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Preparation and characterization of fenofibrate-loaded PLA–PEG microspheres

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Abstract A series of biodegradable block copolymer of poly(lactide)(PLA)/poly(ethylene glycol) (PEG) were prepared by Ring-Opening polymerization of D, L-lactide, using stannous octoate as a catalyst. By nanoprecipitation method, the PLA–PEG can be made into microspheres containing fenofibrate, which is a kind of important cholesterol-lowering drugs. The purpose of this study is to investigate the effect of the copolymer composition on the size, the entrapment and the release behavior of the fenofibrate loaded microspheres. The microspheres can be achieved with small size below 100 nm, better encapsulation efficiencies of more than 55.3% and slower release rates. The release of fenofibrate from microsphere would reach the balance first, when the microsphere prepared by high proportion of hydrophilic PEG block. And the release property of fenofibrate/PLA–PEG microsphere was better than Lipanthyl® (a commercial capsule of fenofibrate). It was observed that the composition of PLA–PEG copolymer played a major role in encapsulation efficiency of microspheres and release rates.

Introduction

Conventional drug delivery provides sharp increase of drug concentration at potentially toxic levels. Following a relatively short period at the therapeutic level, drug concentration eventually drops off until re-administration. The idea of controlled release from polymers dates back to the 1960s through the employment of silicone rubber [1]. The lack of degradability in these systems limits their applicability. As important synthetic biodegradable materials, poly(lactic acid)-poly(ethylene glycol) with amphiphilic character, whose degradation rate can be manipulated by varying the ratio of hydrophilic to hydrophobic segments [2], has appeared to be a particularly promising drug carrier. An interesting property of PLA–PEG particles are their reduced uptake by the mononuclear phagocytic system (MPS) in comparison to that of the unmodified poly(lactic acid)(PLA) particle[3–5].

In recent years, various controlled release drug delivery form: such as microspheres, liposomes, micells, have been investigated to increase drug solubility, to minimize the side effects and improve the efficiency of drugs. Among them, microspheres have attracted a lot of interest. Deng et al. [6], reported PLA–PEG microspheres offered an advantage because of their swelling in the aqueous medium, generating a more stabilizing environment for proteins and minimizing the initial burst release. Ruan and Feng [7] prepared paclitaxel-loaded PLA–PEG-PLA microspheres by oil-in-water single-emulsion solvent extraction/evaporation process. The average particle size of all samples was 13–23 μm. Many researches have been done about drug delivery microspheres [8–13].

In this work, we present synthesis of PLA–PEG, formulation and characterization of PLA–PEG microspheres containing fenofibrate prepared by nanoprecipitation, which has many advantages such as easy operation and avoiding large amount of toxic

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organic solvent. It was demonstrated by Transmission Electron Microscope microscopy and laser light scattering that the particle size did not change significantly with the change of PLA–PEG composition. Subsequently, the entrapment efficiency and the in vitro release behavior of fenofibrate loaded microspheres were measured. The effects of compositions on the fenofibrate release were analyzed.

Experimental

Materials

D, L-lactide was made by our lab, poly ethylene glycol and stannous octoate ($Sn(Oct)_2$) were purchased from Shanghai Chemical Industry (China). All other reagents and solvents were AR grade.

Synthesis of $P_{D,\ L}$LA–PEG

A ring opening polymerization procedure was employed to synthesize the copolymer of PLA–PEG copolymer. Appropriate quantities of the d, l-lactide, PEG and a predetermined amount of $Sn(Oct)_2$ were added to a one-neck flask. Nitrogen was directed through the reaction flask for 30 min by a syringe. Then the reaction flask was evacuated under vacuum for 15 min and sealed. The sealed flask was immersed in a oil bath at 160–170 °C to start the polymerization. The polymerization was stopped after 7 h by pulling the flask out from the oil bath.

Two series of PLA–PEG copolymers were synthesized with LA:PEG-4000 initial feed ratios of 2:1, 3:1, 4:1, 5:1, 8:1, 10:1, 12:1 and prepared with PEG with molecular weights of 2000, 4000, 6000, 8000, 10000.

^{1}H-nuclear Magnetic Resonance (^{1}HNMR)

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

Gel permeation chromatography (GPC)

The molecular weight of the PLA–PEG copolymers was determined at ambient temperature by gel permeation chromatography (GPC) (Waters, USA). Samples were dissolved in tetrahydrofuran (THF) (AR). The number and weight average molecular weights of the polymers were determined relative to polystyrene standards.

