# Preparation, Characterization, and Drug Release Behaviors of Drug-Loaded ε-Caprolactone/L-lactide Copolymer Nanoparticles

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**ABSTRACT:** Copolymers of ε-caprolactone and L-lactide (PCLLA) with different monomer ratio were synthesized by ring opening polymerization, and drug-loaded nanoparticles of poly-ε-caprolactone (PCL), poly-L-lactide (PLLA), and their copolymers were prepared by precipitation method, respectively. The results of differential scanning calorimetry and X-ray diffraction indicated that the copolymerization of PCLLA decreased the crystallinity of the polymers, and the results of transmission electron micrograph and laser light scattering (LLS) revealed that the prepared nanoparticles had a spherical shape, and the size of PCLLA nanoparticles (~ 85 nm) was smaller than that of the PCL and PLLA nanoparticles. The experiment of *in vitro* drug release showed that the drug release rate from PCLLA nanoparticles was slower than that from PCL and PLLA nanoparticles, and the release profile of PCL6/LA4 nanoparticles appeared to follow zero order kinetics. These results suggested that the polymer composition made a great influence on the nanoparticle size and drug release behavior.
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**Key words:** ε-caprolactone/L-lactide copolymer (PCLLA); precipitation method; nanoparticles; nimodipine; release behavior

## INTRODUCTION

Since the development of polymer nanoparticles by Birrenbach and Speiser[1] using nonbiodegradable polymers and by Couvreur et al.[2] using biodegradable polymers, respectively, these solid colloidal systems have been the focus of extensive investigations. Their application has been considered in numerous areas of medicine because of their ability to control drug release and distribution, as well as their biodegradability. Nanoparticles can be prepared either by polymerization of emulsified monomers or by dispersion of preformed polymers,[3] and various methods have been developed to prepare drug-loaded nanoparticles depending on the physicochemical properties of polymers and drugs.[2,4–6]

In 1986, Fessi et al.[5] developed a new method to prepare nanoparticles in an easy and reproducible way based on the precipitation of a polymer by progressively adding the polymer solution into a nonsolvent of the polymer. This method has been applied successfully to various biodegradable polymers such as poly-D,L-lactide (PDLA),[5] polylactide-*co*-glycolide (PLGA),[7] and poly-ε-caprolactone (PCL).[8]

The copolymers of ε-caprolactone and L-lactide (PCLLA) have been widely studied in recent years because they have a number of excellent properties, including biocompatibility, biodegradability, processability, and a broad range of mechanical

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properties from elastomeric to rigid, which make them of great interest for medical applications.[9] Consequently, these copolymers seem to be a potential material for the preparation of nanoparticles applied for controlled drug release.

The aim of this work was to synthesize ε-caprolactone/L-lactide copolymers and prepare drug-loaded nanoparticles of these polymers by the precipitation method. This method is usually suitable for loading lipophilic drugs so that the nimodipine, a second-generation dihydropyridine calcium antagonist with apparent selectivity for cerebral blood vessels,[10] was selected as a model drug to incorporate into the carriers. The physicochemical properties and the drug-release behavior of the PCLLA nanoparticles were investigated and compared with those of the PCL and poly-L-lactide (PLLA) nanoparticles.

## EXPERIMENTAL

### Materials

The poly-ε-caprolactone ($M_n$, 42,500) was purchased from the Aldrich Chemical Co. L-Lactide (L-LA, Aldrich) was purified by recrystallization from dry toluene and ε-caprolactone (ε-CL, Aldrich) was purified by drying over $CaH_2$ and distillation under reduced nitrogen atmosphere. Stannous octoate (Sigma) was used as received. Poloxamer 188 (Pluronic F-68) was obtained from Jinling Petroleum Co., Nanjing, China. Nimodipine was obtained from Xinhua Pharmaceutical Factory, Shandong, China. All the other ingredients were of analytical grade and used without further purification.

### Polymerizations

The homo- and copolymerization were performed in bulk under nitrogen with stannous octoate as the catalyst.[11–13] An amount of $2 \times 10^{-3}$ mol catalyst per mol of monomer was added. After the mixture was thoroughly homogenized, the polymerization reaction was performed at 120°C for 48 h. The polymers were kept in a vacuum chamber at 40°C for 5 days to evaporate the residual monomers.

