Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biomaterials 24 (2003) 3563–3570
Biomaterials
www.elsevier.com/locate/biomaterials

Biodegradable poly(ε-caprolactone)-poly(ethylene glycol) block copolymers: characterization and their use as drug carriers for a controlled delivery system

Shaobing Zhou*, Xianmo Deng, Hua Yang

Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences, P.O. Box 415, Chengdu 610041, People's Republic of China

Received 26 September 2002; accepted 24 March 2003

Abstract

Poly(ε-caprolactone)-poly(ethylene glycol) (PECL)copolymers were synthesized from polyethylene glycol (PEG) and ε-caprolactone(ε-CL) using stannous octoate as catalyst at 160°C by bulk polymerization. The effect of the molecular weight of PEG and the copolymer ratio on the properties of the copolymers was investigated by ^{1}H-NMR, IR, DSC and GPC. PCL and PECL microspheres containing human serum albumin were elaborated by solvent extraction method based on the formation of double w/o/w emulsion. Microspheres were characterized in terms of morphology, size, loading efficiency, and the efficiency of microspheres formation. The results show that the microspheres prepared from PECL-10 and PECL-15 copolymers achieved the highest loading efficiency (about 50%) among all copolymers. These results indicate that the properties of copolymers could be tailored by adjusting polymer composition. It is suggested that these matrix polymers may be optimized as carriers in the protein (antigen) delivery system for different purposes.

Keywords: Biodegradable; Poly(ε-caprolactone); Poly(ethylene glycol); Block copolymer

1. Introduction

A few synthetic polymers, especially linear polyesters such as poly(ε-caprolactone) (PCL), polylactides (PLA), and polyglycolide (PGA) are of great interest for medical applications. In the family of polyesters, PCL occupies a unique position: it is at the same time biodegradable and miscible with a variety of polymers and it crystallizes very readily [1]. A lack of toxicity and great permeability has already found wide use for PCL in medical applications [1].

However, the rather high crystallinity of PCL decreases its compatibility with soft tissues and lowers its biodegradability. These drawbacks may obstruct its application in drug-controlled release systems. This problem might be overcome by copolymerization of ε-caprolactone (ε-CL) with other monomers. It is well known that polyether has the superior properties of nontoxicity, flexibility, hydrophilicity, and biocompatibility. Poly(ethylene glycol) (PEG) is nontoxic and cleared by the US Food and Drug Administration for internal use in the human body [2]. These properties are very useful for a polymer used as a drug delivery system. In recent years, the synthesis of polyester–polyether type block copolymer has attracted much attention, because they can be used in future medical applications in implantation and wound treatment, and as controlled-release drug carriers [3–9]. In the past, we synthesized the ABA-type block copolymers (PELA) comprising poly(D,L-lactide) (A) and poly(ethylene glycol) (B) segments obtained by ring-opening polymerization as described previously [10]. The second component PEG was widely used to improve the biocompatibility of the blood contacting materials. Our further study has confirmed that the hydrophilic domains of PELA copolymers acting as a protein stabilizer or surface modifier of hydrophobic PLA networks could promote the stability of proteins, increase the drug and protein loading efficiency and decrease the amount of emulsifier used in preparation processes [11,12].

*Corresponding author. Tel.: +86-28-852-334-80; fax: +86-28-852-239-78.
E-mail address: shaobingzhou@163.net (S. Zhou).

0142-9612/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0142-9612(03)00207-2

In recent years, Some researchers have reported the synthesis of multiblock copolymers of poly(ε-caprolactone)-poly(ethylene glycol) (PECL). Li et al. reported the synthesis of PECL multiblock copolymers with high molecular weight (M_w: above 20 000) by the polycondensation of PEG bearing two carboxylic end groups and PCL diols in the presence of dicylclohexylcarbodiimide (DCC) as a condensing agent [13,14]. Zhu and coworkers reported the synthesis of PECL triblock copolymers covering a wide range of PEG lengths via anionic polymerization of ε-CL with alkali metal alkoxide derivatives of PEG [15]. Bogdanov et al. reported the synthesis of PECL copolymer by ring-opening polymerization of ε-CL and PEG using stannous octoate as catalyst [16].

In this paper, we successfully synthesized a series of PECL block copolymers with different content and M_w of PEG, such as 6, 4, 2, 1.5 and 1.0 kDa. We characterized the PECL block copolymers by ^{1}H-NMR, IR, GPC, and DSC measurements, etc. Few studies reported the PECL microspheres as a carrier in drug-controlled release system. In the study, we successfully prepared different kinds of PECL microspheres containing human serum albumin (HSA) by a double emulsion w/o/w based on the solvent extraction methods. The microsphere size and distribution, and surface morphology, and the amount of encapsulated HSA (AE) in microspheres and the HSA encapsulation efficiency (EE) of the process and so on, were investigated.

