Preparation and Drug Release Behaviors of 5-Fluorouracil Loaded Poly(glycolide-*co*-lactide-*co*-caprolactone) Nanoparticles

Hanwei Zhang, Jianzhong Bei, Shenguo Wang

BNLMS, State Key Laboratory of Polymer Physics and Chemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

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ABSTRACT: 5-Fluorouracil (5-Fu) loaded poly(glycolide-*co*-lactide-*co*-caprolactone) (PGLC) nanoparticles were prepared by modified spontaneous emulsification solvent diffusion method (modified-SESD method) and characterized by dynamic light scattering, scanning electron microscopy and ^{1}H NMR determination. It was found that the obtained nanoparticles showed near spherical shape and was controllable with the radius range of 30–100 nm. Compared with the nanoparticles prepared by polylactide and poly(lactide-*co*-glycolide) (PLGA) under the similar preparation condition, yield of PGLC nanoparticles was the highest, which reached to about 100%. On the other hand, drug entrapment efficiency of PGLC nanoparticles was also higher than that of PLGA and PLLA nanoparticles. 5-Fu release behavior of PGLC nanoparticles *in vitro* showed that 5-Fu release of PGLC nanoparticles showed a near zero-order release profile, and 5-Fu release rate of PGLC nanoparticles was faster than that of PLLA and PLGA nanoparticles. According to degradation behavior of PGLC nanoparticles, it could be proposed that the kinetic of degradation controlled release played an important role in the release process of PGLC nanoparticles. It revealed that the PGLC nanoparticles could be a promising drug carrier. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 3757–3767, 2007

Key words: nanotechnology; 5-fluorouracil (5-Fu); drug delivery system; polyesters; biodegradation

INTRODUCTION

As an optimal drug action, the most efficient way of a drug delivery system (DDS) is to release the drug to the desired site of the body with a constant rate and decrease or even avoid the side effect at the non-target site. Liposome,[1] micelles,[2] and polymeric nanoparticles[3,4] are promising DDS. Among them, biodegradable polymeric nanoparticles are preferable candidate for DDS.[5] The biodegradable polymeric nanoparticles are colloidal systems with diameter in the range of 10–1000 nm, and the drug can be entrapped in, adsorbed or chemically coupled onto the nanoparticles.[6]

In the past decades, a great number of publications have reviewed many applications of synthetic biodegradable polymers containing different drugs. Among them, biodegradable polyesters, such as polylactide, poly(ε-caprolactone) and poly(lactide-*co*-glycolide) (PLGA), because they have desirable biocompatible and biodegradable properties, particularly have been approved by Food and Drug Administration (FDA) for clinic use, have been widely used in nanoparticles DDS.[4,7–12] However, because of crystallization and low biodegradation rate of these polymers, the clinical application of such polymeric nanoparticles, especially for using as drug carrier to realize rapid and constant drug release, is limited.[13,14]

In previous papers, an improved copolylactone, poly(glycolide-*co*-lactide-*co*-caprolactone) tri-component copolymer (PGLC), was synthesized and proved to use as drug carrier.[15,16] For example, it was used to prepare an eye-implantable cyclosporine (Cs) DDS for sustaining Cs release *in vivo*.[17] Recently, PGLC had been successfully used to fabricate nanoparticles by modified-SESD method.[18] In the present paper, 5-Fu, a sparing water-soluble anticancer drug,[19] was used as model drug to be encapsulated into the PGLC nanoparticles. Physicochemical property of 5-Fu loaded PGLC nanoparticles was studied by measurements of dynamic light scattering system (DLS), scanning electron microscopy (SEM) and ^{1}H NMR. Influences of preparation conditions and polymeric characteristics on yield, drug entrapment efficiency and 5-Fu loading content of the nanoparticles were discussed in detail. Additionally, 5-Fu release

Correspondence to: S. Wang (wangsg@iccas.ac.cn).
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behavior of PGLC nanoparticles *in vitro* was also determined and compared with that of 5-Fu loaded PGLC film, PLLA and PLGA nanoparticles.

MATERIALS AND METHODS

Materials

ε-Caprolactone (CL) (Acros Chemical, N.V) was purified by CaH_2 drying and distillation. L-Lactide (L-LA) and glycolide (GA) (Purac Biochem.) were purified by recrystallization with dried ethyl acetate. Stannous octoate (Sigma, A.R) was used directly without purification. Ethyl acetate was dried by P_2O_5 overnight and then distilled. 5-Fu was provided by Guangzhou Pharmaceutical Factory. All the other reagents were of analytical grade and used without further purification.

Synthesis and characterization of tri-component copolymer PGLC

According to literature method,[16] tri-component copolymer PGLC (molar ratio of GA/LA/CL was 27/63/10) was synthesized by ring-opening polymerization of GA, L-LA, and CL. PLLA and PLGA (molar ratio of LA/GA was 70/30) were also synthesized according to the previous method.[14] All the polymers were purified by dissolving in chloroform and re-precipitating from ethanol.

Molecular weight (M_w, M_n) and polydispersity (PDI) of various polymers were measured by gel permeation chromatography (GPC) (Waters 510 apparatus equipped with Shodex GPC KF-800 columns thermostated) at 35°C. Chloroform was used as eluent at a flow rate of 1.0 mL/min; calibration was performed with polystyrene as standard. ^{1}H NMR spectra were obtained with a Bruker DMX300 spectrometer at room temperature. Glass transition temperature (T_g) of various copolymers was measured by differential scanning calorimeter (DSC) (Du Pont 2100).

Preparation of 5-Fu loaded nanoparticles and control

5-Fu loaded PGLC, PLLA, and PLGA nanoparticles were prepared by the modified-SESD method.[20] Briefly, a predetermined amount of polymer (PGLC, PLLA or PLGA) was dissolved in 1 mL of THF, and an exact volume of ethanol was added into the polymer solution (volume ratio of THF/ethanol = 3/2). Then predetermined amount of 5-Fu was added into the polymer solution to get homogeneous solution. The mixed solution was dropwisely added into 10 mL of Tween 60 aqueous solution (1% w/v) under gentle stirring (about 180–200 rpm) at 25°C for 10 min to form a dispersion system. After the THF and ethanol were thoroughly removed from the dispersion by rotary evaporation at room temperature, the obtained aqueous suspension was filtered by a 0.45-μm-filter membrane to remove precipitates. Finally, the 5-Fu loaded nanoparticles were collected by centrifugation (TDL-40 B, AnTing Instruments Corporation, China) under 100,000 × *g* for 15 min, and washed with distilled water for three times. After lyophilization, the dried 5-Fu loaded polymeric nanoparticles were obtained. Drug-free nanoparticles were prepared by the similar method in the absence of 5-Fu.

As control, 5-Fu loaded PGLC film was prepared by casting the mixed solution with same weight ratio of PGLC/5-Fu in a polytetrafluoroethylene (PTFE) mould. After solvent was removed thoroughly, 5-Fu loaded PGLC film with about 0.1 mm thickness was obtained.

