PCL/Poloxamer 188 Blend Microsphere for Paclitaxel Delivery: Influence of Poloxamer 188 on Morphology and Drug Release

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ABSTRACT: A novel controlled release system, paclitaxel-loaded poly (ε-caprolactone) (PCL)/poloxamer 188 (Pluronic F68, F68) blend microspheres is proposed in the present work. F68 was incorporated into PCL matrices as both a pore-forming agent and a drug releasing enhancer. Paclitaxel-loaded PCL/F68 blend microspheres with different amounts of F68 were prepared by the oil-in water (O/W) emulsion/solvent evaporation method. Characterization of the microspheres followed to examine the particle size, the drug encapsulation efficiency, the surface morphology, and *in vitro* release behavior. The influences of F68 on microsphere morphology and paclitaxel release are discussed. The porosity of the surface of PCL/F68 blend microspheres and the release rate of paclitaxel from the PCL/F68 blend microspheres increased as the initial amount of blended F68 increased. Faster and controlled release was achieved in comparison with the PCL microspheres. Through this study, the developed microporous PCL/F68 blend microspheres could be used as a drug delivery system to enhance and control drug release in the future. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1895–1899, 2007

Key words: poly (ε-caprolactone); blends; poloxamer 188; paclitaxel; porous microspheres

INTRODUCTION

Biodegradable polyesters derived from lactic acid, glycolic acid, and ε-caprolactone have gained much interest in biomedical research for the delivery of various drugs. Poly (ε-caprolactone) (PCL) has attracted attention in the drug delivery systems because of the lack of toxicity and low cost when compared with other biodegradable polyesters, which has led to its application in the preparation of different delivery systems in the form of microspheres, nanospheres, and implants.[1,2] PCL microspheres can be prepared either by PCL alone, or by using copolymers with PCL or PCL blending to obtain the desired release characteristics. Polymer blends represents an alternative means of tailoring the water permeability of the matrices without significantly affecting its mechanical integrity.[3] Blending additives can significantly change drug release from polymer matrices, which mainly depends on additives' hydrophilic nature and solubility as well as interaction with the polymer.[4]

Poloxamer 188 (Pluronic F68, F68) is a poly (ether) with a molecular weight (Mw) of around 8300, containing about 80% poly (ethyl oxide) segment and a 20% poly(propyl oxide) segment. F68 is a FDA approved excipient under the trade name of Poloxamer. It is both water and organic solvent soluble. It has been used in solid dispersions to improve drug solubility.[5,6] In our previous studies, we developed a biodegradable levonorgestrel-releasing implant made of PCL/F68 blend compound, and F68 was incorporated into PCL matrix as a drug-release enhancer.[7] It was demonstrated that once the PCL/F68 matrices are implanted into the body, dispersed F68 molecules will leach out because of body fluids, therefore creating micropores on the capsule wall. The PCL/F68 matrix is biologically safe and nontoxic. The clinical trial for the PCL/F68/levonorgestrel implant is now underway in China. Because of the success of this approach, the investigation into the effect of PCL/F68 blending on entrapment and release of hydrophobic drug from microspheres was attempted in this study. Paclitaxel was used as the model drug.

Paclitaxel ($C_{47}H_{51}NO_{14}$, Mw = 853 Da, Fig. 1) has been proven to exhibit significant activities in clinical trials against a wide spectrum of cancers.[8,9] Since paclitaxel is very water insoluble, the clinically available paclitaxel injection used an emulsifier reagent called Cremophor EL.[10] This has been found to cause serious side effects.[11,12] The primary goal of formulation development for paclitaxel is to elimi-

