent–nonsolvent systems, as shown in Table I. Among these solvent–nonsolvent systems, acetone–water, dichloromethane–ethanol, chloroform–ethanol, and ethyl acetate–ethanol were suitable for PLA–PEG to form particles. Further more, acetone–water induced smaller and more narrowly distributed particles, as shown in Figure 2. This might be attributing to the polar interactions among the solvent, nonsolvent and copolymer. To attain stable nanoparticles, the PLA–PEG copolymer must have adequately dissolved in the solvent and be absolutely undissolved in the nonsolvent system; at the same time, the solvent must have freely dissolved in the nonsolvent, so the solubility parameters of the solvent and nonsolvent were very important. The solubility parameters of every solvent–nonsolvent system are also listed in Table II. In the following experiments, acetone–water was used as the solvent–nonsolvent system.

To find relations between the particle size and O/W, 10 ml of 10 mg/mL PLA–PEG/acetone solution was dropped through 4.5-mm syringes into 1, 2, 4, 5, 7, 10, 12, 15, 17, and 20 mL of distilled water without agitation. Then, the solvents were evaporated naturally. Figure 3 shows the effect of O/W on the sizes of the nanoparticles. Interestingly, when O/W (v/v) was under 2.0, with increasing O/W (v/v), the particle sizes of the nanoparticles decreased continuously. The decrease in O/W (v/v) made phase separation of the oil phase quick, which gave the system a chance to produce larger nanoparticles by the congregation of nanoparticles. At the same time, the solution system comprised more solvent with increasing O/W (v/v), which could increase the solvency of the PLA–PEG copolymer, so some copolymer could partly dissolve in the solvent–nonsolvent system. When the solvent evaporated gradually, the PLA–PEG copolymer dissolved in the solvent–nonsolvent system was separated out and formed small nanoparticles. However, when O/W (v/v) was up to 2.0, large particles appeared; furthermore, the particles formed films quickly with O/W (v/v) values over 2.5, and so nanoparticles could not be prepared. A possible reason was that a large amount of solvent resolved the polymer to a continuous phase, and phase separation did not occur.

To study the effect of the concentration of the PLA–PEG solution on particle size, 10 mL of the PLA–PEG/acetone solution with different concentrations (64.05, 100.95, 159.90, 207.05, 264.40, 308.45, 364.6, and 410.25 mg/mL) was dropped through 4.5-mm syringes into 10 mL of distilled water without agitation. Then, the solvents were evaporated naturally. Figure 4 shows that an increasing concentration of PLA–PEG solution brought a corresponding increase in particle sizes. This could be attributed to an increase in both the amount of PLA–PEG in every solution drop and the viscosity of the oil phase with increasing polymer concentration, which induced poor dispersion and more conglutination.

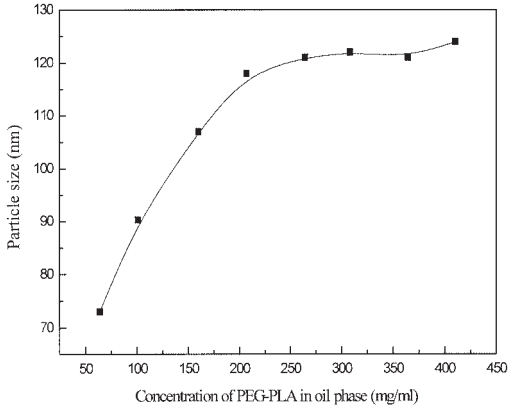


Figure 4 Effect of concentration of PLA–PEG on the sizes of the nanoparticles.

TABLE III
Effect of Operation Method on the Size and Distribution of the PLA–PEG Particles

No.	Operation method	Mean particle size (nm)	SD
A	Solvent phase was dropped into the nonsolvent phase through a syringe	120	31
B	Solvent phase was injected into the nonsolvent phase through a syringe	89.4	39
C	Solvent phase was poured into the nonsolvent phase	2433	989
D	Nonsolvent phase was poured into the solvent phase	123	19
E	Nonsolvent phase was injected into the solvent phase through a syringe	98.6	100
F	Nonsolvent phase was dropped into the solvent phase through a syringe	3218	1524

Effect of operation conditions

Six operation methods were studied to find the formation mechanism of the particles, as shown in Table III and Figure 5. By analysis of particle size and distribution, method A (solvent with polymer drop into nonsolvent) was determined to be suitable for the preparation of the nanoparticles.

As shown in Table IV, different rates of agitation were studied. Figure 6 shows that the particle sizes and distributions of the nanoparticles produced at 0, 500, 1000, and 1500 r/min were very similar for the 10 mL/10 mL system. This could be attributed to the fact that phase separation is a physical–chemical process when the dosage of solvent and nonsolvent is small. The effects of the agitation rate were very small. There was good dispersion without agitation. However, when the dosage of the solvent–nonsolvent system was increased to 40 mL/40 mL, the effect of agitation on the particle sizes and distributions was larger.