Characterization of nanoparticles

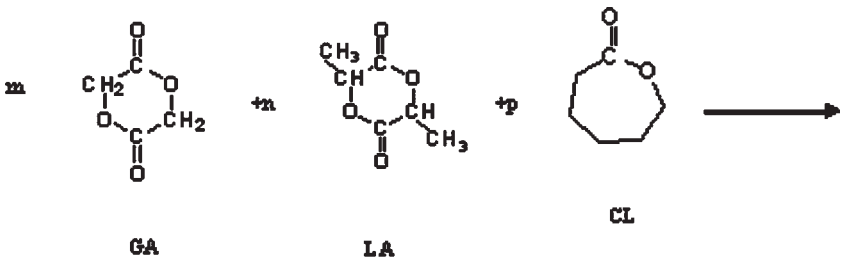
SEM (Hitachi S-530) was used to observe morphology of the nanoparticles. Mean radius and size distribution of the nanoparticles were determined by DLS 90 plus particle size analyzer (Brookhaven Instruments Corporation) at 25°C with angle detection of 90° for 300 s.

Yield, 5-Fu loading content, and entrapment efficiency of nanoparticles

To determine 5-Fu content in the nanoparticles, exactly weighed 5-Fu loaded nanoparticles were dissolved in chloroform. Because 5-Fu could not dissolve in chloroform, distilled water was used to extract 5-Fu and properly dilute to exact volume. Then, the 5-Fu aqueous solution was measured by UV spectrophotometer at the wavelength of 266 nm, and weight of 5-Fu in nanoparticles was calculated according to a calibration curve. After measuring the weight of the 5-Fu loaded nanoparticles and entrapped 5-Fu, yield, 5-Fu loading content and entrapment efficiency were calculated according to the eqs. (1)–(3) respectively:

$$\text{Yield } (\%) = \frac{\text{Weight of 5-Fu loaded nanoparticles-Weight of entrapped 5-Fu}}{\text{Weight of the feeding polymer}} \times 100 \quad (1)$$

$$\text{5-Fu loading content } (\%) = \frac{\text{Weight of 5-Fu in nanoparticles}}{\text{Weight of 5-Fu loaded nanoparticles}} \times 100 \quad (2)$$



$$\sim(1/2GA)_{m1}\text{-}(1/2LA)_{n1}\text{-}CL_{p1}\text{-}(1/2LA)_{n2}\text{-}(1/2GA)_{m2}\text{-}CL_{p2}\sim$$

Scheme 1 Scheme of synthesis of PGLC.

$$\text{5-Fu entrapment efficiency (\%)} = \frac{\text{Weight of 5-Fu in nanoparticles}}{\text{Weight of 5-Fu feeding}} \times 100 \quad (3)$$

In vitro drug release test of nanoparticles

Release test of 5-Fu loaded nanoparticles was carried out as follows, predetermined amount of 5-Fu loaded nanoparticles were placed in a dialysis bag (molecular weight cut-off is 12,000), then the dialysis bag was put into a flask with 20 mL of 0.1*M* phosphate buffer solution (PBS, pH 7.4) and fixed in a shaking-bed under 50 rpm at (37 ± 1)°C. After a predetermined time interval, 0.2 mL of the release medium was taken out and 0.2 mL of fresh PBS was added. The taken out 0.2 mL release medium was diluted by fresh PBS to 10 mL and then UV absorbance of the medium at 266 nm was determined. According to the standard curve of 5-Fu concentration-UV absorbance, 5-Fu concentration in the medium was calculated.

In vitro degradation behavior measurement of nanoparticles

A predetermined amount of 5-Fu free nanoparticles was placed in a dialysis membrane bag (molecular weight cut-off of 12,000), then the samples were incubated in release condition *in vitro.* After a predetermined period, a degraded sample was taken out. A part of the sample suspension was measured by DLS, and the rest of the sample suspension was lyophilized to obtain dried degradation product, and monitored by the changes of molecular weight and chemical composition.

RESULTS AND DISCUSSION

Synthesis and characterization of the tri-component copolymer PGLC

PGLC was synthesized by a ring-opening polymerization of L-LA, GA and CL (feeding mole ratio of LA/GA/CL was 63/27/10), as shown in Scheme 1. PLGA and PLLA were synthesized by the similar method. In order to eliminate effect of molar ratio of LA/GA and molecular weight of the polymer on drug release behavior, molar ratio of LA/GA in PGLC and PLGA was controlled similar to 2.2/1.0, and M_n of all polymers were adjusted similar, as shown in Table I.

GPC curves of PLLA, PLGA and PGLC were measured and shown in Figure 1. It could be seen that all curves showed symmetric and monomodal peaks, and all the peaks appeared at same elution time. It could prove that all polymers were pure with similar molecular weight.

On the other hand, from DSC measurement, it could be found in Table I, there was a clear melt peak at 179°C for PLLA, which meant PLLA was crystal polymer, but there was no melting peak for PLGA and PGLC, which proved they were amorphous polymers. All three polymers had one T_g,

TABLE I
Molecular Weight and Chemical Composition of Polymers

Samples	GPC			Molar ratio[a] GA/LA/CL	T_g (°C)	T_m (°C)	Contact angle (degree)
	M_n	M_w	PDI				
PLLA	31,000	35,000	1.2	0/100/0	60	179	84.6
PLGA	32,000	40,000	1.3	31.0/69.0/0 (1.00/2.23/0)	58	–	74.5
PGLC	33,000	42,000	1.3	27.5/62.5/10.0 (1.00/2.27/0.36)	22.5	–	74.2

[a] Calculated from ^{1}H NMR measurement.

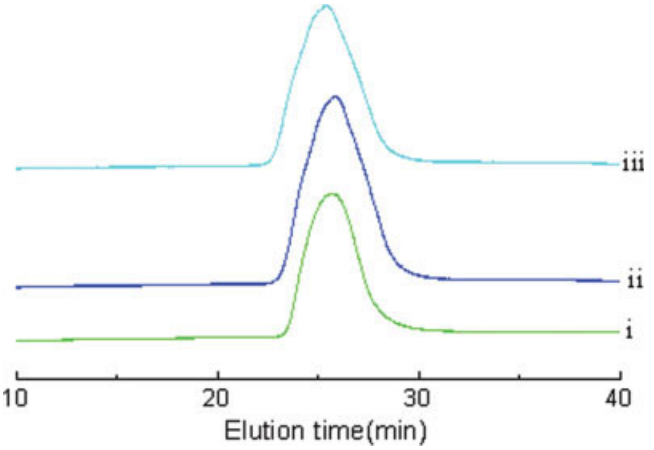


Figure 1 GPC curves of (i) PLLA, (ii) PLGA, and (iii) PGLC.