### Preparation of Nanoparticles

The drug-loaded nanoparticles were prepared by the precipitation method. One hundred milligrams of PCL or PCLLA and 10 mg of nimodipine were dissolved in 20 mL of acetone. Because the PLLA was insoluble in acetone, 100 mg of PLLA was first dissolved in 1 mL of chloroform, then added to 20 mL of acetone with 10 mg of nimodipine. Then the organic phase was dropped into 50 mL of water containing 100 mg of poloxamer 188 under moderate stirring at 50 ~ 55°C. The mixed phase immediately turned milky with bluish opalescence as a result of the formation of nanoparticles. The acetone was removed under reduced pressure and the final volume of the aqueous suspension was concentrated to 20 mL. The suspension was filtered with the paper filter to remove polymer aggregates and crystal particles of free drug.

### Characterization of Polymers and Drug-Loaded Nanoparticles

### Intrinsic Viscosity and GPC Measurement of Polymers

The intrinsic viscosities of the polymers were measured in chloroform at 25°C with an Ubbelohde viscosimeter. Gel permeation chromatography of a copolymer sample was performed at 30°C on a Waters GPC 244 with tetrahydrofuran as the eluent.

### X-Ray Diffraction, DSC, and FTIR

X-ray diffraction diagrams were measured on a Rigaku D/Max-RA diffractometer using Cu-$k\alpha$ radiation (30 KV, 50 mA). The glass transition temperatures ($T_g$), melting temperatures ($T_m$), and differential scanning calorimetry (DSC) thermograms were measured on a Shimadzu DSC-50 at a heating rate of 10°C/min. Fourier transform infrared (FTIR) spectra were obtained by a Nicolet 170SX spectrometer.

### Transmission Electron Micrograph (TEM) and Laser Light Scattering (LLS) Measurements of Nanoparticles

The morphological examination of nimodipine-loaded nanoparticles was performed using a JEOL JEM-100S transmission electron microscope after negative staining with phosphotungstic acid solution (0.5% w/v). The mean particle size and size distribution were measured by the laser light scattering technique using a Coulter® LS 230 Particle Size Analyzer. The measurement range was 0.04–2000 μm. Each sample was diluted with filtered distilled water until the appro-

**Table I Characterization of Homo- and Copolymers of ε-CL/L-LA**

| Sample | Monomer Composition in Feed (wt %) ε-CL | L-LA | [η] (dL/g) | $T_g$ (°C) | $T_m$ (°C) | Appearance |
|---|---|---|---|---|---|---|
| PCL ($M_n$ = 42,500) | 100 | 0 | 1.01 | −60 | 58.4 | Plastic-like, hard |
| PCL6/LA4 | 60 | 40 | 0.95 | −30.5 | — | Gummy, weak |
| PCL4/LA6 | 40 | 60 | 1.05 | −3.7 | — | Tough, elastic |
| PCL2/LA8 | 20 | 80 | 0.92 | 28.1 | 141 | Hard, elastic |
| PLLA | 0 | 100 | 1.03 | 61.1 | 175 | Crystalline, rigid |

priate concentration for measurement was achieved.

### Nanoparticle Yield, Drug-Loading Content, and Entrapment Efficiency

The nanoparticles were separated from the aqueous phase by ultracentrifugation (Ultra Pro™ 80, Du Pont) at 50,000 r/min and 10°C for 1 h. The weight of the nanoparticles was defined as the weight of the resultant nanoparticles sedimentated by ultracentrifugation. The weighed sediment of nanoparticles was dissolved and properly diluted in chloroform. Nimodipine had two strong absorption bands at the wavelength of 237 and 347 nm, respectively, both of which could be used for quantitative analysis and the absorption intensity of 237 nm was stronger than that of 347 nm. Because chloroform showed excessive absorbance at 237 nm, the solution was measured by ultraviolet (UV) spectrophotometer (Shimadzu UV 240) at the wave length of 347 nm and the weight of the drug entrapped in nanoparticles was calculated by a calibration curve.