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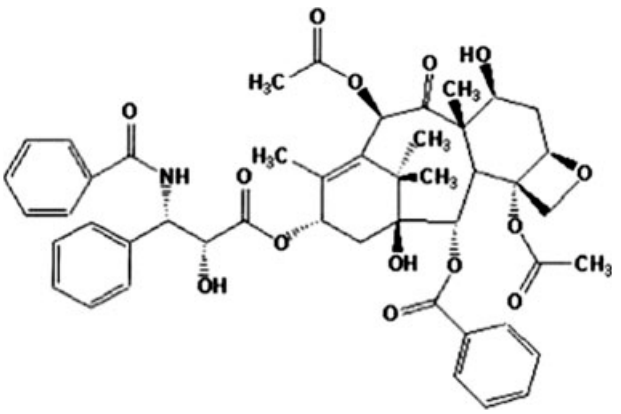

Figure 1 Structure of paclitaxel.

nate the Cremophor EL. Polymeric microspheres may provide an ideal solution to the problem by eliminating the use of such adjuvant.

Therefore, the objective of this study was to investigate paclitaxel-loaded PCL/F68 blend microspheres. In this study, F68 was incorporated into PCL matrices as both a pore-forming agent and a drug releasing enhancer. The degrees of pores were controlled by varying the amount of F68 in the blended matrices. The influences of F68 on microsphere morphology and paclitaxel release will be discussed.

EXPERIMENTAL

Materials

PCL (Mw = 65,000) was purchased from Aldrich (USA). F68 (AR grade) was obtained from BASF (Parsippany, NJ). Paclitaxel was supplied by Guilin Hulang Biochemistry Pharmaceutical. (Guilin, China). Poly (vinyl alcohol) (PVA) (Mw = 30,000–70,000) was from Sigma. Dichloromethane and acetonitrile of HPLC grade were used in this study. All other reagents and solvents were of an analytical grade.

Preparation of microspheres

Paclitaxel-loaded PCL/F68 blend microspheres were fabricated by an oil-in water (O/W) emulsion/solvent evaporation method. Briefly, PCL and different amount of F68 (0, 10, 20, 40%, w/w) were codissolved in dichloromethane (5 mL) with paclitaxel (30%, w/w). The formed solution was poured into 100 mL of PVA solution (2%, w/v) while stirring at 1000 rpm. The resulting emulsion was then stirred overnight at 400 rpm to remove the organic solvent. The final product was recovered by centrifugation at 8000 rpm for 10 min, washed with distilled water, and lyophilized.

Determination of drug content in the microspheres

The amount of encapsulated paclitaxel in the microspheres was determined by HPLC method. Five milligram of paclitaxel-loaded microspheres was dissolved in 2 mL of dichloromethane. A mixture of acetonitrile and distilled water (50 : 50, v/v) was then added. A nitrogen stream was introduced to evaporate dichloromethane until a clear solution was obtained. The HPLC assay was performed on a reverse phase Diamond® C18 column (inner diameter 150 mm × 4.6 mm, pore size 5μm). The mobile phase was a mixture of acetonitrile and water (50 : 50, v/v). The flow rate was 1.0 mL/min and the column effluent was detected at 227 nm with a UV detector. The encapsulation efficiency is defined as the ratio of the amount of the encapsulated drug to that of the drug used for microsphere preparation.[13]

Particle size and surface morphology

The surface morphology of the microspheres was examined by scanning electron microscopy (SEM) (Philips XL-30, Netherlands). The particle sizes of the prepared microspheres were measured by particle size analyzer (Coulter LS-230).

In vitro release study

Five milligram of microspheres were placed into an Eppendorf tube containing 10 mL of 0.01*M* phosphate buffer (pH 7.4) ($n = 3$). Then the samples were placed in a shaking bath (HZQ-C, DongMing electronic, Harbin, China) at 120 rpm and 37°C. At appropriate intervals, the samples were centrifuged at 8000 rpm for 10 min. The supernatant was withdrawn and replaced with an equal volume of fresh release medium. The amount of paclitaxel in supernatants was determined by HPLC method described earlier.

RESULTS AND DISCUSSION

Particle size and encapsulation efficiency

The particle size and encapsulation efficiency of the microsphere samples were listed in Table I. It can be seen that mean particle size of all samples was 15–30 μm.