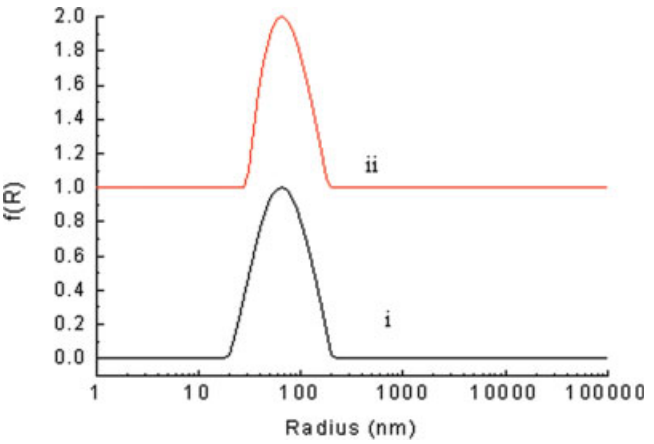


Figure 3 Size distributions of (i) 5-Fu free and (ii) 5-Fu loaded PGLC nanoparticles (mean radius was 68 nm).

where T_g of PLLA and PLGA was 60 and 58°C, respectively, and T_g of PGLC was about 22.5°C, which was much lower than that of PLLA and PLGA. Hydrophilicity of three polymers was identified by contact angle as shown in Table I. It could be considered that hydrophilicity of PGLC and PLGA was improved by introduction of GA component into the polymer.

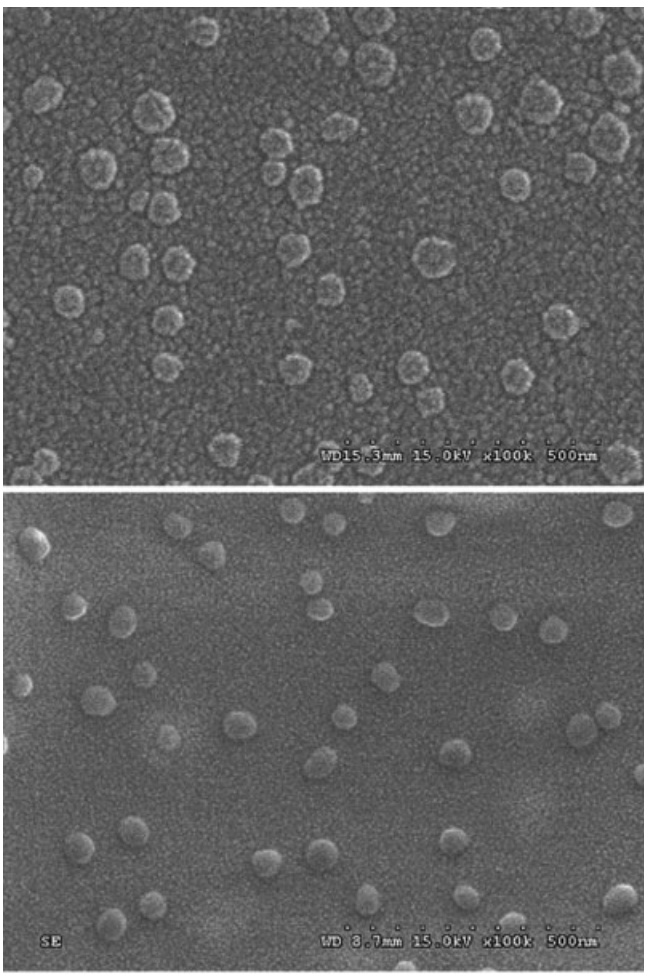


Figure 2 SEM photographs of 5-Fu free (Upper) and 5-Fu loaded PGLC nanoparticles (Down) (×100 k).

Characteristic of PGLC nanoparticles

5-Fu free and 5-Fu loaded PGLC nanoparticles were prepared under same condition. Morphology of 5-Fu free and 5-Fu loaded PGLC nanoparticles was observed by SEM and shown in Figure 2. It could be seen that both 5-Fu loaded and 5-Fu free PGLC nanoparticles had spherical shape. Mean radius and size distribution of the nanoparticles were measured by DLS and listed in Figure 3. It could be seen that two kinds nanoparticles had similar mean radius (about 68 nm) with narrow size distribution. The results of SEM and DLS meant drug encapsulation had no effect on morphology and particles size of the PGLC nanoparticles.

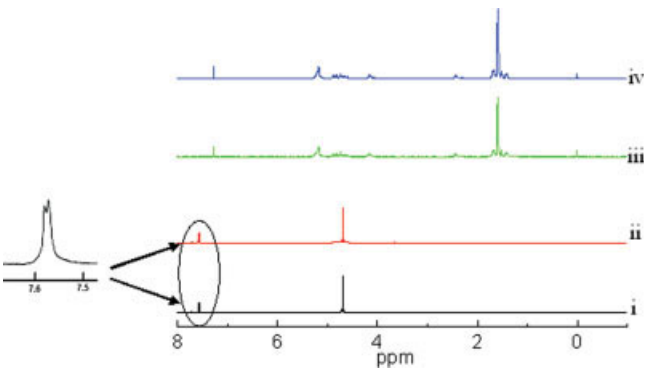


Figure 4 ^{1}H NMR spectra of (i) pure 5-Fu, (ii) D_2O phase of 5-Fu loaded PGLC nanoparticles, (iii) $CDCl_3$ phase of 5-Fu loaded PGLC nanoparticles, and (iv) 5-Fu free PGLC nanoparticles.

