Polymer Bulletin 59, 117–125 (2007)
DOI 10.1007/s00289-007-0747-z

Polymer Bulletin

Preparation of carbon dioxide/propylene oxide/ε-caprolactone copolymers and their drug release behaviors

Dongming Peng, Kelong Huang (✉), Yanfei Liu, Suqin Liu, Hong Wu, Han Xiao

College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, China
E-mail: kelonghuang@yahoo.com.cn; Fax: +86-731-8879850

Received: 27 December 2006 / Revised version: 12 February 2007 / Accepted: 22 February 2007
Published online: 9 March 2007 –

Summary

Poly (propylene-*ram*-ε-caprolactone carbonate) (PPCL) and poly (propylene carbonate) (PPC) were synthesized by ring-opening copolymerization from carbon dioxide, propylene oxide (PO) and ε-caprolactone (CL) using a polymer-supported bimetallic complexes (PBM) as catalyst. PPC and PPCL microspheres containing a 5-alpha reductase inhibitor, finasteride were elaborated by a conventional oil-in-water (O/W) emulsion-solvent evaporation method. The effects of polymer used on microspheres morphology, size, drug loading, encapsulation efficiency and drug release behaviors were examined. *In vitro* drug release of these microcapsules was performed in a pH 7.4 phosphate-buffered solution. A prolonged *in vitro* drug release profile was observed. The release profiles of finasteride from PPC and PPCL microcapsules were found to occur with a burst release followed by a gradual release phase. Drug release rates were dependent upon the properties of the polymer in the microspheres, the higher hydrolytic activity of polymer provided faster release rate.

Keywords

polycarbonates; microspheres; drug delievery systems

Introduction

In recent years, biodegradable polymers have found increasing applications in the pharmaceutical industry as matrices for drug delivery systems [1]. Controlled-release microspheres prepared from biodegradable polymers have been extensively investigated. Such delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity and improved patient compliance and convenience [2]. Aliphatic polycarbonate represents one family of biodegradable materials used for biomedical applications such as drug carriers and implant materials because of their good biocompatibility, low toxicity, and biodegradability [3-4]. Recently, aliphatic polycarbonates have been explored in the search and design of new polyester-related structures for medical applications [5].

In order to improve the thermal properties and degradability of polycarbonates, the third monomer such as γ-butyrolactone [6], ε-caprolactone [7] and maleic anhydride [8] was introduced into the copolymerization of CO_2 and epoxides. Poly-ε-caprolactone (PCL) is a biodegradable, biocompatible and semicrystalline polymer with slow degradation. This has led to its application in the preparation of long-term delivery systems in the form of microspheres, nanospheres and implants [9]. PCL has been extensively investigated for use as implantable or injectable biodegradable carriers for the controlled release of active agents [10]. However, applications of PCL may be limited for its hydrophobic character and high crystallinity [11]. This problem may be overcome by copolymerization of ε-caprolactone with other monomers [12-13], and thus they may find much wider applications [14].

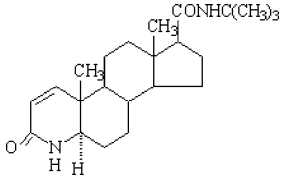
Finasteride is a 5-alpha reductase inhibitors, is recognized as a successful therapy for benign prostatic hyperplasia (BPH) [15], hair loss in men (androgenetic alopecia) and the most common cause of hair loss [16]. In addition, finasteride is also used in the prevention of prostate cancer [17]. However, patients with benign prostatic hyperplasia need a long-term treatment with finasteride at least 6 months [18], the possible side effects may exist [19]. The use of extended release products offers potential advantages like sustained blood levels, attenuation of adverse effects and improved patient compliance. Hence, its formulation in controlled release form is important in pharmaceutical study.

In the present study, four kinds of PPC and PPCL copolymers were synthesized and developed as drug carrier materials, finasteride was used as a hydrophobic model drug, and the different kinds of microspheres were prepared by the O/W emulsion-solvent evaporation method. Their *in vitro* drug release profile was performed in a pH 7.4 phosphate-buffered solution.

Materials and methods

Materials

The gift sample of finasteride was supplied by Shanghai Asia Pioneer Prarmaceutical Co., Ltd, China, used as received, and the structure of finasteride is shown in Scheme 1. ε-Caprolactone was purchased from Daicel and distilled before use. Carbon dioxide (purity more than 99.5%) was purchased from Hunan Special Gas Factory (China). The other reagents and solvents were analytical-grade and were used as received.