Preparation of fenofibrate loaded microspheres

To prepare microspheres, PLA–PEG copolymer and fenofibrate dissolved in 10 ml of acetone, and then the solution was injected in a dropwise through a syringe into water. After coagulation, acetone was evaporated naturally for more than two days.

Particle size analysis for microspheres

The particle sizes and distributions of all microspheres were determined by LS230 Laser particle analyzer (Culter, American).

Transmission Electron Microscope (TEM)

Cu halftone was immerged to the microspheres-water system, and then dyed by 4% (wt) phosphotungstic acid. Morphology of prepared microspheres was examined using the H-600 TEM (Hitachi, Japan).

Determination of the entrapment efficiency

For determine the Entrapment Efficiency of fenofibrate, a known amount of fenofibrate was dissolved in ethanol and UV absorbance value at 286 ± 1 nm was measured. Serially diluted concentration of fenofibrate in ethanol was used to construct a calibration curve. $Y = -0.05379 + 46.17614X$ ($r = 0.99749$)

A known amount of fenofibrate-loaded microspheres were incubated in ethanol at 60 °C for 2 h, filtrate. Fenofibrate content was measured spectrophotometrically (at 286 ± 1 nm).

Encapsulation efficiency (%) = (Weight of fenofibrate loaded)/(Weight of fenofibrate input) × 100

In vitro release

A known amount of fenofibrate loaded microspheres were introduced into dialysis bags and put them into 0.5% sodium dodecyl sulfate (SDS) which was continuously stirred by Dissolution Tester at 37 °C. At certain time intervals, samples were removed from the release medium, fenofibrate content was measured spectrophotometrically (at 296 ± 1 nm).

Results and discussion

Composition of PLA–PEG copolymer

The chemical compositions of the copolymers determined by NMR (Fig. 1) corresponded closely to the

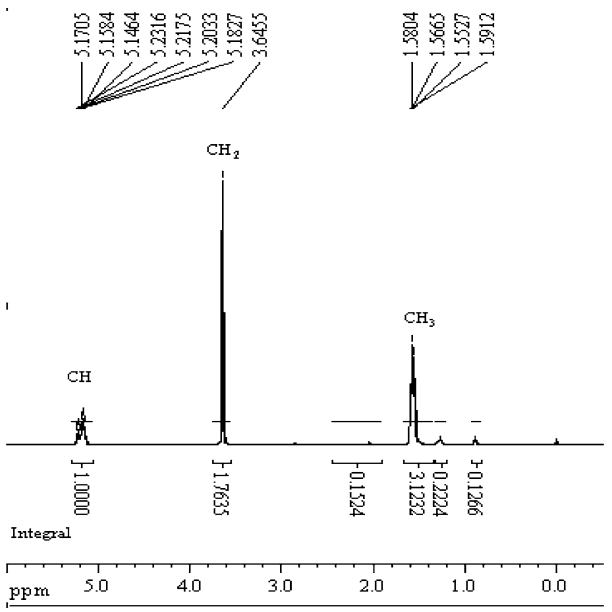

Fig. 1 ^{1}H-NMR spectrum of PLA–PEG copolymer (LA:-PEG = 4:1, PEG4000)

percentage of the monomers used in the polymerization reaction.

The peaks at 1.56 ppm (CH_3) and 5.18 ppm (CH) can be attributed to PLA blocks, and peaks at 3.64 ppm (CH_2) are characteristic of main chain methylene units in the PEG blocks, and the area of these three peaks were 1837.7 (CH_3), 565.2(CH), 957.2(CH_2). The area of the CH and CH_2 peaks were used for calculation of the PLA/PEG block weight ratio by the following equations:

$$\begin{aligned}\text{Block number ratio} &= (\text{the number of PLA blocks})/(\text{the number of PEG blocks})\\ &= (4 \times \text{area of CH peaks})/(\text{area of } CH_2 \text{ peaks})\\ &= 4 \times 565.2/957.2\\ &= 2.36\end{aligned}$$

$$\begin{aligned}\text{Block number ratio} &= (\text{the number of PLA blocks})/(\text{the number of PEG blocks}\\ &= \text{Block number ratio} \times \text{molecular weight of LA block}/\text{molecular weight of EG block}\\ &= 2.36 \times 72/44\\ &= 3.86\end{aligned}$$

This block weight ratio (3.86) is close to the weight ratio of $_{D,L}$-lactide and PEG before reaction (4.00) (Table 1).