The nanoparticle yield, drug-loading content, and drug entrapment efficiency are presented in eqs. (1), (2), and (3), respectively:

$$\text{Nanoparticle yield (\%)} = \frac{\text{Weight of nanoparticles}}{\text{Weight of polymer and drug fed initially}} \times 100 \quad (1)$$

$$\text{Drug loading content (\%)} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}} \times 100 \quad (2)$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of drug fed initially}} \times 100 \quad (3)$$

### *In Vitro* Drug Release Studies of Nanoparticles

*In vitro* release studies were performed as follows: Five milliliters of nanoparticle suspensions (corresponding to 2.5 mg of nimodipine) was placed in a dialysis membrane bag, tied, and dropped into 200 mL of a phosphate buffer solution media (0.1*M,* pH 7.4). The entire system was kept at 37°C with continuous magnetic stirring. At selected time intervals, 4 mL of aqueous solution was withdrawn from the release medium. The phosphate buffer solution had little absorbance at 237 nm and the drug concentration in this release medium was relatively dilute, so the solution was assayed spectrophotometrically for nimodipine at 237 nm, the stronger absorption band, using a UV spectrophotometer. The release of drug was determined by a calibration curve.

## RESULTS AND DISCUSSION

### Characterization of Polymers

To compare the physicochemical properties of nanoparticles prepared with different polymers, a number of homo- and copolymers were synthesized by ring opening polymerization using the same amount of stannous octoate as the catalyst. Polymers, monomer compositions in feed, polymer intrinsic viscosity, $T_g$ and $T_m$, and physical appearance are presented in Table I. As shown in Table I, all the samples had a similar intrinsic viscosity. The number average molecular weight ($M_n$) of PCL6/LA4 was 39,800 with a relative low polydispersity, D = 1.74, determined by gel permeation chromatography (GPC).

Figure 1 shows the X-ray diffraction diagrams of PCL, PLLA, and their copolymers. The diagram of PCL(a) showed two distinctive peaks at angles of 21.54 and 23.75 degrees 2$\theta$. The PLLA(e)