Scheme 1. Chemical structure of finasteride

Synthesis of copolymers

In this study, copolymers PPC and PPCL were produced by copolymerization of carbon dioxide, propylene oxide and ε-caprolactone using a polymer-supported bimetallic complexes (PBM) as catalyst as described previously [7]. Briefly, required

amount of PBM catalyst, toluene, propylene oxide (PO), and CL were added into an FYX-0.3 stainless steel autoclave in the absence of oxygen. The molar ratios of PO to CL were 3:0, 3:1, 3:2, and 3:3 (for PPC, PPCL31, PPCL32 and PPCL33, respectively). The autoclave was then pressurized to ±4 MPa with a CO_2 cylinder. The reaction mixture was stirred magnetically at 60°C for 24 h. When the reaction was finished, the resulting viscous mixture was removed, washed and dried. Finally it was purified, and dried till constant weight. Their characterization was also detailed in our earlier work [7]. ^{1}HNMR spectra were recorded on a Varian Inova-400 spectrometer with $CDCl_3$ as solvent, and the molar fractions of CO_2, PO and CL were calculated by integrating area. The glass transition temperature (T_g) of the copolymers was determined by different scanning calorimetry (DSC) in a TA DSC-Q10. The temperature range was from -20°C to 100°C and the heating rate was 10°C/min in nitrogen atmosphere. Intrinsic viscosity [η] measurements were carried out in benzene at 35°C using an Ubbelohde viscometer. The molecular weight of copolymers was determined by gel permeation chromatography (GPC, Waters 1515/2414, USA) operating with chloroform and calibrated with polystyrene standards.

Hydrolytic degradation of copolymers

The hydrolysis tests were performed as described previously [7]. Briefly, the films of copolymers were prepared by the cast method, and after dried, the films were immersed in vials filled with a phosphate-buffered saline solution (PBS, 0.1M, pH 7.4) at 37°C for predetermined time. The degradability of the copolymers was determined through total weight loss of the films over a certain time.

Preparation of microspheres

PPC, PPCL31, PPCL32 and PPCL33 were used as drug carriers, and finasteride was used as a model drug. The oil-in-water emulsion evaporation technique was applied to the fabrication of hydrophobic drug loaded microspheres. Briefly, 0.15 g finasteride was dispersed in 10 mL of a 5 wt % polymer CH_2Cl_2 solution by sonication. The organic phase was then emulsified with agitation in an aqueous phase consisting of 200mL of a 0.2% (w/v) solution of polyvinyl alcohol (PVA). Stirring was continued for 5 h at a predetermined rate at room temperature, until CH_2Cl_2 was completely evaporated. The obtained microcapsules were collected by centrifugation, washed in distilled water, and then dried in *vacuo*.

Size and morphology of microspheres

The morphology of the microspheres was observed with a light microscope (XSP-2C, China) and a scanning electron microscope (SEM) (KYKY2800, China). The particle size and distribution of the microcapsules were measured with a laser diffraction particle size analyzer (Malven, Mastersizer 2000, British).

The drug-loading content and the encapsulation efficiency of microspheres

To determine the drug-loading content and the encapsulation efficiency, a weighted quantity of microspheres was dissoloved in CH_2Cl_2, and then finasteride was extracted into methanol. The amount of finasteride was determined by HPLC. The chromatograph used was equipped with a Waters 515 solvent delivery pump and a

2487 UV-detector. The separation was achieved by reversed phase column C_{18} (Diamosil 4.6mm×200mm 5μ). The detection wavelength was 210 nm. The mobile phase used was water-acetonitrile-tetrahydrofuran in the ratio of 8:2.5:1 and the flow rate was 1.0 mL/min. The drug-loading content in microspheres, given as a percentage, indicates the amount (mg) of finasteride encapsulated per 100 mg of microspheres. And the encapsulation efficiency of the process indicates the percentage of finasteride encapsulated with respect to the total amount used for the preparation of microspheres.

Wide-angle X-ray diffraction analysis

To clarify the structure of the microspheres, the wide-angle X-ray diffraction (WXRD) measures of finasteride, placebo microspheres and drug-loaded microspheres were performed at room temperature using a Rigaku D/max 2550 VB^+ 18Kw X-ray diffractometer.

In vitro release studies

Release studies of finasteride from PPC and PPCL microspheres were determined by adding 15 mg of finasteride equivalent microspheres to 50 ml of phosphate buffered saline (0.1 M pH 7.4) in a shaking water bath at 37°C. The sample (2 mL) was collected at different intervals and replaced with fresh medium, the amount of released drug was estimated from the samples by HPLC as the aforementioned method.