Table 1 The number average molecular weight of PLA–PEG4000 with different feed ratio

LA:PEG feed ratio	–CH	$-CH_2$	N(PLA)/N(PEG)	M(PLA)/M(PEG)	Mn
4:1	1.0000	1.7635	2.7463	3.7264	18924
5:1	1.0000	1.4233	2.8033	4.5872	22367
8:1	1.0000	0.8831	4.2526	6.9588	31853
10:1	1.0000	0.7094	5.6569	9.2568	41045
12:1	1.0000	0.5509	7.1200	11.6509	50622
15:1	1.0000	0.4391	8.8810	14.5325	62148

There is one peak produced by GPC. The number average molecular weight of PLA–PEG copolymer obtained from GPC is 5196 g mol^{-1},weight average molecular weight is 8421 g mol^{-1}.

Particle size of fenofibrate loaded microspheres

Effect of the PLA/PEG block weight ratio

To study the effect of the PLA/PEG block weight ratio on particle size and distribution, PLA–PEG copolymers were synthesized using the different initial feed ratio of LA:PEG-4000 (4:1, 5:1, 8:1, 12:1, 15:1).

The size of fenofibrate/PLA–PEG microspheres increased with decreasing amount of PEG in copolymers (Table 2, Fig. 2). It has been shown that at same PEG molecular weight, the lower PEG initial feed ratio, the longer copolymer chain and the higher molecular weight of PLA–PEG copolymer these conditions were better for the entrapment of fenofibrate and formed bigger microspheres. However, it did not observe the significant change. The size of fenofibrate loaded PLA–PEG microspheres were about 100 nm and the distribution was narrow.

Effect of the molecular weights of PEG

At the same feed ratio of LA:PEG, the effect of the molecular weights of PEG on particle size of fenofibrate loaded microspheres also was discussed. As the molecular weight of PEG in the block copolymers increased, the diameter of microspheres slightly increased (Fig. 3, Table 3).

Table 2 Effect of the feed ratio of LA:PEG4000 on particle diameter of microsphere

LA: PEG4000 feed ratio	Particle diameter (nm)	SD (nm)
4:1	83.1	31
8:1	88.8	40
12:1	95.9	60
15:1	96.7	52

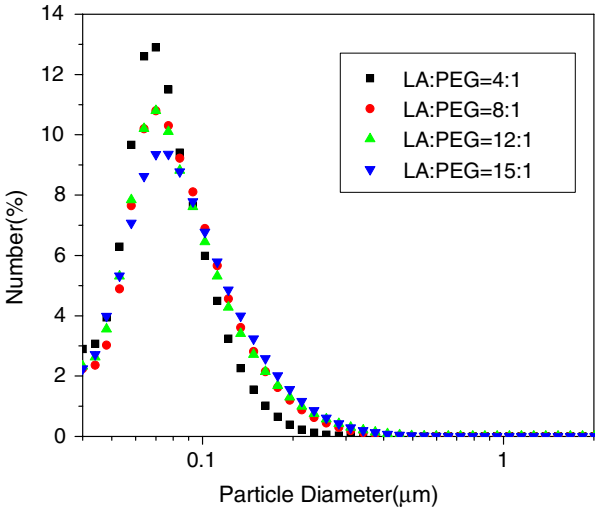

Fig. 2 Effect of the feed ratio of LA:PEG4000 on particle size

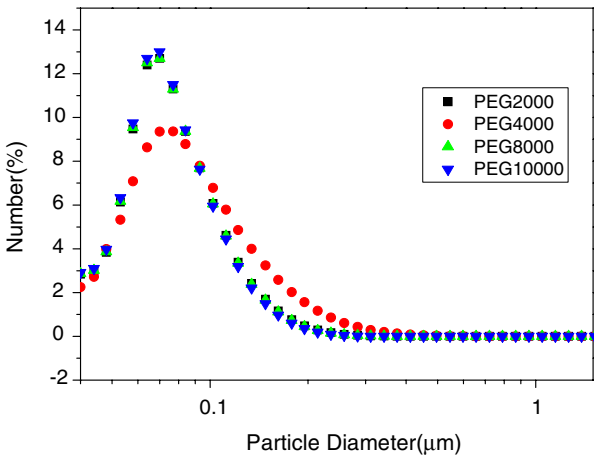

Fig. 3 Effect of molecular weights of PEG on particle size of microspheres (LA:PEG = 5:1)

Apparently, the effect of materials used on the particle size was not significant. It suggested that the size of microspheres was largely controlled by the nanoprecipitation process during microspheres preparation.