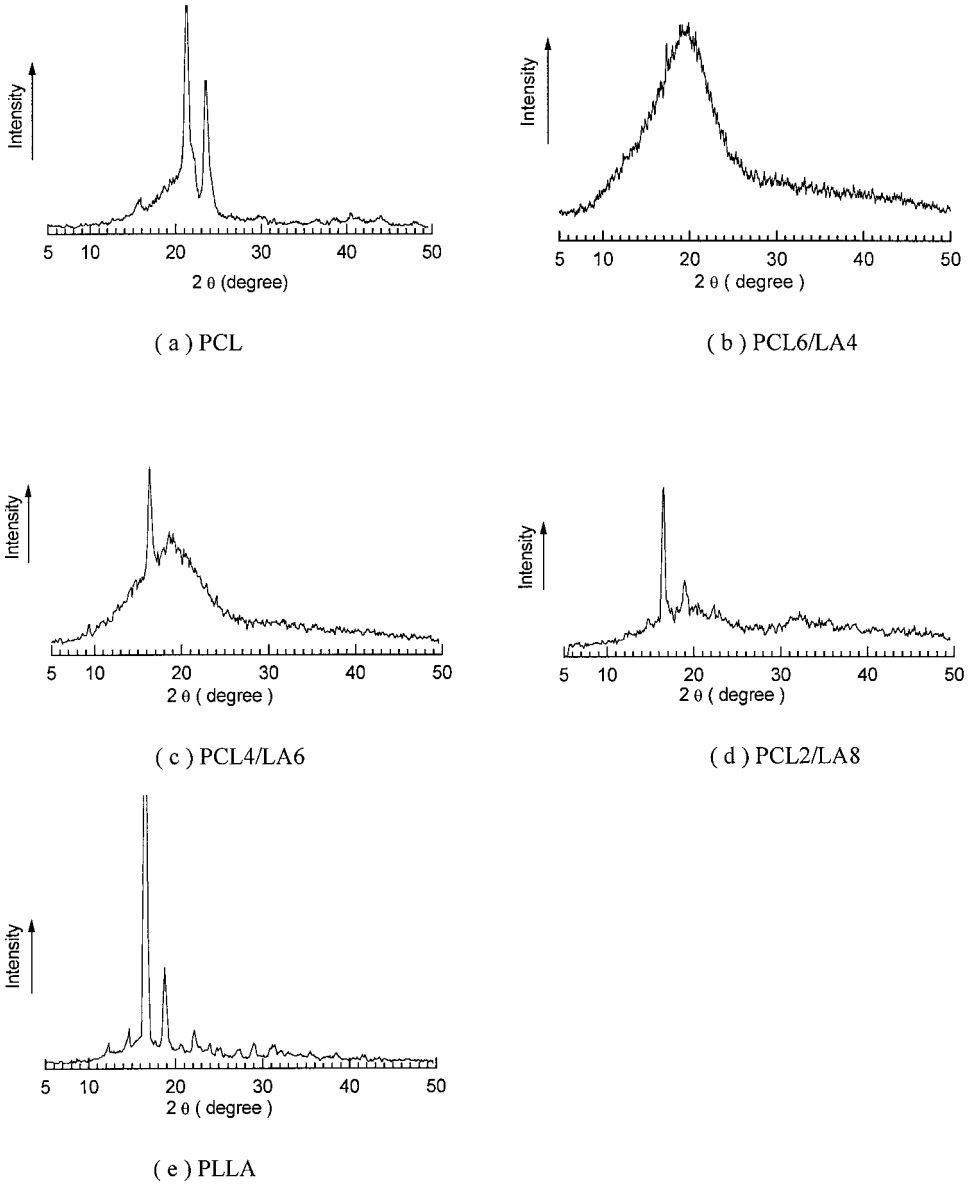


**Figure 1** X-ray diffraction diagrams of (a) PCL, (b) PCL6/LA4, (c) PCL4/LA6, (d) PCL2/LA8, and (e) PLLA.

showed a large number of crystalline diffraction peaks and the two sharpest and most intense ones were at 16.59 and 18.97 degrees $2\theta$. As for the copolymers, PCL2/LA8(d) showed two less intense crystalline peaks at 16.7 and 19.04 degrees $2\theta$. PCL4/LA6(c) showed a wide amorphous peak and a weak crystalline one at 16.55 degrees $2\theta$, and PCL6/LA4(b) only showed a wide amorphous peak, suggesting that PCL4/LA6 and PCL6/LA4 were present in an amorphous state. From these results, it could be concluded that the crystallinity of the polymer was closely related to the polymer composition and could be adjusted by varying the monomer ratio during the polymerization process. The $T_g$ and $T_m$, and the X-ray diffraction results were in good agreement with reports in the literature.[11–13]

## Morphology and Size of Drug-Loaded Nanoparticles

The TEM photographs of the nanoparticles prepared by the precipitation method are shown in Figure 2. TEM photographs showed that most of