No significant differences were observed between particle sizes of all the samples. Apparently, the effect of the amount of F68 on the particles size was not significant, suggesting that the particle size was largely controlled by the emulsion process during microsphere preparation. The encapsulation efficiency of 100% PCL microspheres was nearly 90%, which was the same as that of PCL/F68 blend microspheres with different amounts of F68, meaning that the addition of F68 had not decreased the amount of paclitaxel encapsulated. In general, the

TABLE I
Particle Size, Encapsulation Efficiency of Paclitaxel-Loaded Microspheres with Different Amounts of F68

Sample	Formulation (F68 weight and drug loading, w/w)	Particle size (μm)	Encapsulation efficiency (%)
Pm	PCL/30% paclitaxel (without F68)	16.26 ± 7.62	89.23
PFm1	PCL/10% F68/30% paclitaxel	24.32 ± 10.68	90.12
PFm2	PCL/20% F68/30% paclitaxel	21.62 ± 9.83	88.35
PFm3	PCL/40% F68/30% paclitaxel	18.75 ± 7.33	87.66

high encapsulation efficiency of hydrophobic drugs such as paclitaxel is relatively easy with hydrophobic polymers, since the loss of the drug to the water phase is less likely to occur in comparison with hydrophilic drugs.[13]

Surface morphology

SEM measurements were undertaken to investigate homogeneity of PCL/F68 blends and the effects of different amounts of F68 on microsphere morphology. The homogeneity of the PCL/F68 blends was evident from the SEM micrographs in Figures 2 and 3, which showed that no obvious phase separation occurred and micropores formed by F68 distributed evenly. This is understandable since F68 has a high molecular weight and is both organic and water soluble, such feature of F68 is favorable to form a molecular dispersion in the lipophilic PCL matrices when dissolved in dichloromethane during preparation process.

Figure 3 showed that the SEM micrographs of porous surface of PCL/F68 blend microspheres formed by various amounts of F68. The PCL microspheres possessed smooth surfaces without any existing pores. In contrast, the surface of PCL/F68 blend microspheres was coarse with uniform micropores. From SEM, it can be seen that most of the pores had a diameter of about 0.5–1.5 μm, which was much larger than that of the drug molecules. Our previous studies had demonstrated that hydrophilic additives, particularly if leachable or during swelling, could act as pore-forming agents to produce porous structure