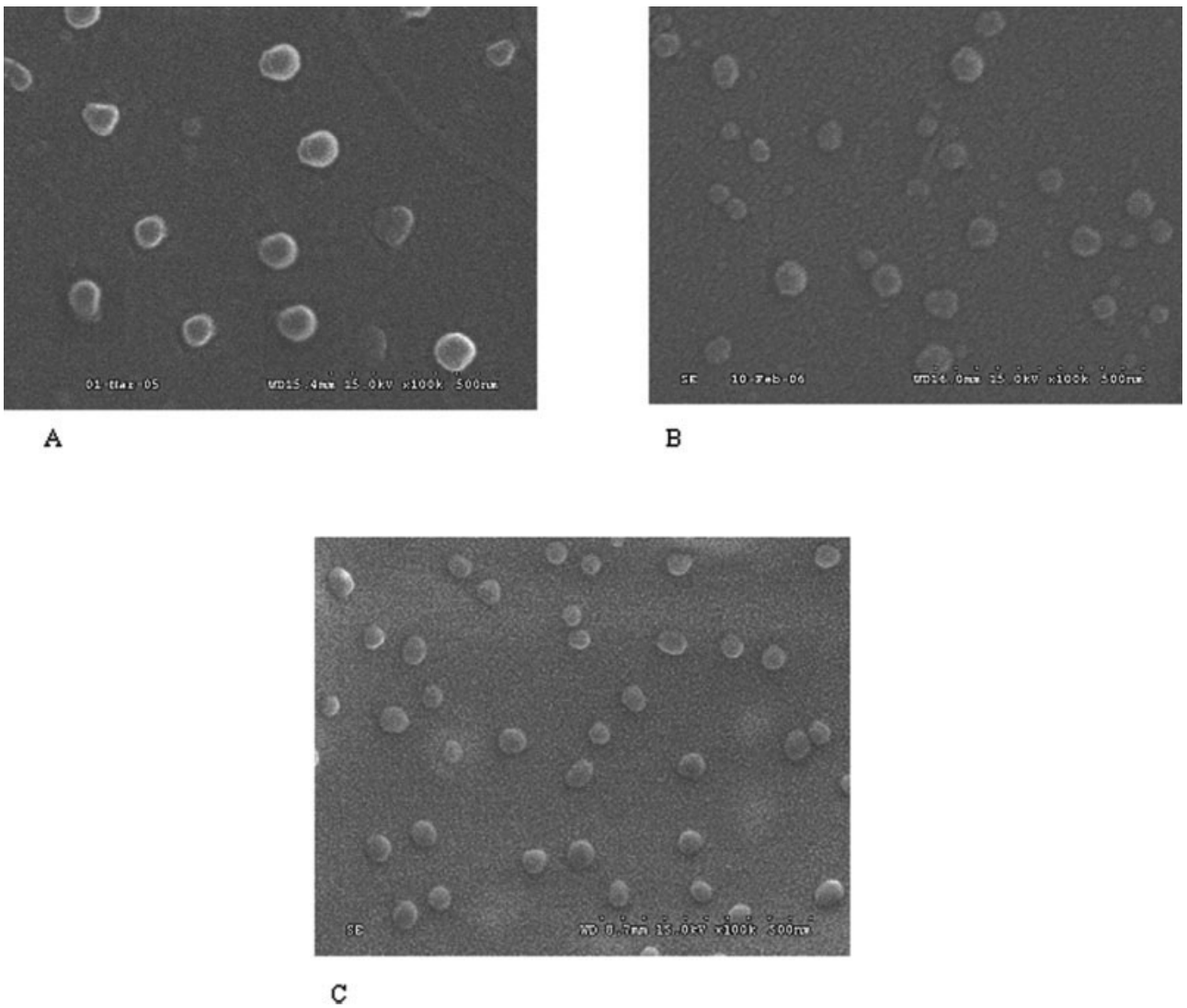


Figure 5 SEM photographs of 5-Fu loaded (A) PLLA, (B) PLGA, and (C) PGLC nanoparticles (×100 k).

Chemical component of 5-Fu loaded nanoparticles was characterized by ^{1}H NMR measurement. First, 5-Fu loaded nanoparticles dissolved into $CDCl_3$, and then D_2O was added. Since 5-Fu could not dissolve in $CDCl_3$, so it was extracted and dissolved in D_2O. Then, ^{1}H NMR was used to measure both the $CDCl_3$ phase (Fig. 4, iii) and D_2O phase (Fig. 4, ii) for determining chemical component of the 5-Fu loaded nanoparticles. In this work, the pure 5-Fu (i) (D_2O as solvent) and 5-Fu free PGLC nanoparticles ($CDCl_3$ as solvent) were also measured and also shown in Figure 4. It could be seen there was similar proton signal[21] at 7.5–7.6 ppm for D_2O phase of 5-Fu loaded nanoparticles (ii) and pure 5-Fu (i). As well as there was similar proton signals in spectra of $CDCl_3$ phase of 5-Fu loaded nanoparticles (iii) and 5-Fu free PGLC nanoparticles (iv).[16] The above result meant that 5-Fu loaded PGLC nanoparticles composed of both 5-Fu and PGLC, and 5-Fu had not changed during the nanoparticles fabrication procedure.

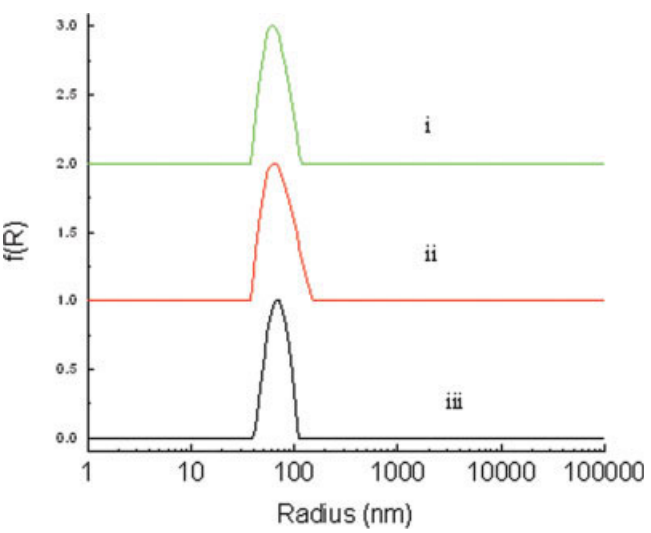


Figure 6 Size distributions of different polymeric 5-Fu loaded (i) PGLC, (ii) PLGA, and (iii) PLLA nanoparticles.

TABLE II
Yield, 5-Fu loading and Entrapment Efficiency of 5-Fu in Various Polymeric Nanoparticles

5-Fu loaded nanoparticles	Mean radius (nm)	Yield (%)	5-Fu entrapment efficiency (%)	5-Fu loading[a] (%)
PLLA	72	41.3	34.1	19.8
PLGA	67	63.2	29.5	12.3
PGLC	68	~100	43.7	11.6

[a] Feeding dose of 5-Fu/Polymer = 30/100 (w/w).

Effect of polymer on yield, 5-Fu loading content, and entrapment efficiency of nanoparticles

5-Fu loaded PGLC, PLLA and PLGA nanoparticles were prepared under same feeding dose (5-Fu/polymer = 30/100 w/w), and SEM images and size distribution of three kinds nanoparticles were shown in Figures 5 and 6, respectively. It could be seen that all 5-Fu loaded nanoparticles had regular spherical shape and smooth surface. According to the results of DLS (Fig. 6), it could be also found that three kinds nanoparticles had narrow and unimodal size distribution. Mean radius of PGLC nanoparticles was about 68 nm, which was similar to that of the PLGA nanoparticles (67 nm) and PLLA nanoparticles (72 nm) as shown in Table II.

The yield, 5-Fu loading content and 5-Fu entrapment efficiency of various nanoparticles were also determined and summarized in Table II. It could be seen that the yield of 5-Fu loaded PLLA nanoparticles and PLGA nanoparticles was 41.3 and 63.2% respectively, while the yield of PGLC nanoparticles was much higher than that of PLLA and PLGA nanoparticles, even reached about 100%. Additionally, 5-Fu loading efficiency of three polymeric nanoparticles was also different. The loading efficiency of PLLA nanoparticles were the highest, about 19.8%, and the 5-Fu loading efficiency of PLGA and PGLC nanoparticles were similar, about 12%. On the other hand, it was found that 5-Fu entrapment efficiency of PGLC nanoparticles was much higher than that of PLGA and PLLA nanoparticles, and was about 42.4%.