2. Materials and methods

2.1. Materials

PEG ($M_w = 6$ kDa) and polyvinyl alcohol (PVA, 88% hydrolyzed, $M_w = 130$ kDa) were purchased from Guangzhou Chemical Reagents Department. PEG with 4 kDa was purchased from Guangzhou Medicine Station Chemical Reagents Company. PEG with 2, 1.5, and 1.0 kDa were produced by Gaonan Chemical Factory of Shanghai Pudong. All these PEG monomers were purified by precipitation from chloroform solution into ether and dried to constant weight under vacuum at 30°C before use.

ε-CL(from Aldrich) was dried over calcium hydride for 48 h at room temperature and then distilled under reduced pressure prior to polymerization. Stannous octoate (SnOct) (from Sigma Corp.) was used as received.

HSA was purchased from Institute of Blood-Transfusion, Chinese Academy of Medical Science. All other chemicals and solvents were of reagent grade or better.

2.2. Polymerization procedure

The reactants were prepared by introducing under a nitrogen atmosphere a known volume of caprolactone monomer into a glass ampoule previously dried containing a pre-weighted amount of PEG. One drop of SnOct was added. The ampoule was connected to a vacuum line, evacuated, sealed off and placed in oil bath at 160°C. A slow and progressive viscosity increase of the bulk homogeneous mixture was always observed during the polymerization and the copolymers was achieved over 4 h. After cooling at room temperature, the ampoule was opened, and the resulting block copolymers were dissolved in methylene chloride and precipitated in excess of cold methanol/hexane. They were dried at 40°C under vacuum.

2.3. Characterization of the block copolymers

^{1}H-NMR spectra were obtained with $CDCl_3$ as solvent and TMS as internal standard, using a Bruker AM 300 apparatus at 25°C. The actual PEG content of PECL was calculated from the integral height of hydrogen shown in ^{1}H-NMR. IR spectra were recorded on a Nicolet MX-1 IR spectrometer. The samples were prepared by casting films from acetone solution onto KBr plates. Average M_w and its distribution were determined by gel permeation chromatography (GPC, waters ALC/GPC 244,USA) operating with THF and calibrated with polystyrene standards. Intrinsic viscosity was measured with an Ubbelohde viscometer on 0.2%(g/dl) solution of polymer at 30°C in benzene, and the molecular weight (Mv) was calculated using the equation [17]: $[\eta] = 9.94 \times 10^{-5} Mv^{0.82}$. The transition temperatures and melting temperatures of polymers were measured by DSC (SEIKO EXSTAR6000) under a flow of nitrogen at a scanning rate of 10°C/min. The thermograms covered from –80 to 80°C.

2.4. Preparation of HSA-loaded polymer microspheres

PECL and PCL microspheres containing HSA were prepared by the solvent extraction based on the formation of a modified double emulsion $w_1/o/w_2$ reported previously [18]. Briefly, the w_1 phase, containing an aqueous solution of HSA protein was dispersed into the organic phase consisting of the polymer dissolved in dichloromethane and ethyl acetate (40.0 mg/ml), using a high speed stirrer for 60 s at room temperature. The primary water-in-oil emulsion was immediately added to the external aqueous phase (100 ml of 2.0% PVA solution) and further emulsified again by a high-speed homogenizer.

The organic solvent was extracted by adding 100 ml of 6% isopropanol and the mixture was stirred at a moderate speed at ambient temperature for 3 h. After complete removal of organic solvent, the microspheres were collected by centrifugation (Tomy Seiko Co., Japan). The resultant microspheres were rinsed with distilled water and centrifuged three more times, then lyophilized overnight and stored at 4°C.

2.5. Characterization of microspheres

The microsphere size and distribution were determined with a laser diffraction particle size analyzer (MALVEN, MASTERSIZER 2000, British). The microspheres were evaluated for surface morphology by scanning electron microscopy (SEM, Amray) and the efficiency of microspheres forming (EMF) was obtained by counting regular spherical microspheres of at least 200 particles shown in SEM.

The amount of HSA entrapment was measured by placing 100 mg of microspheres in 1.5 ml of dichloromethane and extracting the HSA three times with 1.5 ml of double distilled water. The amount of HSA protein was measured by the Bradford's protein assay as described previously [19]. The HSA content of the extraction solution was determined, compared with a standard curve of data obtained by assaying known concentrations of HSA solutions. The amount of encapsulated HSA (AE) in microspheres, given as a percentage, indicates the amount (mg) of HSA encapsulated per 100 mg of microspheres. And the encapsulation efficiency (EE) of the process indicates the percentage of HSA encapsulated with respect to the total amount used for the preparation of microspheres.

3. Results and discussion

3.1. Characterization of PECL copolymers

PECL copolymer was synthesized and the structure of copolymer had been characterized in a previous study [15]. The homopolymer of PCL and copolymers of ε-CL and PEG were polymerized in bulk at 160°C using stannous octoate as the catalyst. Triblock copolymers (ABA) are formed by ring opening polymerization of ε-CL with biofunctional HO–PEG–OH.