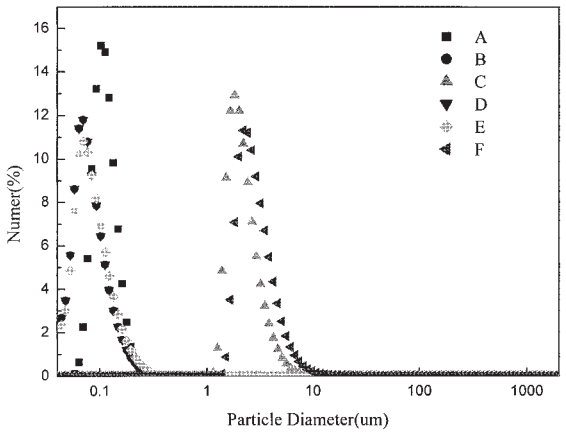


Figure 5 Effect of operation method on the sizes and distributions of the nanoparticles: (A) the solvent phase was dropped into the nonsolvent phase through a syringe, (B) the solvent phase was injected into the nonsolvent phase through a syringe, (C) the solvent phase was poured into the nonsolvent phase, (D) the nonsolvent phase was poured into the solvent phase, (E) the nonsolvent phase was injected into the solvent phase through a syringe, and (F) the nonsolvent phase was dropped into the solvent phase through a syringe.

To study the effect of syringe size on the particle sizes and distributions, 10 mL of the 10 mg/mL PLA–PEG/acetone solution was dropped through 4.5-mm syringes into 5, 7, 10, 12, 15, 17, and 20 mL of distilled water without agitation. Then, the solvents were evaporated naturally. Figure 7 and Table I show the effect of the size of the syringe on the sizes of the PLA–PEG nanoparticles. With a larger syringe size, the size of the PLA–PEG nanoparticles was larger. This could be attributed to the fact that the syringe was the primary dispersing region. A small-size syringe created good dispersion and produced small nanoparticles.

TABLE IV
Effect of the Rate of Agitation on the Sizes of the PLA–PEG Microspheres

No.	O/W (v/v; mL/mL)	Rate of agitation (r/min)	Mean particle size (nm)	SD
R-1	10/10	0	120	31
R-2	10/10	500	106	36
R-3	10/10	1000	117	31
R-4	10/10	1500	113	32
R-5	40/40	0	85.7	37
R-6	40/40	1000	80.9	30

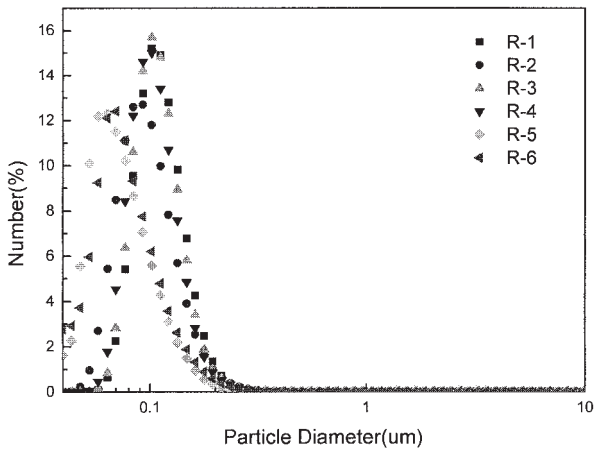


Figure 6 Effect of the rate of agitation on the sizes and distributions of the nanoparticles. For operation parameters, see Table IV.

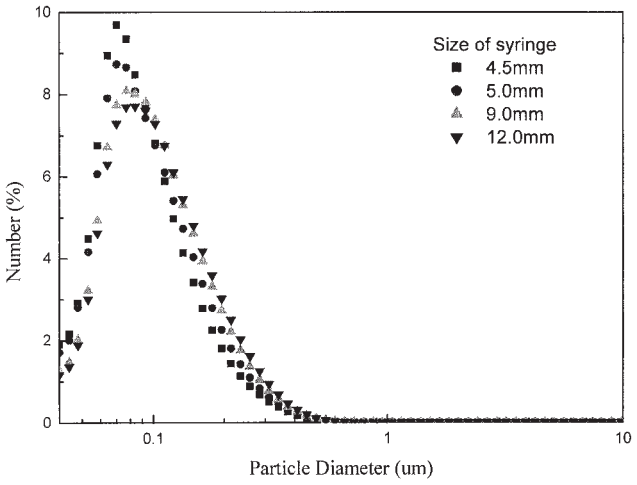


Figure 7 Effect of the size of the syringe on the sizes and distributions of the nanoparticles.

The PLA–PEG/acetone solution (10 mL of 10 mg/mL solution) was dropped through a 4.5-mm syringe into 10 mL of distilled water at different temperatures without agitation. The products were all evaporated naturally. As shown in Table VI, the temperatures of the oil and water phases had unclear effects on the sizes of the nanoparticles at 0–56°C (the boiling point of acetone is 56°C). With the increasing oil or water phase temperature, the sizes of the nanoparticles changed constantly. The size differences between these prepared particles were all less than 12.4 nm. Differences in the size distribution for these nanoparticles were less than 30 nm.