Morphology of fenofibrate loaded microspheres

Effect of the PLA/PEG block weight ratio

TEM photographs (Fig. 4) show microspheres have narrow dispersion and are less than 100 nm, which further proves that the microspheres of PLA–PEG copolymer were prepared. The size of the microspheres prepared with different feed ratio of PLA–PEG copolymer was within the range of 80–100 nm.

Table 3 Effect of the molecular weights of PEG on particle size of fenofibrate loaded microspheres(LA:PEG = 5:1)

M_W of PEG	Particle diameter (nm)	SD (nm)
2000	84.3	33
8000	83.9	32
10000	82.5	30

The microspheres prepared with less content of PEG appeared to be more spherical. With the PEG content increased, the chain length and the molecular weight ofcopolymers increased. It was helpful to entrap the drug and microspheres looked smoother and more round. The microspheres prepared with PLA–PEG (4121) was notably larger and more spherical than those prepared with PLA–PEG (4041, 4051).

Thomas et al. [14] reported PLA microspheres had microporous structures and the open porous structures in PLA microspheres. It seems that hydrophilic PEG made the microspheres smoother and nonporous. Ruan and Fengs [7] reported that increasing the amount of PEG in the synthesis of the PLA–PEG–PLA copolymer could increase the amount of PEG on the microsphere surface, leading smoother and better biocompatibility.

Effect of the molecular weights of PEG

Figure 5 shows the TEM photographs of the shape of microspheres prepared with different PEG molecular weight. The spheres tended to conglutinated together with decreasing PEG molecular weight and with the increasing of PEG molecular weight, the molecular weight of copolymer increased and the size of microspheres increased.

The PEG molecules adsorbed on the surface of the microspheres prevented the coalescence of microspheres. Therefore, it appears that PEG coating can ensure better stabilization.

Encapsulation efficiency of fenofibrate loaded microspheres

Effect of the PLA/PEG block weight ratio

There were many experimental parameters such as composition of PLA–PEG copolymer, the drug concentration, the copolymer concentration, volume of water and oil phase and the temperature of water and oil phase influence the encapsulation efficiency of fenofibrate loaded microspheres. However the effect of the composition of PLA–PEG copolymer on the encapsulation efficiency is conclusive.

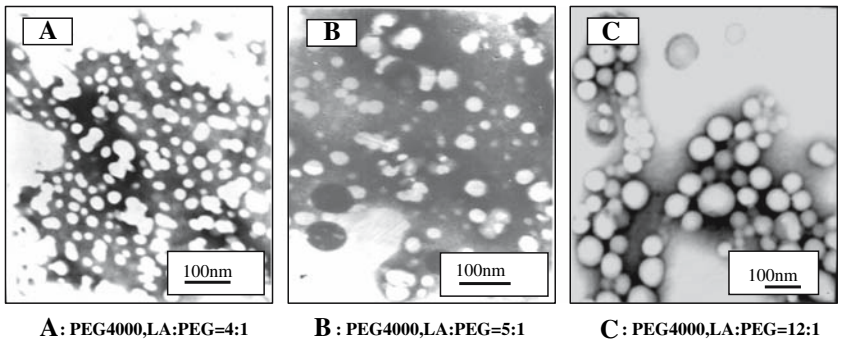

Fig. 4 TEM morphology of different feed ratio of LA:PEG4000 of fenofibrate loaded microspheres