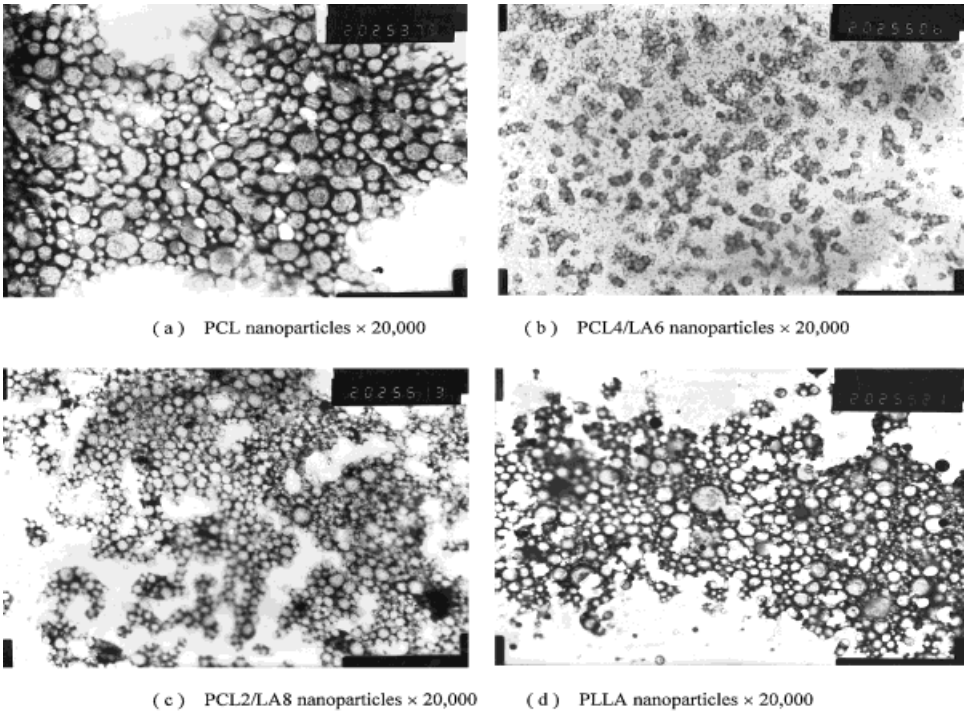


**Figure 2** Electron transmission microphotography of: (a) PCL nanoparticles, (b) PCL4/LA6 nanoparticles, (c) PCL2/LA8 nanoparticles, and (d) PLLA nanoparticles.

the drug-loaded nanoparticles had a discrete spherical shape with a diameter less than 200 nm. In addition, from the TEM photographs, it could be observed that the size of PCLLA copolymer nanoparticles was smaller than that of PCL and PLLA homopolymer nanoparticles.

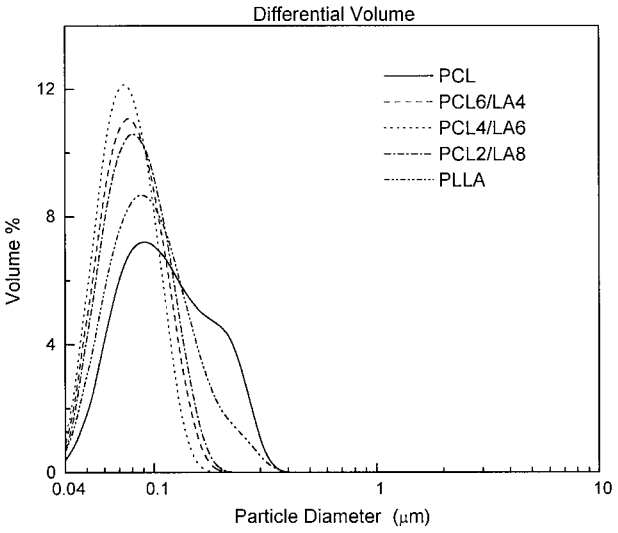


**Figure 3** Size distribution of PCL, PLLA, and PCLLA nanoparticles.

The results of particle size characterized by LLS are shown in Figure 3 and Table II. The mean diameters of all the kinds of nimodipine-loaded nanoparticles were less than 150 nm. The size of PCLLA copolymer nanoparticles (~ 85 nm) was smaller than that of the PCL (132 nm) and PLLA (111 nm) homopolymer nanoparticles, and the PCLLA nanoparticles also had a narrower particle size distribution. These results were in agreement with the observation of TEM and indicated that the polymer composition had a great influence on the mean particle size.

According to the mechanism of nanoparticle formation suggested in the literature,[7,8,14] once

**Table II Mean Particle Size of Nimodipine-Loaded PCL, PLLA, and PCLLA Nanoparticles**

| Sample | Mean Diameter ± SD (nm) |
|---|---|
| PCL | 132 ± 64.1 |
| PCL6/LA4 | 86.6 ± 27 |
| PCL4/LA6 | 81.3 ± 22.8 |
| PCL2/LA8 | 85.9 ± 29 |
| PLLA | 111 ± 52 |

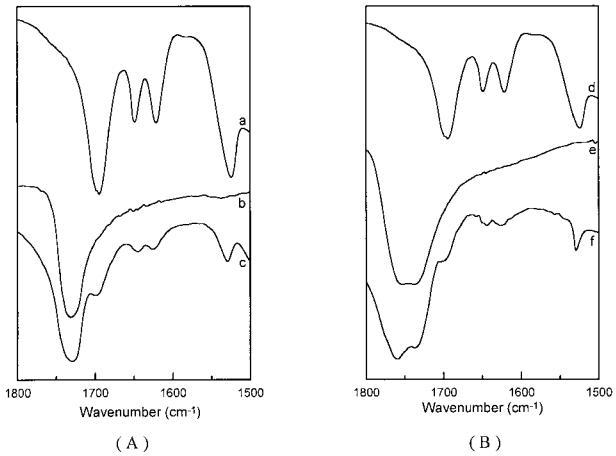


**Figure 4** Infrared spectra of (A) (a) nimodipine, (b) PCL, and (c) nimodipine-loaded PCL nanoparticles; (B) (d) nimodipine, (e) PCL4/LA6, and (f) nimodipine-loaded PCL4/LA6 nanoparticles.