Results and discussion

The PPC and PPCL copolymers with different compositions were synthesized by adjusting the feeding ratios of monomers. The main properties of the obtained copolymers for drug carrier use were summarized in Table 1.

Table 1. Main properties of various copolylactones for drug carrier use

Sample	PO:CL (molar ratio)	Composition[a] (mol %)			$[\eta]^b$ (dL/g)	$M_w{}^c$ ($\times 10^4$)	$T_g{}^d$ (ºC)
		f_{CO_2}	f_{CL}	f_{PO}			
PPC	3:0	42.17	0.00	57.83	0.463	3.86	29.1
PPCL31	3:1	37.3	6.64	56.06	0.582	5.12	33.5
PPCL32	3:2	37.2	10.21	52.59	0.684	6.71	41.6
PPCL33	3:3	38.1	7.92	53.98	0.625	5.73	38.7

[a] Calculated from ^{1}HNMR measurements using $CDCl_3$ as solvent.
[b] Measured in benzene at 35°C using an Ubbelohde viscometer.
[c] Measured by gel permeation chromatography (GPC) using polystyrene as standard.
[d] Determined by different scanning calorimetry (DSC) on a TA DSC-Q10 instrument.

Degradability of the copolymers

Hydrolytic degradation is caused by the reaction of water with labile bonds, typically ester bonds, in the polymer chain [5]. The degradability behavior of the copolymers synthesized from various PO:CL ratios was shown in Figure 1.

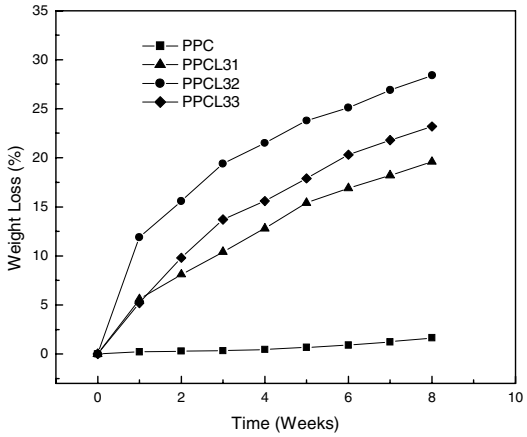


Figure 1. The degradability behavior of the PPC and PPCL copolymers

Figure 1 revealed that the hydrolytic activities of PPCL copolymers were much stronger than that of PPC. This could be due to the introduction of the hydrolytic ester units that in CL. It can be seen that PPCL32 had the best degradability which contained the most molar fraction of CL (10.21 %). The higher the molar fraction of CL, the higher the hydrolytic activity was. The results are in agreement with the conclusions from our earlier studies [7]. In this tests, the water uptake of PPCL did not exceed 8 %, and the molecular weight of the samples at various degradation stages decreased slightly. After hydrolytic degradation in pH 7.4 PBS for 8 weeks, the weight loss of PPCL32 was about 30%. The degradation and erosion of polymers are complicated, we supposed that the process was as follows: water penetrated into the polymer matrix, and hydrolytic degradation was caused by the reaction of water with ester bounds in the polymer chain, which induced chain scission to form oligomers and monomers. Progressive degradation changed the microstructure of the bulk through the formation of pores, via which oligomers and monomers were released, leading to the weight loss of polymers films. The water uptake of the copolymers was low, thus the water penetration was slow compared to the erosion process, and mass loss was confined to the surface layers of the samples. The degradation products were diffused to the degradation medium before measuring the molecular weight, so the molecular weight of copolymers changed slightly. Therefore, we infer that the degradation mainly occurs on the surface of polymer.

Morphological characterization of microspheres

The optical microscopy (Figure 2(a)) of the drug-loaded microspheres in latex revealed the spherical geometry. Further studies using scanning electron microscopy provided a better understanding of the morphological characteristics of the microspheres. The microspheres obtained by both batches were spherical, smooth and individually homogeneously distributed, and the morphology of PPC microspheres was similar to that of PPCL microspheres. SEM image of dry sample of PPCL32 microspheres was shown in Figugre 2(b), the particles were spherical in shape with smooth surfaces. The microspheres diameter was about 4 μm.