IR and proton NMR spectra for a typical PECL copolymer with a 10% content and M_w 2.0 kDa of PEG are shown in Figs. 1 and 2, respectively. It is clearly seen in Fig. 1 that the PECL copolymer exhibits peaks characteristic of both PEG and PCL. The absorption band at 1729 cm^{-1} is attributed to the C=O stretching vibrations of the ester carbonyl group. The absorption bands at 1177 and 1240 cm^{-1} are attributed to the characteristic C–O–C stretching vibrations of the repeated $–OCH_2CH_2$ units of PEG and the –COO-bonds stretching vibrations, respectively. The absorption band at 3442 cm^{-1} is assigned to terminal hydroxy groups in the copolymer from which PEG homopolymer has been removed. All the C–H stretching bonds are centered at 2946 and 2867 cm^{-1}. All these signals indicate that the PECL block copolymer may be formed [15].

In order to further confirm the formation of block copolymer, an ^{1}H-NMR spectrum is made and shown in Fig. 2. Peaks at 1.42, 1.62, 2.34, and 4.09 ppm are assigned to methylene protons of $–(CH_2)_3–$, $–OCCH_2–$, and $–CH_2OOC–$ in PCL units, respectively. The sharp single peak at 3.66 ppm is attributed to the methylene protons of homosequences of the PEG oxyethylene units. The very weak peak at 4.3 ppm is attributed to the

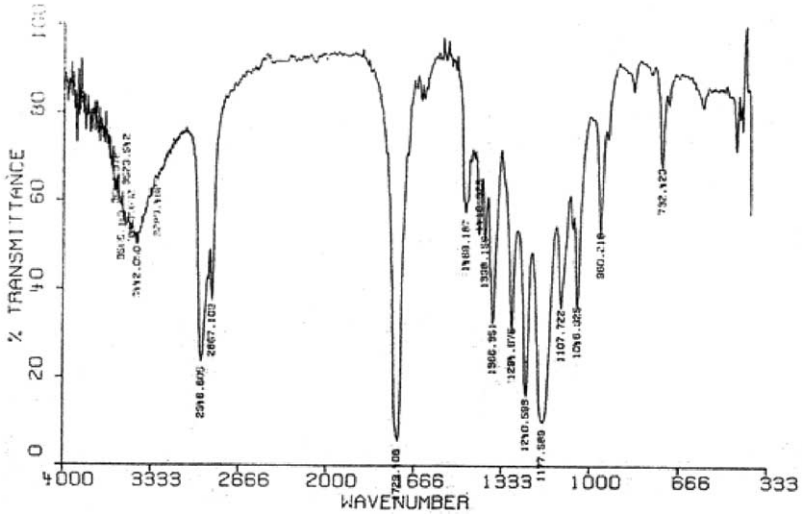

Fig. 1. FTIR spectrum of PECL-3 copolymer.

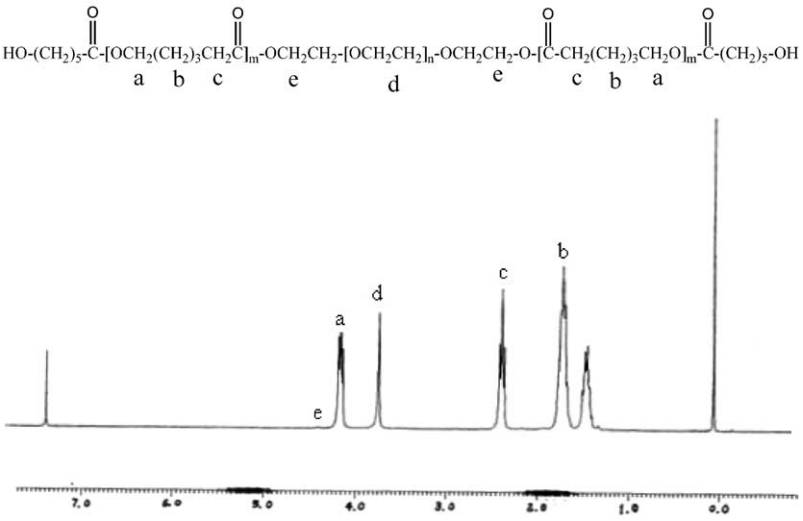

Fig. 2. ^{1}H-NMR spectrum of PECL-3 copolymer.