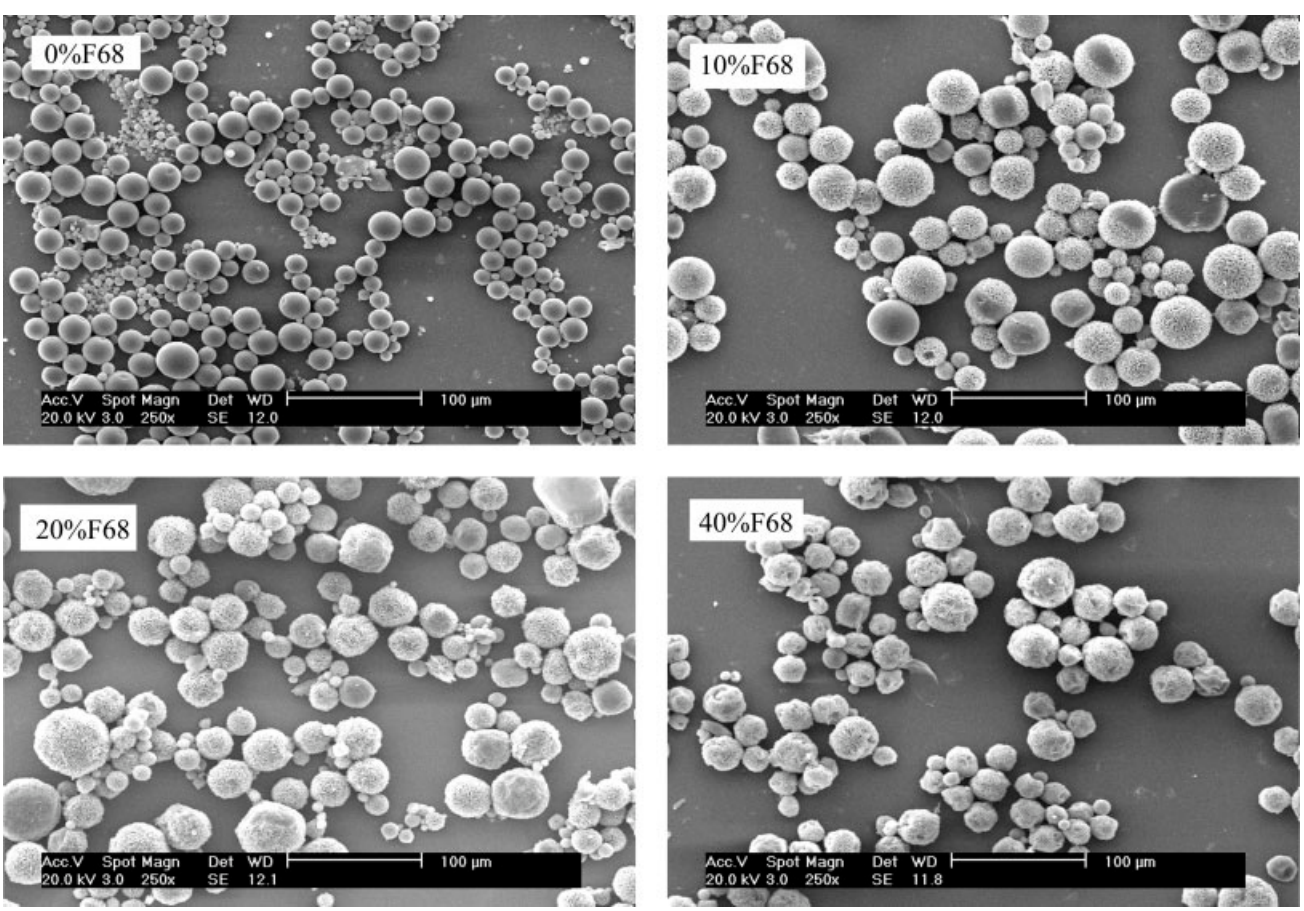

Figure 2 SEM micrographs of PCL/F68 blend microspheres formed by various amounts of F68.