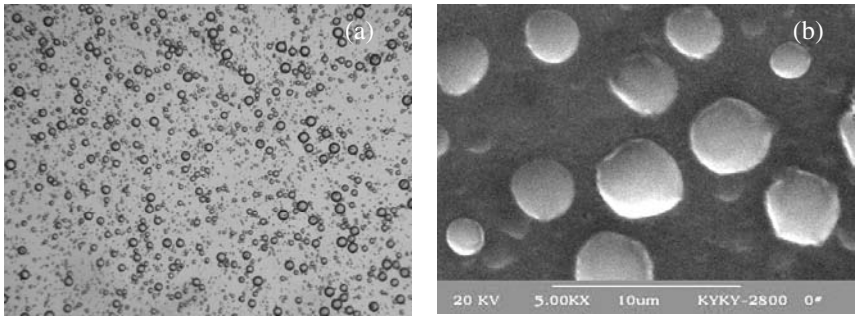


Figure 2. (a) Light micrograph (magnification=1000 times), and (b) SEM image of finasteride-loaded PPCL32 microspheres

Size and encapsulation efficiency of microspheres

The five kinds of PPC and PPCL microspheres containing finasteride from various copolymers were elaborated by O/W emulsion solvent evaporation method under the same conditions. The drug loading, encapsulation efficiency and size of microspheres were summarized in Table 2.

Table 2. Properties of the drug-loaded microspheres prepared by various copolymers

Copolymer	Drug loading Φ (%)	Encapsulation efficiency θ (%)	Mean diameter (μm)	Size range (μm)
PPC	9.36	67.49	4.82	0.1-15
PPCL31	12.45	83.36	3.95	0.1-12
PPCL32	12.84	85.13	3.48	0.1-10
PPCL33	11.67	78.50	3.62	0.1-10

As seen from Table 2, all microspheres formulations had particle size smaller than 15μm diameter. The mean diameter of the microspheres ranged from 3.62 to 4.82 μm, and tended to decrease with increasing content of CL units. The drug-loading content and entrapment efficiency were affected by the properties of the copolymers. The microcapsules prepared from PPCL32 copolymers achieved the highest drug-loading content (12.84%) and entrapment efficiency (85.13%) among the four copolymers.

Wide-angle X-ray diffraction (WXRD) analysis

The WXRD spectra recorded for the pure finasteride, placebo microspheres, and finasteride-loaded microspheres are presented in Figure 3. These studies are useful to investigate crystallinity of the drug in the obtained microspheres. The WXRD of the finasteride samples yielded a typical pattern of crystalline substances. In our earlier report, PPC and PPCL were amorphous [7]. X-ray diffraction pattern obtained for placebo PPCL32 microspheres was also amorphous. The characteristic crystalline peaks of finasteride were not observed in finasteride-loaded microspheres, but instead only peaks observed in the placebo were seen. In view of the WXRD results mentioned above. This indicated that drug was dispersed at the molecular level in the polymeric matrix and hence no crystals were found in the drug-loaded matrices [20].

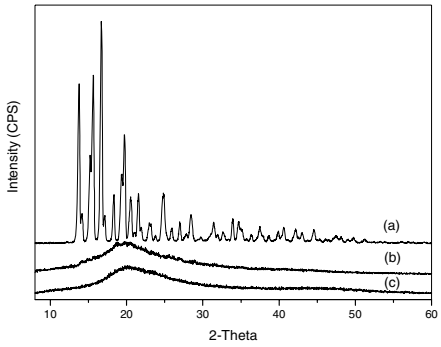


Figure 3. The wide-angle X-ray diffraction spectra of (a) pure finasteride, (b) finasteride-loaded PPCL32 microspheres, and (c) placebo PPCL32 microspheres

In vitro release of finasteride

The release of a drug is a rather complicated process, which is affected by many factors, such as the properties of polymer matrix, the size and structure of the delivery system, drug loading content, and so on. *In vitro* release behaviors of finasteride from the four kinds of microspheres were studied in pH 7.4 PBS. Figure 4 shows the percent release of finasteride from all samples of microspheres against incubation time in pH 7.4 PBS.