the organic droplets contact water, the diffusion of acetone from the organic to aqueous phase produces very small droplets and makes the polymer molecules aggregate and precipitate instantaneously. Furthermore, these small droplets will break up into smaller and smaller ones until the polymer precipitates completely, which leads to the formation of nanoparticles. In the case of this study, the decrease of the mean particle size of copolymers might be attributed to the copolymerization of ε-CL and L-LA, reducing the crystallinity of the polymer and modifying the solubility of the polymer in acetone. This might make the acetone droplets break up into finer nanodroplets before the polymer precipitation.

### Drug-Loading Properties of Nanoparticles

Figure 4(a and b) shows the FTIR spectra over the range 1500–1800 $cm^{-1}$ for the PCL and PCL4/LA6 nanoparticles, respectively. The polymer spectra showed the carbonyl band for PCL at 1732 $cm^{-1}$ and for PCL4/LA6 at 1753 and 1738 $cm^{-1}$. The nimodipine spectrum showed the characteristic bands at 1695 $cm^{-1}$ of carbonyl bond, at 1640 $cm^{-1}$ of the C═C stretch, at 1620 $cm^{-1}$ of the aromatic C═C stretches and at 1520 $cm^{-1}$ of the —$NO_2$ group. The characteristic bands of the nimodipine and the polymer could be observed in the drug-loaded nanoparticles spectra without distinct shifts for both PCL and PCL4/LA6 nanoparticles. These results indicated that the drug had been entrapped in the polymer matrix and there were no chemical interactions between nimodipine and polymer.

The X-ray diffraction diagrams of nimodipine, polymers, and nimodipine-loaded nanoparticles are shown in Figure 5. The crystalline nimodipine showed a large number of sharp diffraction peaks, whereas these characteristic peaks were not detected for the nimodipine-loaded nanoparticles of PCL and PCL4/LA6. These results coincide with those obtained by DSC. Figure 6 shows the DSC thermograms for nimodipine, polymers, and nimodipine-loaded nanoparticles. The thermogram of nimodipine showed an endothermic peak at 125°C corresponding to its melting temperature and this endothermic peak was also not detected in the thermograms for both PCL and PCL4/LA6 nanoparticles. From these results, it could be concluded that the nimodipine was dispersed molecularly as amorphous state in both polymer matrixes.

The nanoparticle yield, drug loading content, and entrapment efficiency of nimodipine-loaded PCL, PLLA, and PCLLA nanoparticles are shown in Table III. All the samples had high nanoparticle yield, drug loading content, and entrapment efficiency except PLLA nanoparticles. The poor solubility of PLLA in acetone and addition of chloroform in the organic phase might result in the low nanoparticle yield and drug-loading content.

### *In Vitro* Release Behavior of Drug-Loaded Nanoparticles

Figure 7 shows the release profiles of nimodipine from PCL, PLLA, and PCLLA nanoparticles. It can be observed that the homopolymer nanoparticles released the drug much faster than the copolymer nanoparticles and the PLLA nanoparticles had the fastest release rate in all the samples. The drug release rate seemed to be reduced as the weight ratio of ε-CL/L-LA of the polymer approached one. In addition, it was notable that the release curve of PCL6/LA4 was near to a straight line, suggesting that the drug release from PCL6/LA4 nanoparticles appeared to approximate a zero order release. These results indicated that the drug release rate was also related strongly to the composition of the polymer matrix.