Table 1
Polymerization of ε-CL with different ratio of PEG (M_w = 60 kDa), and characterization of copolymers[a]

Sample no.	Feed (PEG wt%)	Calculated(NMR) (PEG wt%)[b]	Mv[c] ($\times 10^{-4}$)	$M_{n,NMR}$[d] ($\times 10^{-4}$)	$M_{n,GPC}$[e] ($\times 10^{-4}$)	M_w/M_n
PCL	0	0	9.96	—	17.8	1.22
PECL-5	5.0	4.2	7.67	14.2	16.5	1.26
PECL-10	10.0	9.4	3.87	7.0	8.4	1.37
PECL-15	15.0	13.8	3.04	4.4	5.2	1.29
PECL-30	30.0	26.5	2.61	2.9	4.0	1.41
PECL-50	50.0	43.2	1.23	1.4	2.2	1.36

[a] Reaction was carried out at 160°C in the presence of $Sn(Oct)_2$ in bulk.
[b] Estimated from the integral height of hydrogen shown in ^{1}H-NMR Spectrum.
[c] $[\eta] = 9.94 \times 10^{-5} Mv^{0.82}$ (at 30°C in benzene).
[d] Number-average molecular weight calculated from ^{1}H-NMR Spectrum.
[e] Number-average molecular weight measured by GPC (calibrated with polystyrene standards).

methylene proton of PEG end unit. These results are consistent with those obtained from PECL triblock copolymers prepared by the ring opening polymerization of ε-CL with alkali metal alkoxide derivatives of poly(ethylene glycol) [15]. The M_w of the PECL copolymers and the PEG/PCL block ratios in the triblock copolymers were all calculated by comparing CL methylene (4.09 ppm) and PEG methylene (3.66 ppm) integrations as reported previously [15,20]. The PEG contents estimated from ^{1}H-NMR spectrum and the amount added before polymerization are compared in Tables 1 and 2, which indicate that they are almost identical. It can be seen from Tables 1 and 2 that both the amount and M_w of PEG reacted with ε-CL monomers have a great effect on the M_w of the resulting PECL copolymers. Similarly, it was found that the M_w's of copolymers increased with the increase of the M_w's of PEG from 1000 to 6000. The result is consistent with conclusions from a previous study [21]. It also may be proposed that initiators with high M_w have less activity than those with low M_w. Thus, it is possible to control the M_w and composition of copolymers simply by changing the PEG M_w and the ratio of monomer to PEG. It is very significant to design polymer for biomedical applications.

The copolymers had narrrow M_w distributions with M_w/M_n ratios varying from 1.22 to 1.51 (Tables 1 and 2). From GPC curve in Fig. 3, it can be seen that only a single peak exists, which suggests the monodispersion of M_w and the absence of any homopolymer of CL and PEG monomers. These indicate that no transesterification and/or backbiting reactions occurred during the copolymerization [22].

In order to check the dependence of the thermal behaviour of the copolymers on their composition, DSC measurements were employed. The glass transition (T_g), and the melting temperatures (T_m) of PECL copolymers and PCL homopolymer are collected in Table 3. Thermograms of copolymers with increasing M_w and increasing content of PEG units together with a

Table 2
Polymerization of ε-CL with different M_w of PEG (wt% = 10%), and characterization of copolymers[a]

Sample no.	M_w of PEG	Feed (PEG wt%)	Calculated (NMR) (PEG wt%)[b]	Mv[c] ($\times 10^{-4}$)	$M_{n,NMR}$[d] ($\times 10^{-4}$)	$M_{n,GPC}$[e] ($\times 10^{-4}$)	M_w/M_n
PECL-10	6000	10.0	9.4	3.87	7.0	8.4	1.37
PECL-1	4000	10.0	9.2	2.81	4.3	5.1	1.41
PECL-2	2000	10.0	8.8	1.20	2.2	2.9	1.38
PECL-3	1500	10.0	8.1	0.85	1.8	2.6	1.51
PECL-4	1000	10.0	8.3	0.63	1.2	1.5	1.42

[a] Reaction was carried out at 160°C in the presence of $Sn(Oct)_2$ in bulk.
[b] Estimated from the integral height of hydrogen shown in ^{1}H-NMR Spectrum.
[c] $[\eta] = 9.94 \times 10^{-5} Mv^{0.82}$ (at 30°C in benzene).
[d] Number-average molecular weight calculated from ^{1}H-NMR Spectrum.
[e] Number-average molecular weight measured by GPC (calibrated with polystyrene standards).

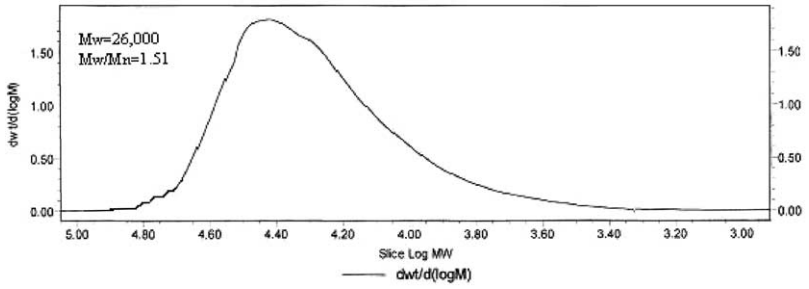

Fig. 3. CPC curve of PECL-3 copolymer.