Effect of the dosage of span-80

Figure 8 shows that the addition of span-80 led to the production of smaller particles. In fact, span-80 was added as a surfactant. When the dosage of span-80 was more than 0.0024 g/mL, the particle sizes were similar, so only a little span-80 was enough to produce good dispersion. It was obvious that span-80 reduced the surface tension of water and made the particles more stable by adsorbing on the surface of the particles. When the dosage of span-80 reached a limit, called the *critical micelle concentration,* the continued increase in the dosage of span-80 caused very few changes in the surface tension and particle sizes, so smaller nanoparticles with narrow distributions were prepared by the addition of a little span-80.

TABLE V
Effect of the Size of the Syringe on the Sizes of the PLA–PEG Microspheres

No.	PLA–PEG concentration (g/mL)	O/W (v/v; mL/mL)	Size of the syringe (mm)	Mean particle size (nm)	SD
S-1	0.04	10/10	4.5	106	62
S-2	0.04	10/10	5	112	66
S-3	0.04	10/10	9	120	71
S-4	0.04	10/10	12	125	80

TABLE VI
Effect of the Temperatures of the Oil and Water Phases on the Sizes of the PLA–PEG Nanoparticles

Temperature of the oil phase (°C)	Temperature of the water phase (°C)	Mean particle size (nm)	SD
0	0	90.9	16
0	10	89.1	14
0	20	88.6	15
10	0	93.8	15
10	10	93.6	16
10	20	90.1	16
10	30	90.6	16
10	40	98.5	15
10	50	101	16
20	0	88.8	15
20	10	100	17
20	20	92.0	15
20	30	95.1	15
20	40	93.9	16
20	50	100	16
35	0	95.1	15
35	10	94.8	17
45	0	92.3	15
45	10	93.5	17
56	0	92.7	15
56	10	101	16

Morphologies of the PLA–PEG nanoparticles

Figure 9 shows the morphologies of the nanoparticles prepared without and with span-80 by TEM. The particles had narrow dispersions and were less than 100

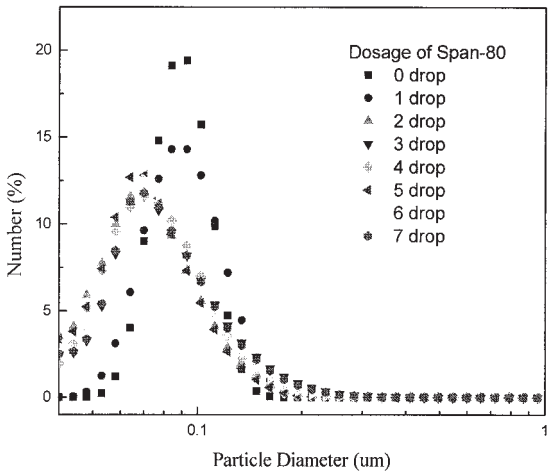


Figure 8 Effect of the dosage of span-80 on the sizes and distributions of the nanoparticles.

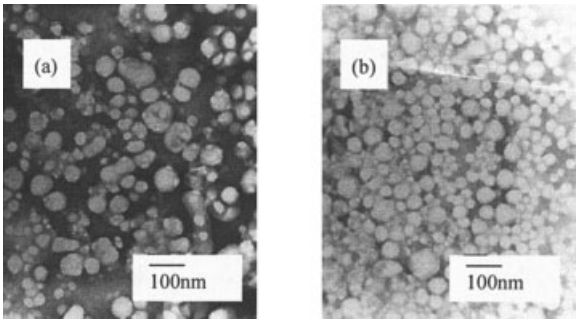


Figure 9 Transmission electron micrographs of PLA–PEG nanoparticles: (a) without span-80 and (b) with span-80.

nm, which further proved that nanoparticles of the PLA–PEG copolymer were prepared. When the nanoparticles prepared without and with span-80 were compared, we found that the particles conglutinated together without span-80 [Fig. 9(a)]; with the addition of span-80, the nanoparticles were smoother and more round [Fig. 9(b)]. As a kind of surfactant, the addition of span-80 can prevent the congregation of particles.

CONCLUSIONS

In this study, nanoparticles based on a PLA–PEG copolymer were prepared by nanoprecipitation. The effects of different parameters on the sizes and distributions of the nanoparticles were discussed. For the PLA–PEG matrix, acetone–water was the best solvent–nonsolvent system, and stable, more narrowly distributed nanoparticles were attained with the acetone–water system. With increasing O/W (v/v), nanoparticle size decreased continuously. Increasing the concentration of the PLA–PEG solution brought about a corresponding increase in the particle sizes. A small syringe produced good dispersion and small nanoparticles. The temperature of the oil and water phases produced unclear effects on the sizes of the nanoparticles. The addition of span-80 led to smaller particles. As shown by TEM, the nanoparticles of the PLA–PEG copolymer were further proved, and the nanoparticles prepared with span-80 were smoother and more round.

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