The release of a drug from the polymer nanoparticles is a rather complicated process and can be affected by many factors, such as the polymer degradation, molecular weight, crystallinity, glass transition temperature, the binding affinity

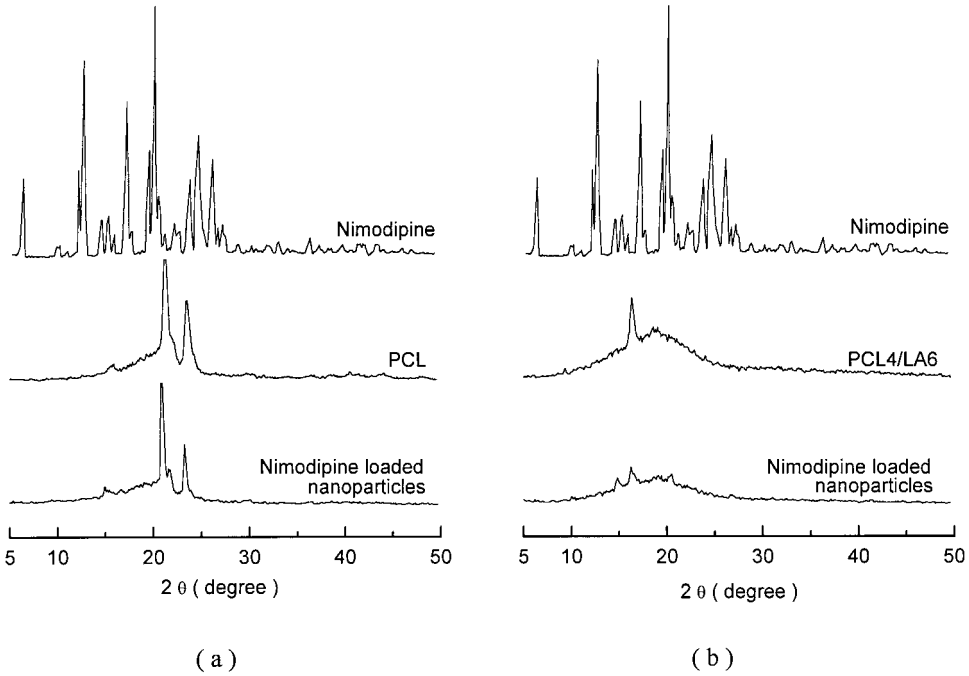


**Figure 5** X-ray diffraction diagram of (a) nimodipine, PCL, and nimodipine-loaded PCL nanoparticles; (b) nimodipine, PCL4/LA6, and nimodipine-loaded PCL4/LA6 nanoparticles.

between the polymer and the drug, and so on, but one or two of them would mainly determine the drug-release behavior of the nanoparticles.[15]

According to the report by Malin et al.,[16] polymer degradation did not substantially occur for all of the PCL, PLLA, and PCLLA systems during

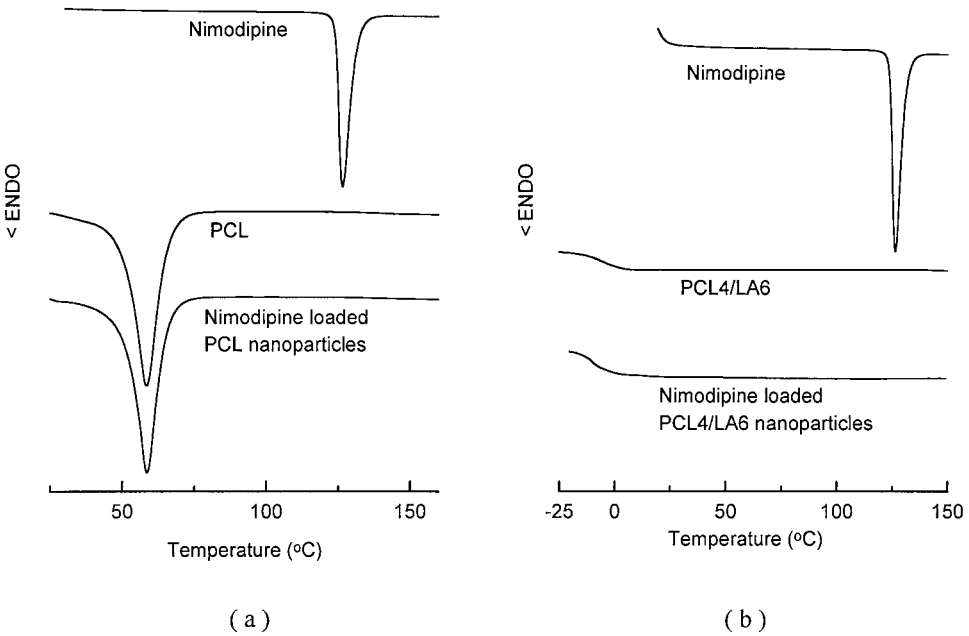


**Figure 6** DSC thermograms of (a) nimodipine, PCL, and nimodipine-loaded PCL nanoparticles; (b) nimodipine, PCL4/LA6, and nimodipine-loaded PCL4/LA6 nanoparticles.