Table 3
The glass transition and melting temperatures of PECL copolymers and PCL homopolymer

Sample no.	T_g[a] (°C)	$T_{m,PEG}$[b] (°C)	$T_{m,PCL}$[c] (°C)
PCL	−63.0	—	62.5
PECL-5	−62.1	—	62.0
PECL-10	−61.8	28.9	61.0
PECL-15	−60.0	29.5	59.5
PECL-30	−59.9	31.5	56.2
PECL-50	—	37.0	54.4
PECL-1	−62.0	31.7	62.1
PECL-2	−61.0	30.0	63.0
PECL-3	−58.9	28.6	59.7
PECL-4	−56.5	24.5	55.6

[a] Glass transition temperature (T_g) for PCL and PECL copolymers.
[b] Peak melting temperatures ($T_{m,PEG}$) for PEG units in PECL copolymers.
[c] Peak melting temperatures ($T_{m,PCL}$) for PCL units in PECL copolymers.

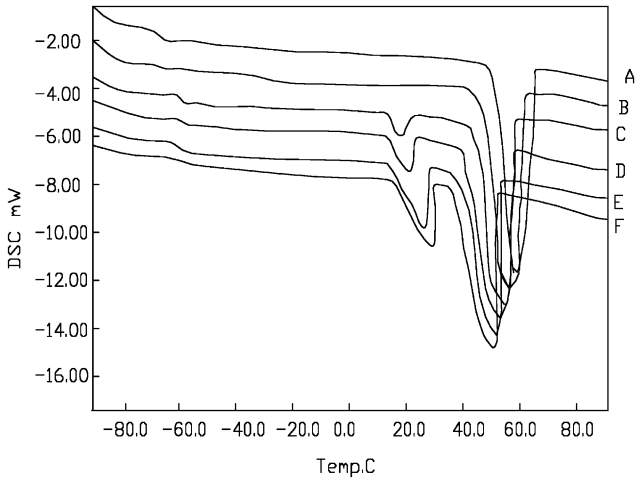

Fig. 4. DSC curves of PCL homopolymer and PECL copolymers: (A) PCL, (B) PECL-5, (C) PECL-10, (D) PECL-15, (E) PECL-30, (F) PECL-50. The experimental conditions were the same as those in Table 3.

reference mixture of ε-CL units and PCL homopolymer are shown in Figs. 4 and 5, respectively. It can be seen from Table 3 that the T_g's of all copolymers are shifted slightly to higher temperature in comparison to the T_g of PCL homopolymer. Furthermore, T_g's tend to decrease with increasing PCL/PEG ratios. The result resembles the conclusion reported previously [23]. This is mainly due to the increase in mobility of ε-CL units in the copolymer chain, in which the five methylene groups act as soft segments. From Table 3, we also can see that T_g's increase slightly with decreasing M_w of PEG units in copolymers. The reason was very complex. It may be that the low M_w of PEG unit in PECL copolymer can increase pliancy of polymer chain more easily than the

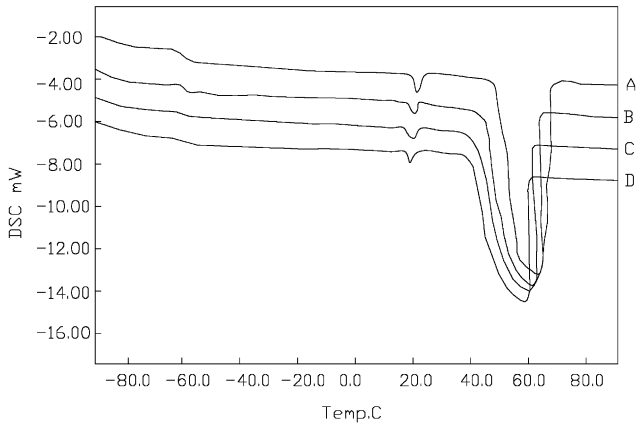

Fig. 5. DSC curves of PECL copolymers: (A) PECL-1, (B) PECL-2, (C) PECL-3, (D) PECL-4. The experimental conditions were the same as those in Table 3.

high M_w of PEG units. It can also be seen from Table 3 that $T_{m,PEG}$'s for PEG units in PECL copolymers increase and work up to the T_m of crystalline PEG homopolymer of comparable molecular mass, and $T_{m,PCL}$'s for PCL units in PECL copolymers decrease with decreasing PCL/PEG ratios, thus indicating a melting-point depression. The result is in very good agreement with previous study [24]. The observed melting depression of PEG units may be explained in the following way. The sequence which crystallizes first, namely PCL unit, has a tendency to freeze the following whole structure, thus imposing either imperfect crystallization or smaller crystallites onto the PEG unit. With decreasing M_w of PEG, both $T_{m,PEG}$'s and $T_{m,PCL}$'s decrease. It is well known that M_w of polymer has a great effect on its melting temperature. This is mainly due to the decrease of M_w for each unit.