To explain the above experimental results, the mechanism of the modified-SESD method should be explained. When the polymer (PLLA, PLGA or PGLC) in mixed solution of THF/ ethanol was dispersed into aqueous medium, the ethanol preferentially diffused out of the droplets, and made the perturbation of the interface spontaneously to produce a large interfacial area, which led to form nanosize quasi-emulsion droplets of the polymer solution. After THF further diffused out of the droplets, the droplets were solidified to form stable nanoparticles.[20]

However, because of the differences among the polymers, the yield, 5-Fu loading content and 5-Fu entrapment efficiency of relevant nanoparticles were different. Due to the high hydrophobicity, crystallization and T_g of PLLA, organic solvent was much difficult to diffuse out of the PLLA droplets. So during formation process of the PLLA nanoparticles, large numbers of droplets congregated together to form polymeric deposition. On the other hand, since crystal PLLA chain could effectively prevent leakage of 5-Fu from the nanoparticles, PLLA nanoparticles had the lowest yield but the highest drug loading content. While PLGA and PGLC were amorphous copolymer, and were more hydrophilic than PLLA, which favored the diffusion of THF out of the polymeric droplets and solidification of nanoparticles, so yield of PLGA and PGLC nanoparticles increased greatly. Moreover, because T_g of PGLC was 25°C, which was even lower than the preparation temperature, PGLC polymer chain was at rubbery state, which flavored the rapid diffusion of THF and the nanoparticles formation, so yield of PGLC nanoparticles was much higher than that of PLGA or PLLA nanoparticles. While for entrapment efficiency, since

TABLE III
Summarization of Yield and 5-Fu Loading Content of Nanoparticles Prepared Under Different Temperature

Preparation temperature (°C)	PLLA nanoparticles		PLGA nanoparticles		PGLC nanoparticles	
	Yield (%)	5-Fu loading* (%)	Yield (%)	5-Fu loading* (%)	Yield (%)	5-Fu loading[a] (%))
0	20.3	27.7	41.5	21.5	53.7	20.7
10	24.3	24.2	53.7	18.4	67.5	17.6
20	30.9	21.3	65.3	12.7	94.4	12.5
30	45.7	14.3	80.7	10.3	~100	9.5
50	75.1	9.3	91.7	6.7	~100	5.4

[a] Feeding dose of 5-Fu/polymer was 30/100 (w/w).

TABLE IV
Yield, 5-Fu Loading Content and Entragment Efficiency of 5-Fu Loaded Nanoparticles Prepared by Different PGLC Concentration

PGLC concentration (mg/ml)	Mean radius (nm)	Yield (%)	Entrapment efficiency (%)	5-Fu loading[a] (%)
5	36	~100	30.5	8.4
20	68	~100	43.7	11.6
50	100	68.5	48.8	18.1

[a] Feeding dose of 5-Fu/PGLC was 30/100 (w/w).

the highest yield nanoparticles and high loading content of PGLC nanoparticles, their 5-Fu entrapment efficiency was much higher than that of PLGA and PLLA nanoparticles.

Effect of the preparation temperature on characteristics of nanoparticles

5-Fu loaded nanoparticles were prepared under different preparation temperature, and the results were listed in Table III. It could be seen that yield of various polymeric nanoparticles increased gradually with enhancement of the preparation temperature. On the other hand, 5-Fu loading content of the nanoparticles decreased greatly. At 0°C, the yield of PLLA, PLGA, and PGLC nanoparticles was 20.3, 41.5, and 53.7% respectively. However, when the temperature increased to 20°C, the yield of PGLC nanoparticles was about 95% and 5-Fu loading content was about 12.5%. Yield of the PLGA and PLLA nanoparticles also increased to about 65 and 30% respectively. With the preparation temperature increasing to 50°C, yield of PLLA and PLGA nanoparticles were reached about 71 and 92% respectively, but their 5-Fu content decreased greatly to 9.3 and 6.7% respectively.

It was obvious that with preparation temperature increasing, the diffusivity of organic solvent in the droplets was greatly improved. THF was easier to diffuse out of the polymeric droplets and resulted in the enhancement of nanoparticles' yield. On the other hand, the enhancement of temperature also improved the diffusivity of 5-Fu, so with temperature increasing, more and more 5-Fu would leak out. Besides, it was generally known that high temperature has great side effect on drugs or bioactive molecules entrapped in the nanoparticles. Therefore, it is unreasonably to increase yield of nanoparticles by enhancement of preparation temperature.

According to above experiments, it could be proved that T_g of the polymers and preparation temperature would greatly affect the yield and drug loading content of nanoparticles. Compared with PLLA or PLGA, because of low T_g, the yield of PGLC nanoparticles could be kept high even at rather low preparation temperature, which was a major advantage for PGLC as a drug carrier.

Effect of copolymer concentration on the characteristics of nanoparticles

Three PGLC concentrations in mixed solution, 5, 20, and 50 mg/mL, were chosen to prepare nanoparticles in same proportion of 5-Fu (5-Fu/PGLC = 3/10 wt/wt). Yield, 5-Fu loading content, and entrapment efficiency of PGLC nanoparticles prepared by different PGLC concentration were shown in Table IV. It was obvious that with PGLC concentration increasing, the mean radius of nanoparticles increased gradually. At same time, both 5-Fu loading content and encapsulation efficiency of nanoparticles increased gradually, but the yield of the nanoparticles decreased gradually. When PGLC concentration increased to 50 mg/mL, yield of nanoparticles was only about 70%. DLS curves of nanoparticles prepared by different PGLC concentration were shown in Figure 7. All 5-Fu loaded nanoparticles showed a unimodal size distribution, but with PGLC concentration increasing, size distribution of PGLC nanoparticles would become wider and wider, which were similar to the drug-free nanoparticles.[18]

Since higher copolymer concentration (which meant high viscosity of the polymer solution) would reduce

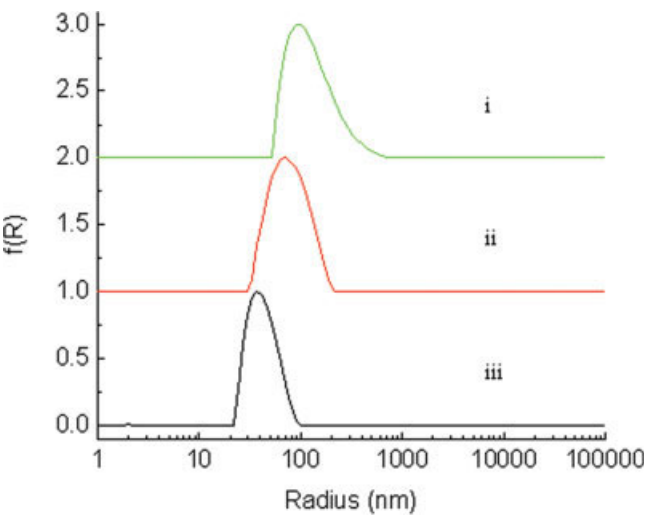


Figure 7 Size distributions of 5-Fu loaded nanoparticles prepared by different PGLC concentration. (i) 50 mg/mL; (ii) 20 mg/mL; (iii) 5 mg/mL.