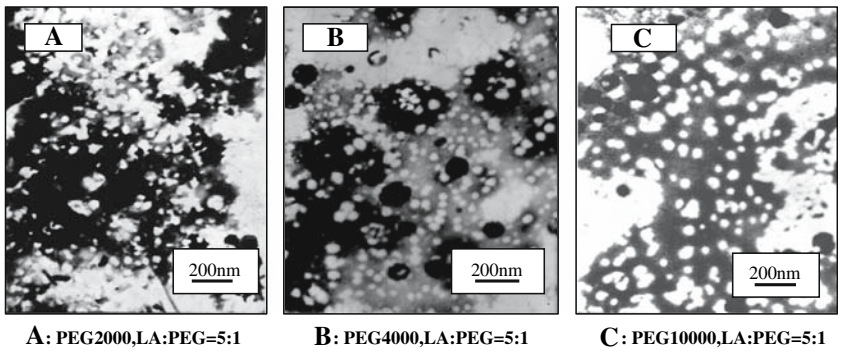

Fig. 5 TEM morphology of different molecular weight of PEG of fenofibrate loaded microspheres (LA:PEG = 5:1)

Figure 6 shows the effect of the PLA–PEG block weight ratio on encapsulation efficiency. The encapsulation efficiency of microsphere samples was 22.6–55.3%.

It was observed that as PLA–PEG4000 block weight ratio increased, the encapsulation efficiency increased accordingly. With the increasing of PLA–PEG4000 block weight ratio, the hydrophilicity of copolymer decreased. The PLA–PEG hydrophobic structure became a kind of the obstacle against drug leakage into the aqueous phase during microspheres formation.

Effect of the molecular weights of PEG

The effect of different PEG molecular weight on the encapsulation efficiency was studied. The copolymers were synthesized by PEG2000, PEG4000, PEG6000, PEG8000 and PEG10000 at same feed ratio LA:-PEG = 5:1.

Figure 7 shows that the encapsulation efficiency of fenofibrate loaded PLA–PEG microspheres are higher when the PEG molecular weight is in the range of 4000–8000, because the appropriate PEG chain length

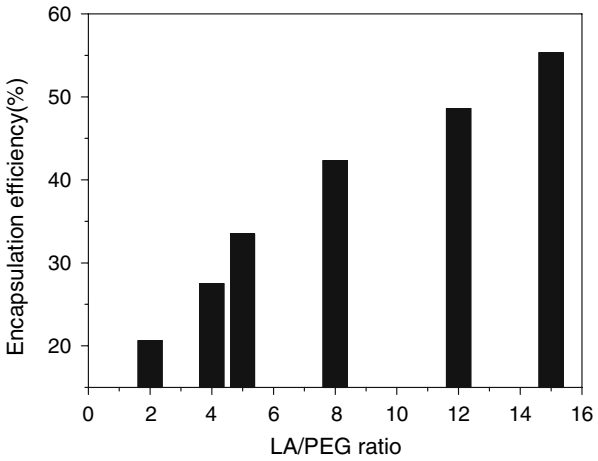

Fig. 6 Effect of the feed ratio of LA:PEG4000 on percent of encapsulation efficiency of fenofibrate loaded microspheres

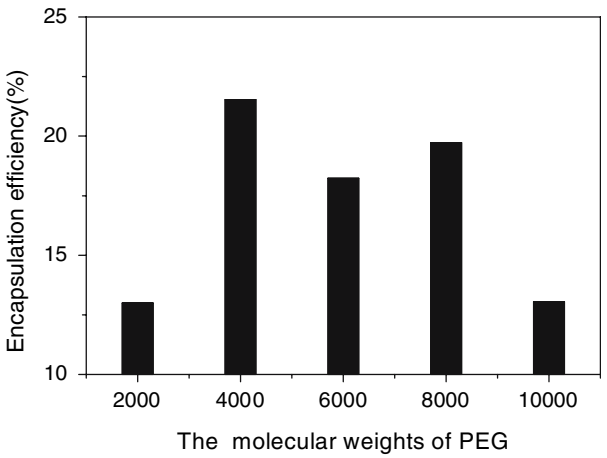

Fig. 7 Effect of the molecular weights of PEG on encapsulation efficiency of fenofibrate loaded microspheres (LA:PEG = 5:1)

improve the hydrophilicity and flexible of copolymers, which result to better entrapment of the drug by copolymer. When the molecular weight of PEG below 2000, the chain of copolymer was flexible, but the chain of PEG was short and the hydrophilicity of copolymer was not well improved to have high encapsulation efficiency. In contrast the molecular weight above 10,000, the copolymer chain was less flexible and it was bad for the drug entrapment.