Fig. 4 illustrates the DSC patterns for PCL homopolymer and PECL copolymers with changing PCL/PEG ratios. All the DSC curves display only one T_g (about -60°C) being close to T_g of PCL homopolymer, which probably could prove the immiscibility of the PEG and PCL constituents. The T_g's of copolymers are shifted slightly to higher temperature in comparison to the T_g of PCL homopolymer. The DSC curves exhibit two endotherms, and the T_m's are listed in Table 3. The higher temperature endotherm from 54°C to 64°C can be attributed to melting of the PCL crystal phase. The low temperature peaks in the range of 20–40°C correspond to the melting of the PEG crystal phase. It can be seen from Fig. 4 that the melting peaks and melting temperatures of PEG units tend to turn strong with increasing content of PEG units in copolymers. However, the melting temperatures of PCL units are shifted slightly to lower temperature with increasing content of PEG units in copolymers. The reason is in agreement with those reported previously [20]. It may be

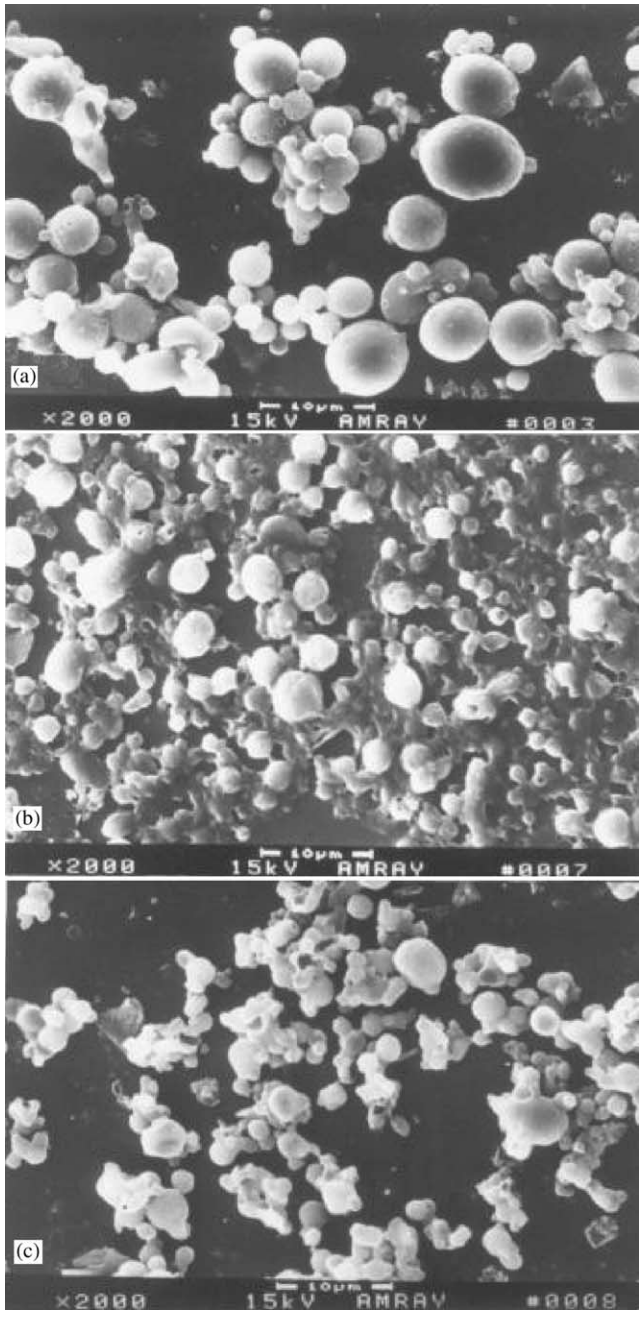

Fig. 6. The dispersion pattern and morphology determined by scanning electron microscope of (a) HSA/PECL-10, (b) HSA/PECL-30, (c) HSA/PECL-3 microspheres.

that the series with high PEG content forms the crystalline structure of the dominant PEG block, which prevents crystallization of the PCL block.

Fig. 5 exhibits the DSC curves for PECL copolymers of increasing M_w of PEG units in copolymers. The T_g's and T_m's are listed in Table 3. All the DSC curves show only one glass transition temperature and two endotherms. Their analyses are the same as those obtained from Fig. 4. The T_m's of PEG units and PCL units are influenced by M_w of PEG units. It is well known that the

Table 4
The characteristics of PECL and PCL microspheres

Sample no.	Mean diameter (μm)	Size range (μm)	HSA entrapment (%)	Loading efficiency (%)	EMF (%)
PCL	5.4	0.5–12	25.8	0.42	90
PECL-5	4.2	0.5–10	35.7	0.56	92
PECL-10	2.0	0.5–8	48.9	0.78	97
PECL-15	1.6	0.2–8	50.1	0.86	97
PECL-30	1.1	0.1–5	42.6	0.67	90
PECL-50	0.5	0.05–3	39.8	0.60	85
PECL-1	2.0	0.3–8	45.2	0.74	91
PECL-2	1.8	0.3–8	40.9	0.63	81
PECL-3	1.9	0.3–8	37.8	0.58	78
PECL-4	1.7	0.2–8	35.4	0.55	70

melting temperature falls with decreasing M_w of PEG. So it results in T_m's decreasing of PEG units and PCL units in copolymers.