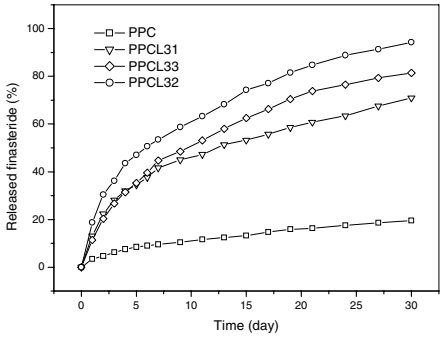


Figure 4. The release profiles of finasteride from microspheres prepared from various copolymers in pH 7.4 PBS

As shown in Figure 4, the release rates of finasteride from the microspheres were dependent upon the properties of the polymer in the microspheres. After release for 30 days, PPCL32 microspheres with the highest hydrolytic activity showed 94.23% drug release which was far higher than the 19.61% release of finasteride from PPC microspheres. Release of finasteride from PPCL31 and PPCL33 microspheres were 70.96% and 81.42%, respectively. Because the difference of the morphology and the drug loading content between the four kinds of microspheres was slight, an effect of the morphology and the drug loading content of the microspheres on the release of finasteride was avoided. The difference of the copolymers' component was

considered, and that was the difference brought out by CL. In view of the results mentioned above. It showed that release profiles of finasteride were highly polymer-dependent. This suggests that the hydrolytic activity of polymer was a main factor that controlled the rates of drug release. The higher hydrolytic activity of polymer provided faster release rates. The finasteride release profiles of all samples microspheres were found to occur with an initial burst release phase followed by a slower release phase. The release involved two different mechanisms, that is, diffusion of finasteride molecules and degradation of the polymer matrix. The burst release of the finasteride was associated with those finasteride molecules dispersing close to the microspheres surface, which diffused out in the initial incubation time. Most commonly, a higher drug loading results in a faster initial release rate. The finasteride was dispersed in the polymeric matrix at the molecular level, and the gradual release rate of finasteride from the microspheres was depended on the erosion or degradation of the matrix. So, the faster the degradation of copolymers, the faster was the finasteride release from the microspheres.

Conclusion

The utility of PPC and PPCL copolymers to encapsulate and control the release of finasteride, *via* microspheres, was investigated in the present study. The finasteride-loaded microspheres were elaborated by solvent evaporation method based on an O/W emulsion. The microspheres had a spherical, smooth morphology and a mean diameter of approximately 4 μm, and were shown to retard finasteride release in pH 7.4 PBS. The microspheres had a long release period of over 4 weeks. Because of the existence of the CL ester unit, PPCL had stronger degradability than PPC. The finasteride release rate of the PPCL microspheres was much faster than that of the PPC microspheres. The present results suggest that the PPCL microspheres could be used as controlled release devices, and the drug release period of it would be much shorter than that of PPC microspheres prepared under the same conditions.

Acknowledgements. This work was supported by National High Technology and Development Research '863' Program of China (No.2001AA218011) and the Hunan Provincial Science Foundation for Distinguished Young Scholars of China (No. 06JJ10003)

References

1. Freiberg S, Zhu XX (2004) Int J Pharm 282: 1
2. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM (1999) Chem Rev 99: 3181
3. Rokicki G (2000) Prog Polym Sci 25: 259
4. Zhu KJ, Hendren RW, Jensen K, Pitt CG (1991) Macromolecules 24: 1736
5. Edlund U, Albertsson AC (2001) Adv Polym Sci 157: 67
6. Lu LB, Huang KL (2005) Polym Int 54: 870
7. Liu SQ, Xiao H, Huang KL, Lu LB, Huang QY (2006) Polym Bull 56: 53
8. Liu YF, Huang KL, Peng DM, Wu H (2006) Polymer 47: 8453
9. Sinha VR, Bansal K, Kaushik R, Kumria R, Trehan A (2004) Int J Pharm 278: 1
10. Goodwin CJ, Braden M, Downes S, Marshall NJ (1998) J Biomed Mater Res 40: 204
11. Causa F, Netti PA, Ambrosio L, Ciapetti G, Baldini N, Pagani S, Martini D, Giunti A (2006) J Biomed Mater Res 76A(1): 151
12. Ferruti P, Mancin I, Ranucci E, Felice CD, Latini G, Laus M (2003) Biomacromolecules 4: 181

13. Zhou SB, Deng XM, Yang H (2003) Biomaterials 24: 3563
14. Yen MS, Kuo SC (1998) J Appl Polym Sci 67: 1301
15. Roehrborn CG (2003) Rev Urol 5 Suppl 5: S12
16. Heinzl S (1999) Med Monatsschr Pharm 22(4): 124
17. Coltman CA, Thompson IM, Feigl P (1999) Eur Urol 35(5-6): 544
18. Tkachuk VN, Al'-Shukri SKh, Kornienko VI, Kuz'min IV (1998) Urol Nefrol (Mosk) 4: 37
19. Sudduth SL, Koronkowski MJ (1993) Pharmacotherapy 13(4): 309
20. Guyot M, Fawaz F (1998) Int J Pharm 175: 61