Release behavior of fenofibrate loaded microspheres

Effect of the PLA/PEG block weight ratio

As shown in Fig. 8, the fenofibrate loaded PLA–PEG microspheres resulted in significantly slower release of the drug from the microspheres compared with that from Lipanthyl®. As the PEG content increased, the rate and amount of fenofibrate release also increased. It seems the hydrophilic PEG segment would facilitate the drug release. The drug release is diffusion controlled as the drug can travel through the pores formed during sphere hardening. When feed ratio of LA:PEG increased, the shell of microspheres become thicker, which slower the diffusion of drug. The amount of fenofibrate released during the period of rapid release was affected by the composition of the microspheres, increasing when the PEG content of microspheres increased. This would suggest that in the case of microspheres with a high PEG content, either the fraction of drug content close to the surface was higher or the drug could diffuse out faster or both. The amount of released fenofibrate reaches 50% after 40 h.

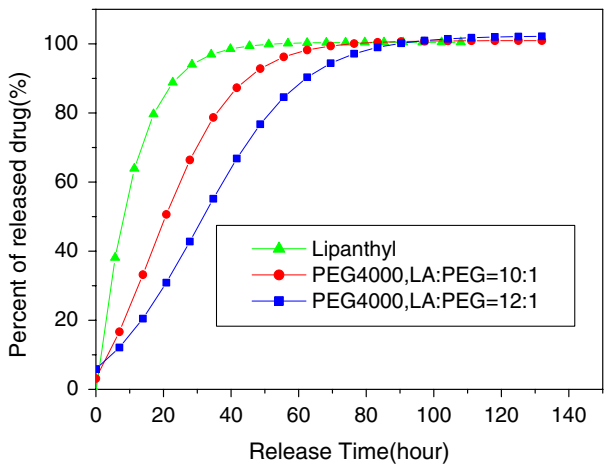

Fig. 8 Effect of the feed ratio of LA:PEG4000 on release property of fenofibrate loaded microspheres

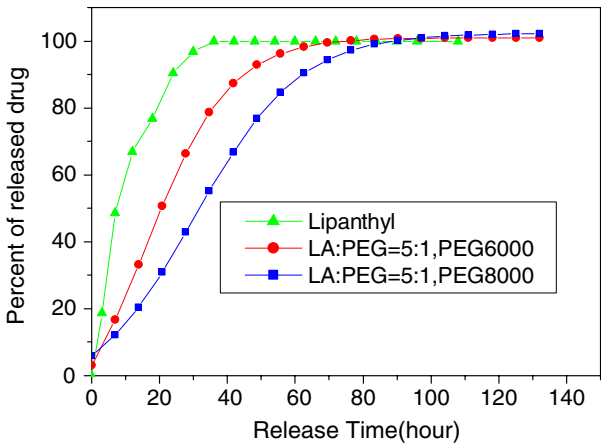

Fig. 9 Effect of the molecular weights of PEG on release property of fenofibrate loaded microspheres (LA:PEG = 5:1)

Moreover, the shapes of the release profiles of PLA–PEG microspheres are similar.

Effect of the molecular weights of PEG

The rate and amount of release likely depends on the hydrophilicity of the microsphere's material because the copolymer matrix acts as a barrier across which the loaded fenofibrate diffuses to the external medium (Fig. 9). Microspheres that more hydrophilic are expected to more easily dissolved in buffer solution and to cause higher release rates. Compared to Lipanthyl® (micronsied fenofibrate), released completely in nearly 30 hours. The fenofibrate loaded PLA–PEG microspheres showed more excellent release behavior. Further in vivo studies are needed in order to fully elucidate the biodistribution and pharmacokinetics of fenofibrate.

Conclusion

Fenofibrate is believed to have a broad spectrum of lipid-lowering activity and reduce the total cholesterol level and triglycerides [15]. The release file of commercial fenofibrate, Lipanthyl®, is not ideal. The possibility of achieving better performance of fenofibrate loaded PLA–PEG microspheres was investigated by studying the effect of LA:PEG feed ration and PEG molecular weight of copolymer on the properties of microspheres. The entrapment efficiency and release behavior in vitro of microspheres depended on the composition of PLA–PEG microspheres, suggesting that the desired formulation of fenofibrate loaded PLA–PEG microspheres should be optimized in order to meet the specific needs of the application.

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