3.2. Characterization of microspheres

The PECL copolymers and PCL hopolymer microspheres containing HSA were prepared by a double emulsion $w_1/o/w_2$ based on the solvent evaporation method under the same condition. The surface morphology of all microspheres was determined by SEM. The scanning electronic micrograph of PECL microspheres containing HSA is shown in Fig. 6. The AE, EE, EMF, the mean diameter and their size distribution of PCL and PECL microspheres containing HSA are summarized in Table 4. Fig. 6a shows that PECL-10 microspheres containing HSA had smooth spherical surface structure and there was no evidence of collapsing. Fig. 6b displays that PELA-30 microspheres containing HSA appear to adhere to each other. This may be as a result of the hydrophilicity of PECL copolymers increasing with increasing content of PEG units in copolymers. Fig. 6c exhibits that PECL-3 microspheres containing HSA appear to be pitted and some irregular shapes are mingled with them. This may be due to the M_w of PECL which greatly affects the quality of the formed microspheres under the experimental conditions. The copolymer with higher M_w is more beneficial to form solid microspheres than that with lower M_w during the solvent evaporation process. As seen from Table 4, the results indicate that only PECL-10 and PECL-15 had good EMF (>95%) compared to PCL and other PECL microspheres that the M_w and ratios of PCL/PEG of copolymers have a great effect on the EMF. Except for PCL and PECL-5 microspheres, the size of PECL microspheres containing HSA was less than 2.0 μm, which is critical for microspheres to reach the immunization-related tissues following oral administration [25]. Furthermore, the size of the microspheres of copolymers tends to decrease with increasing content of PEG units. Nanoparticls could be prepared on the basis of PECL-50 copolymer. This may be as a result of the improvement of PECL hydrophilicity. As seen from Table 4, all the PECL microspheres resulted in high HSA loading efficiency compared with PCL microspheres. It may be a result of the existence of certain amount of hydrophilic PEG segments in the polymers chains, which improved the affinity of polymer with protein molecules. The microspheres prepared from PECL-10 and PECL-15 copolymers achieved the highest EE (about 50%) among all the copolymers. This may be on account of the hydrophilicity and the M_w of PECL that greatly affects the quality of the formed microspheres under the experimental conditions. The copolymer with higher M_w is more beneficial to form solid microspheres with regular shape than that with lower M_w during the solvent evaporation process. It is beneficial to improve the EE of water-soluble protein for polymers with appropriate hydrophilicity [11,12,26]. From the above-mentioned results, we could draw a conclusion that PECL-10 and PECL-15 microspheres containing HSA would act as a suitable antigen delivery system with appropriate particle size (about 2.0 μm), particle size distribution (0.5–8 μm) and high protein loading efficiency.

4. Conclusions

In conclusion, a series of PECL block copolymers were synthesized by ring opening polymerization. The M_w of PEG and the ratios of PCL/PEG had a great effect on the M_w and composition of the resulting copolymers. PCL and PECL microspheres containing HSA were prepared by a double emulsion w/o/w based on solvent extraction methods. All PECL copolymers could form microspheres containing HSA. Furthermore, all the PECL microspheres achieved high HSA loading efficiency compared with PCL microspheres. These materials might be used as drug delivery carriers in

medical applications. The properties of HSA-loaded microspheres were influenced by the M_w and hydrophilicity of polymer. However, it is clear that more detailed investigations are necessary to clarify the effect of polymer matrix on protein stability and antigen immunogenicity during microspheres preparation and antigen releasing procedure, the in vitro degradation mechanism and influence factors and drug release profiles.

Acknowledgements

This work was supported by the National Science Foundation of China (grant number 20004009) and the National 973 project.