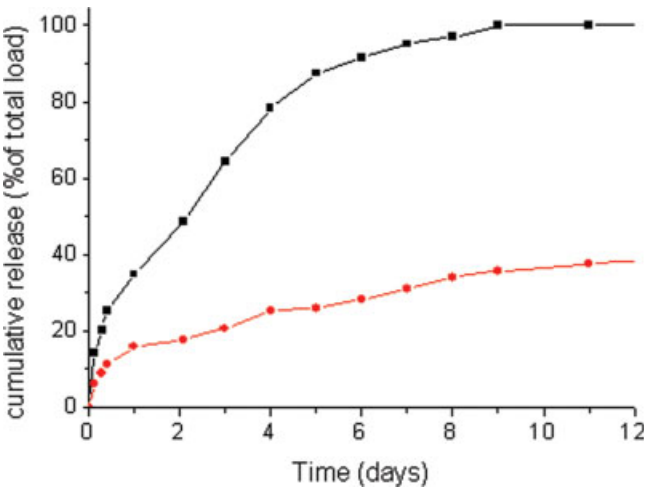


Figure 8 Comparison of 5-Fu release from 5-Fu loaded PGLC (-■-) nanoparticles and (-●-) film.

diffusion of 5-Fu into aqueous phase, and at same time, higher copolymer concentration was also favor in forming nanoparticles with larger size, both of which would retain the leakage of 5-Fu effectively.[22] So nanoparticles prepared by higher PGLC concentration would have higher drug loading content and entrapment efficiency.

In vitro drug release behaviors

Comparison of in vitro drug release behaviors between 5-Fu loaded PGLC nanoparticles and 5-Fu loaded PGLC film

5-Fu loaded PGLC film (thickness of the film was about 0.1 mm) was used as the control. Mean radius of 5-Fu loaded PGLC nanoparticles was about 68 nm. 5-Fu loading content of all the samples was adjusted to 11–12%, and 5-Fu release curves of PGLC nanoparticles and film in the release medium were shown in Figure 8. It could be seen that the release behavior of PGLC nanoparticles exhibited great differences from that of PGLC film. According to the 5-Fu release profile of PGLC nanoparticles, at the initial stage (about 0.5 day), about 25% of the total drug was released from the nanoparticles, which could assume that this portion of 5-Fu was deposited on the surface of nanoparticles, and could get access to aqueous medium rapidly. Then, the release rate slowed down until it became constant. The release time of PGLC nanoparticles lasted for about 8–9 days. On the other hand, the release rate of film was much slower than that of the nanoparticles. When all 5-Fu entrapped in PGLC nanoparticles released out, only less than 40% of total 5-Fu entrapped in film released out. It could be concluded that because of the huge surface and small size, the drug release behavior of drug-loaded nanoparticles was entirely different from traditional DDS.[15]

Effect of nanoparticles size on *in vitro* drug release behavior of 5-Fu loaded PGLC nanoparticles

5-Fu loaded PGLC nanoparticles with three different mean radii (the mean radius was 36, 68, and 100 nm respectively, and 5-Fu loading content was about 11–12%) were prepared by changing PGLC concentration and feeding content of 5-Fu in organic solution. Their release behaviors were studied and shown in Figure 9. It could be seen that all three samples displayed a rapid and sustain release behavior. Nevertheless, it could be found that nanoparticles with larger size had lower release rate. For nanoparticles with mean radius of 36 nm, the release time lasted for about 6 days, but it took about 10–11 days for all entrapped 5-Fu released from PGLC nanoparticles with the largest mean radius. It could be explained that the larger nanoparticles had bigger cores and smaller surface area, so the drug incorporated in the larger nanoparticles diffused across the polymer matrix into the aqueous medium more slowly than that of the smaller nanoparticles. So the smaller the mean radius of the nanoparticles was, the larger the surface area of the nanoparticles, the faster drug release rate of the nanoparticles was.

Effect of polymers on *in vitro* drug release behavior of 5-Fu loaded polymeric nanoparticles

5-Fu loaded nanoparticles prepared by PLLA and PLGA were used as control samples. Mean radii of all samples were 68–70 nm, and 5-Fu loading content was 11–12%. 5-Fu release curves of various nanoparticles were shown in Figure 10. It could be seen that 5-Fu release rate of three kinds nanoparticles were in

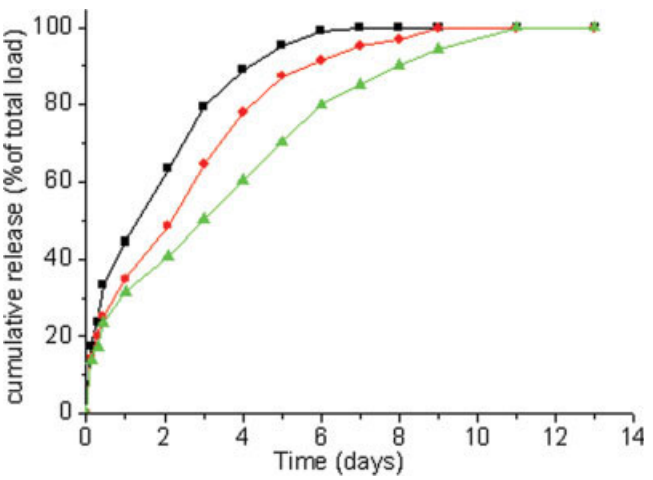


Figure 9 Comparison of 5-Fu release from different-sized PGLC nanoparticles. Mean radium: -▲- 100 nm; -●- 68 nm; -■- 36 nm.

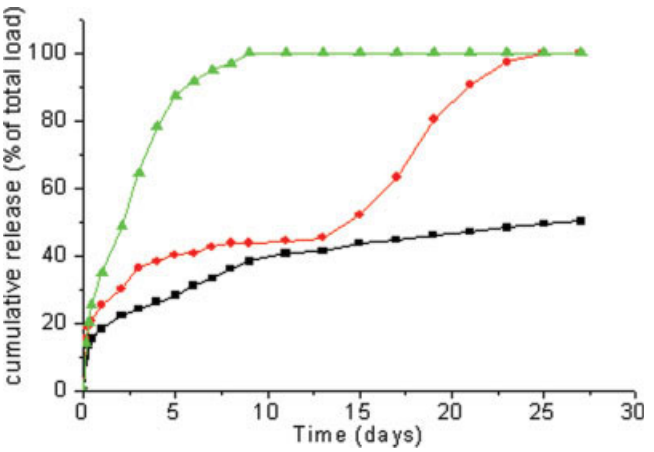


Figure 10 Comparison of 5-Fu release from different polymeric nanoparticles. -▲- 5-Fu loaded PGLC nanoparticles; -●- 5-Fu loaded PLGA nanoparticles; -■- 5-Fu loaded PLLA nanoparticles.