the monitored period. Therefore, the drug-release rate might not be largely affected by the polymer degradation in this study. Izumikawa et al.[17] have reported that the crystallinity of the polymer matrixes greatly affect the drug release rate. As shown in Figures 1 and 7, the higher the crystallinity of the polymer matrix was, the faster was the nimodipine release from the nanoparticles. From these results, we could also consider that the crystallinity of the polymer played an important role in the drug release of the nanoparticles. The fastest release rate of PLLA nanoparticles might be attributed to the high crystallinity of the PLLA matrix. The high crystallinity could lead to forming a microchannel structure and high surface area in the polymer matrix, and make the drug easily released from the nanoparticles.[17] On the other hand, the relatively low hydrophobicity of PLLA compared with that of PCL and PCLLA might also make the lipophilic drug, nimodipine, fast released. The faster release rate of PCL nanoparticles might also be attributed to its crystalline matrix and the other factor affecting the release behavior might be the low $T_g$ of PCL ($T_g = -60°C$). It has been reported that a polymer matrix, having low $T_g$, is highly permeable to low molecular weight drugs.[18] In contrast to the homopolymer nanoparticles, the PCLLA copolymer nanoparticles had a compact amorphous matrix and their release rate was determined by the slow diffusion of the drug from the matrix. This might be the main reason for the low drug-release rate of the PCL6/LA4 and PCL4/LA6 nanoparticles.

**Table III Nanoparticle Yield, Drug Loading Content, and Entrapment Efficiency of Nimodipine-Loaded PCL, PLLA, and PCLLA Nanoparticles**

| Sample | Drug Loading Content (%) | Entrapment Efficiency (%) | Nanoparticle Yield (%) |
|---|---|---|---|
| PCL | 8.4 | 88.2 | 95.5 |
| PCL6/LA4 | 8.6 | 91.1 | 96.3 |
| PCL4/LA6 | 8.7 | 91.1 | 95.2 |
| PCL2/LA8 | 8.1 | 75.1 | 84.3 |
| PLLA | 3.7 | 19.5 | 47.8 |

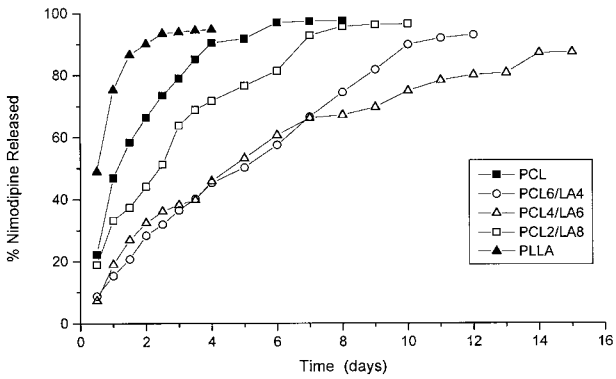


**Figure 7** Nimodipine release profiles from PCL, PLLA, and PCLLA nanoparticles.

## CONCLUSIONS

A series of drug-loaded nanoparticles of PCLLA, PCL, and PLLA have been successfully prepared by the precipitation method. The size and size distribution of the prepared nanoparticles and the drug release behavior are affected significantly by the composition of the polymer matrix. The release rate from the copolymer PCLLA nanoparticles is lower than that from the homopolymer PCL and PLLA nanoparticles. The drug release from PCL6/LA4 nanoparticles appears to approximate zero order release. These results show that the copolymers of ε-caprolactone and L-lactide are promising materials for preparing nanoparticles as drug carrier and the release rate from nanoparticles can be adjusted by changing the composition of polymer matrix.

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