References

[1] Hiljanen-vainio M, Karjalainen T, Seppala. Biodegradable lactone copolymers. 1. characterization and mechanical behavior of ε-caprolactone and lactide copolymers. J Appl Polym Sci 1996; 59:1281–8.
[2] Zhu KJ, Lin XZ, Yang SL. Preparation, characterization and properties of polylactide-poly(ethylene glycol) copolymers: a potential drug carrier. J Appl Polym Sci 1990;39:1–9.
[3] Berglund F. Renal excretion and volume of istribution of poly(ethylene glycol)(PEG)in the rat and the dog. Acta Physiol Scand 1968;74:20A–1A.
[4] Marcotte N, Polk A. Goosen. Kinetics of protein diffusion from a poly(D,L-lactide)reservoir. Pharm J Sci 1990;79:407–10.
[5] Suh H, Jeong BM, Rathi R, Kim SW. Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly(ethylene oxide)-poly(lactide/glycolide)nanospheres. Biomed J Mater Res 1998;42(2):331–8.
[6] Jeong BM, Bae YH, Lee DS, Kim SW. Biodegradable block copolymers as injectable druf-delivery system. Nature 1997; 388:860–2.
[7] Jeong BM, Lee DS, Shon JI, Bae YH, Kim SW. Thermoreversible gelation of poly(ethylene oxide)bildegradable polyester block copolymers. Polym J Sci A 1999;37:751–60.
[8] Jeong BM, Bac YH, Kim SW. Thermoreversible gelation of PEG–PLGA–PEG triblock copolymer aqueous solutions. Macromolecules 1999;32:7064–9.
[9] Choi SW, Choi SY, Jeong BM, Kim SW, Lee DS. Thermoreversible gelation of poly(ethylene oxide) biodegradable polyester block copolymers. J Polym Sci A 1999;37(14):2305–17.
[10] Deng XM, Xiong CD, Cheng LM. Synthesis and characterization of block copolymer from lactide and poly(ethylene glycol). J Polym Lett 1990;28:411–6.
[11] Deng XM, Li XH, Yuan ML, et al. Optimization of preparative conditions for poly DL-lactide-polyethylene glycol microspheres with entrapped *Vibrio cholera* antigens. J Control Release 1999;58:123–31.
[12] Deng XM, Zhou SB, Li XH, Zhao J, Yuan ML. In vitro degradation and release profiles for poly-dl-lactide-poly(ethylene glycol) microspheres containing human serum albumin. J Control Release 2001;71:165–73.
[13] Ei SM, Garreau H, Vert M, Petrova T, Manolova N, Rashkov I. Hydrolytic degradation of poly(oxyethylene)-poly(caprolactone) multiblock copolymers. J Appl Polym Sci 1998;68:989–98.
[14] Petrova T, Manolova N, Rashkov I, Li S, Vert M. Synthesis and characterization of poly(oxyethylene)-poly(caprolactone) multiblock copolymers. Polym Int 1998;43:419–26.
[15] Zhu ZX, Xiong CD, Zhang LL, Deng XM. Synthesis and characterization od poly(ε-caprolactone)-poly(ethylene glycol) block copolymer. J Polym Sci A 1997;35:709–14.
[16] Bogdanov B, Vidts A, Bulcke VD, Verbeeck R, Schacht E. Synthesis and thermal properties of poly(ethylene glycol)-poly(ε-caprolactone) copolymers. Polymer 1998;39:1631–6.
[17] Lundberg RD, Koleske JV, Wischmann KB. Lactone polymers. III. Polymerization of ε-caprolactone. J Polym Sci 1969;7: 2915–30.
[18] Ogawa Y, Yamamoto M, Okada H, Yashiki T, Shimamoto T. A new technique to efficiently entrap leuprolide cetate into microcapsules of polylactic acid or copoly(lactic/glycolic)acid. Chem Pharm Bull 1988;36:1095–103.
[19] Braford M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
[20] Lee JW, Hua FJ, Lee DS. Thermoreversible gelation of biodegradable poly(ε-caprolactone) and poly(ethylene glycol) multiblock copolymers in aqueous solution. J Control Release 2001;73:315–27.
[21] Zhu ZX, Xiong CD, Zhang LL, Yuan ML, Deng XM. Preparation of biodegradable polylactide-poly(ethylene glycol) copolymer by lactide reacted poly(ethylene glycol). Eur Polym J 1999;35:1821–8.
[22] Peter J, Ester M, Peter V, Jennie H, Peter J, Jan F. Melt block copolymerization of ε-caprolactone and lactide. J Polym Sci A 1997;35:219–26.
[23] Fukuzaki H, Yoshida M, Asano M, Kumakura M. Synthesis of low-molecular-weight copoly(l-lactic acid/ε-caprolactone) by direct copolycondensation in the absence of catalysts, and enzymatic degradation of the polymers. Polymer 1990;31:2006–14.
[24] Cerrai P, Tricoli M, Andruzzi F, Paci M. Polyether–polyester block copolymer by non-catalysed polymerization of ε-caprolactone with poly(ethylene glycol). Polymer 1989;30:338–43.
[25] Singh M, Singh O, Talwar GP. Biodegradable delivery system for a birth control vacvcine: immunologyenicity studies in rats and monkeys. Pharmacol Res 1995;12:1796.
[26] Li XH, Zhang YH, Yuan RH, Deng XM. Influence of process parameters on the protein stability encapsulated in poly dl-lactide-poly(ethylene glycol)microspheres. J Control Release 2000;68: 41–52.