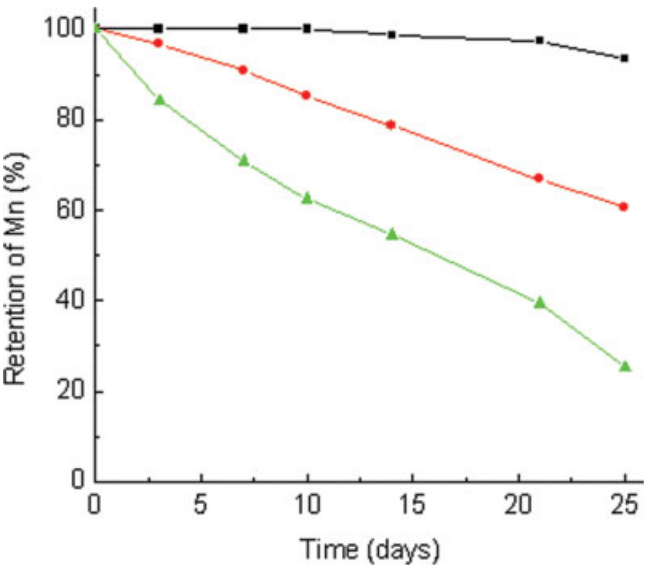


Figure 11 Dependence of molecular weight (M_n) change of various polymeric nanoparticles on degradation time. -▲- PGLC nanoparticles; -●- PLGA nanoparticles; -■- PLLA nanoparticles.

the order of PGLC > PLGA >> PLLA. During the first 0.5 day, about 25, 21, and 15% of total 5-Fu content in PGLC, PLGA and PLLA nanoparticles were released respectively. It indicated that some 5-Fu was deposited on the surface of nanoparticles, and majority of 5-Fu was entrapped into the nanoparticles. Then, three kinds nanoparticles showed entirely different release behavior. For PGLC nanoparticles, most entrapped 5-Fu was released with a rapid and constant release behavior. 5-Fu release curve of PLGA nanoparticles could be divided into three stages obviously. After burst release stage, 5-Fu was released slowly, and about 25% of 5-Fu entrapped was released out gradually during 13–14 days. Then the rest 5-Fu in PLGA nanoparticles was released with a rapid and sustaining release behavior for about 6 days. While the release rate of PLLA nanoparticles was much slower than that of PLGA or PGLC nanoparticles.

It was well known that drug release behavior of nanoparticles also depended on characteristics of the polymer used. To further explore drug release mechanism of various nanoparticles, degradation behavior of the nanoparticles was studied in an environment that was similar with the 5-Fu release environment.

M_n change of three kinds of nanoparticles was determined as shown in Figure 11. It was clear that PGLC, PLGA, and PLLA nanoparticles had different degradation rate. After degradation for 20 days, PLGA and PGLC nanoparticles lost about 35 and 60% of original M_n respectively, while PLLA nanoparticles only lost about 5% of original M_n. So it could be concluded that the degradation rate of nanoparticles was in the order of PGLC > PLGA >> PLLA.

Changes of the chemical composition of PLGA and PGLC nanoparticles in the degradation process were also detected by ^{1}H NMR and shown in Table V. It could be seen that the content of components in PLGA and PGLC nanoparticles changed gradually during the degradation process. However, GA detached more rapidly than other components because of its better hydrophilicity. Therefore, molar ratio of other components (either the LA units in PLGA or the CL and LA units in PGLC) increased gradually. Although it was very difficult to measure weight loss of the nanoparticles, according to previous research on the degradation of PGLC,[16] the changes of the chemical composition in the copolymers were caused by dissolution of the oligomers in the copolymers, which resulted in weight loss of the copolymer. So according to the previous research and present experimental results, it could be concluded that the gradual weight loss of the nanoparticles would appear during the degradation process.

Mean radius of nanoparticles during degradation process was determined by DLS as shown in Figure 12. Obviously, mean radii of three kinds nanoparticles

TABLE V
Composition Changes of Different Nanoparticles During Degradation in Release Medium

Degradation time (days)	PLGA nanoparticles [lactyl]/[glycotyl]	PGLC nanoparticles [glycotyl]/[lactyl]/[caproxyl]
0	69.0/31.0	27.5/62.5/10.0
7	69.0/31.0	26.0/63.0/11.0
14	69.8/30.2	25.2/63.5/11.3
21	70.3/29.7	25.3/63.5/11.4

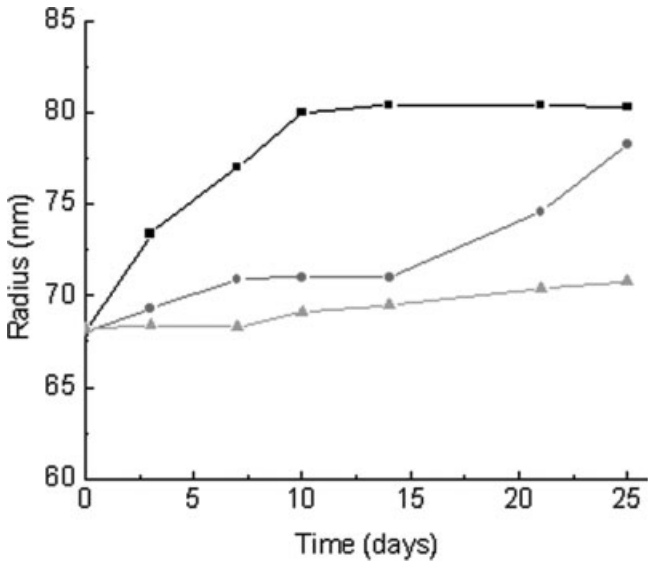


Figure 12 Dependence of size change of various polymeric nanoparticles on degradation time. -■- PGLC nanoparticles; -●- PLGA nanoparticles; -▲- PLLA nanoparticles.

changed different. For PGLC nanoparticles, the mean radius increased from 68 to 80 nm during 10 days because of the swelling of the nanoparticles. While the mean radius of PLGA nanoparticles changed slightly during the first 15 days, their mean radius increased from 68 to 78 nm during the following 10 days. For PLLA nanoparticles, their mean radius changed slightly during the whole degradation process. More interesting, it could be found there were close relationships between 5-Fu release curves (Fig. 10) and mean radius changes of the nanoparticles (Fig. 12). Briefly, with swelling of the nanoparticles, 5-Fu entrapped in the nanoparticles released rapidly. For PLGA nanoparticles, after 15 days of degradation in release medium, the whole nanoparticles began swelling, and most 5-Fu entrapped released rapidly. While the swelling of PGLC nanoparticles took place as soon as they were put into the release medium, and at same time, 5-Fu entrapped was released with a rapid and constant rate.

From above-mentioned experimental results, it could be concluded that polymeric degradation should play the main role in swelling of the nanoparticles. Briefly, M_n decreasing and weight loss of polymer made the nanoparticles become loose gradually. Thus, the water could permeate into the nanoparticles easily and resulted in the swelling of nanoparticles. It was obvious that the movement of 5-Fu became easier in such swelled nanoparticles, and could easily diffuse outside rapidly.[23] However, different nanoparticles had their own special characteristics. The amorphous state, improved hydrophilicity and low T_g (22.5°C) of PGLC would favor water diffusion into PGLC nanoparticles, and led in the rapid degradation and swelling of the nanoparticles. Therefore, obvious swelling and degradation of PGLC nanoparticles took place during the first week. So, PGLC nanoparticles showed a rapid release rate at the beginning stage. With 5-Fu release going on, although the reduction of 5-Fu concentration in carrier would slow release rate, the degradation of PGLC nanoparticles could decrease the diffusion resistance to the drug in carrier. So, the main 5-Fu release of PGLC nanoparticles kept a constant drug release rate, and the main release profile was in near zero-order release, which indicated that degradation controlled release kinetic would play an important role in the release procedure of PGLC nanoparticles.[22,24] On the other hand, PLGA was also at amorphous state, which also favored the degradation of PLGA nanoparticles. However, the high T_g (58°C) of the PLGA made its nanoparticles have glassy cores, which could prevent 5-Fu to escape from the nanoparticles and the water diffuse inside effectively at the beginning stage, and only a portion of 5-Fu deposited at the surface could get access to aqueous medium at this stage. During the original stage, the diffusion controlled release mechanism would play the main role. After 15 days of degradation, M_n decreasing and weight loss made the PLGA nanoparticles loosing and swelling, 5-Fu remained inside the PLGA nanoparticles could release quickly. At this stage, the degradation controlled release mechanism played the leading role. While for PLLA nanoparticles, because of crystallinity and low degradability, the movement of 5-Fu in PLLA nanoparticles was further limited, so 5-Fu release from PLLA nanoparticles was mainly controlled by drug diffusion just similar to the un-degradation drug carriers.

CONCLUSIONS

In this report, a kind of nanoparticle DDS was developed by using tri-component copolymer PGLC. The 5-Fu loaded PGLC nanoparticles were prepared by modified-SESM method. The obtained nanoparticles have spherical shape and mean radius is in the range from 30–100 nm. It was found that both the preparation conditions and the physical property of the copolymers influenced the nanoparticles yield, 5-Fu loading content, and encapsulation efficiency. The obtained results show, compared with PLLA or PLGA nanoparticles, yield of the PGLC nanoparticles was much higher at low preparation temperature, and their 5-Fu loading content was also high. More important, 5-Fu release behavior of PGLC nanoparticles was also studied, and it could be found that PGLC nanoparticles could realize a near zero-order release, which was different from PLLA

and PLGA nanoparticles. According to the results of degradation experiments, 5-Fu release behavior of PGLC nanoparticles was mainly determined by degradation of PGLC nanoparticles. To conclude, the PGLC nanoparticles seem to be a promising DDS.

References

1. Hou, D. Z.; Xie, C. S.; Huang, K. J.; Zhu, C. H. Biomaterials 2003, 24, 1781.
2. Gupta, A. K.; Madan, S.; Majumdar, D. K.; Maitra, A. Int J Pharm 2000, 209, 1.
3. Pignatello, R.; Bucolo, C.; Spedalieri, G.; Maltese, A.; Puglisi, G. Biomaterials 2002, 23, 3247.
4. Liu, M. X.; Dong, J.; Yang, Y. J.; Yang, X. L.; Xu, H. B. Eur J Pharm Biopharm 2005, 41, 375.
5. Soppimath, K. S.; Aminabhavi, T. M.; Kulkami, A. R.; Rudzinski, W. E. J Control Release 2001, 70, 1.
6. Labhasetwar, V. Pharm News 1997, 4, 28.
7. Kreuter, J. In Colloidal Drug Delivery Systems; Kreuter J., Ed.; Marcel Dekker: New York, 1994; p 221.
8. Gombatz, W.; Pettit, D. K. Rev Bioconjugate Chem 1995, 6, 332.
9. Berton, M.; Allemann, E.; Stein, C. A.; Gurny, R. Eur J Pharm Biopharm 1999, 9, 163.
10. Mainardes, R. M.; Evangelista, R. C. Int J Pharm 2005, 290, 137.
11. Ravi, K.; Bakowsky, U.; Lehr, C. M. Biomaterials 2004, 25, 1771.
12. Chawla, S. J.; Amiji, M. M. Int J Pharm 2002, 249, 127.
13. Ignatius, A. A.; Claes, L. E. Biomaterials 1996, 17, 831.
14. Wu, X. S. In Encyclopedic Handbook of Biomaterials and Bioengineering, Part A: Materials; Wise, D. L., Trantolo, D. J., Altobelli, D. E., Yaszemski, M. J., Gresser, J. D., Schwartz, E. R., Eds.; Marcel Dekker: New York, 1995, p 1015.
15. Cai, Q.; Bei, J. Z.; Wang, S. G. J Biomed Sci Polym Ed 2000, 11, 273.
16. Cai, Q.; Bei, J. Z.; Wang, S. G. Polym Adv Technol 2002, 13, 105.
17. Wang, S. G.; Cai, Q.; Bei, J. Z.; Shi, W. Y.; Xie, L. X. Key Eng Mater 2005, 288, 125.
18. Zhang, H. W.; Cui, W. J.; Bei, J. Z.; Wang, S. G. Polym Degrad Stabil 2006, 91, 1929.
19. Haller, D. G. Eur J Cancer 1995, 31A (7/8), 1255.
20. Murakami, H.; Kobayashi, M.; Takeuchi, H.; Kawashima, Y. Powder Technol 2000, 107, 137.
21. Wang, X. Y.; Lin, J.; Zhang, X. M.; Liu, Q.; Xu, Q.; Tan, R. X.; Guo, Z. J. J Inorg Biochem 2003, 94, 186.
22. Zhang, L. Y.; Hua, Y.; Jiang, X. Q.; Yang, C. Z.; Lu, W.; Yang, Y. H. J Control Release 2004, 96, 135.
23. Allen, C.; Maysinger, D.; Eisenberg, A. Colloids Surf B 1999, 16, 3.
24. Zuleger, S.; Lippold, B. C. Int J Pharm 2001, 217, 139.