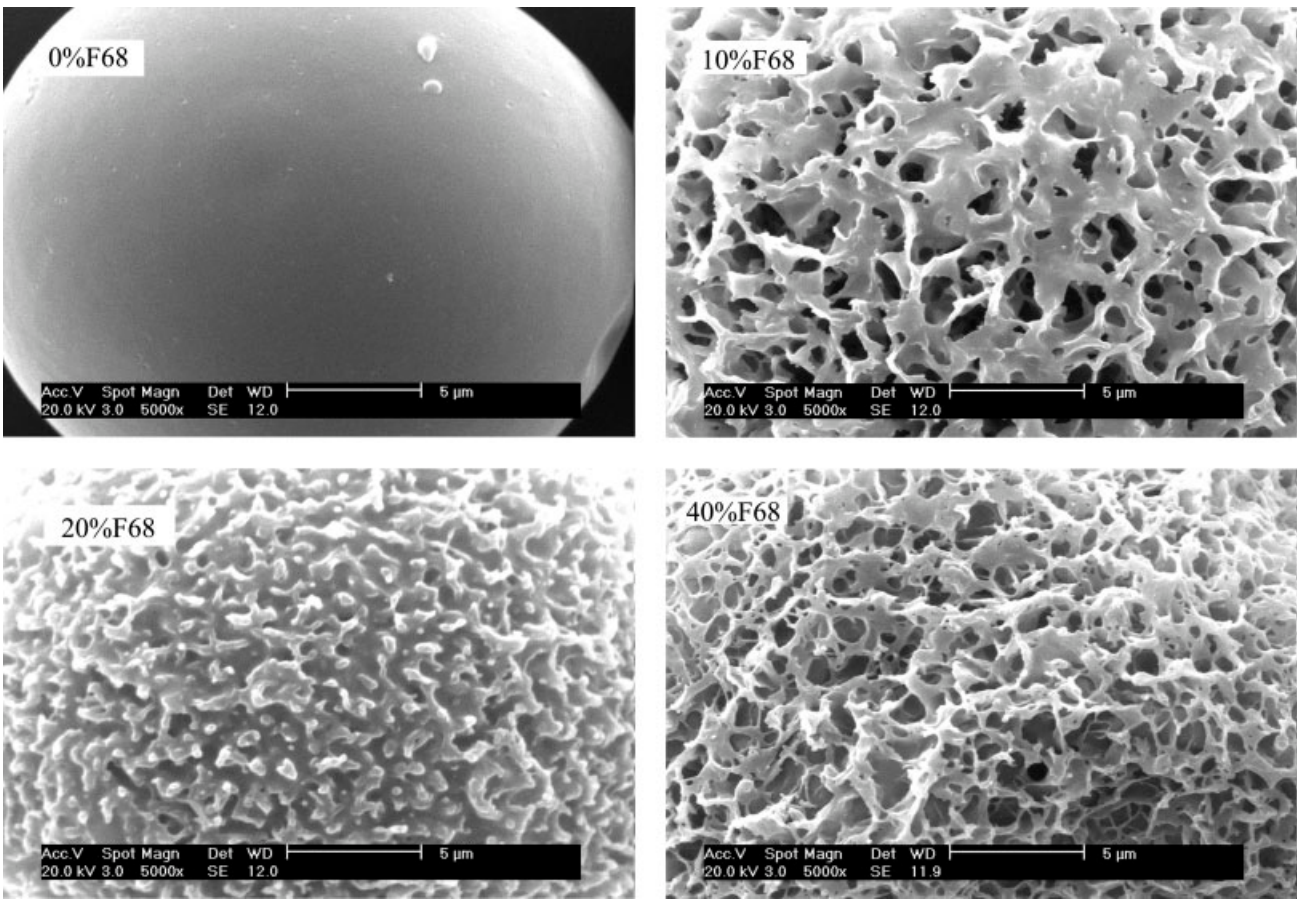

Figure 3 SEM micrographs of porous surface of PCL/F68 blend microspheres formed by various amounts of F68.

when contacted with an aqueous medium.[14] As mentioned earlier, F68 is both organic and water soluble. So the pores in the surface of PCL/F68 blend microspheres could be attributed to the hydrophilicity of F68. F68 leached out due to the water phase during the emulsification and solvent-removal process, therefore creating porous structure in the surface of the PCL/F68 microspheres. Theoretically, the porosity of surface of PCL/F68 blend microspheres was proportional to the amount of blended F68 initially. This result was consistent with the observation from SEM, which showed that the number of micropores increased as the initial amount of blended F68 increased.

In vitro release study

Effects of various amount of F68 (0, 10, 20, and 40%, w/w) on the paclitaxel release behaviors from PCL/F68 blend microspheres were shown in Figure 4. As shown in Figure 4, when blended 20 and 40% of F68, the paclitaxel-loaded PCL/F68 blend microspheres resulted in positively faster release of the drug from the microspheres when compared with that from PCL microspheres. This might be, at least partially, due to the porous morphology of the PCL/F68 blend microspheres formed by F68 when it leached out from the matrices in aqueous medium. According to Kuu et al. the porous structure can be occupied by release medium.[15] The pore structure presumably attenuated the barrier properties of PCL matrices for drug diffusion and was the main route for drug release, although further studies are needed to substantiate this.

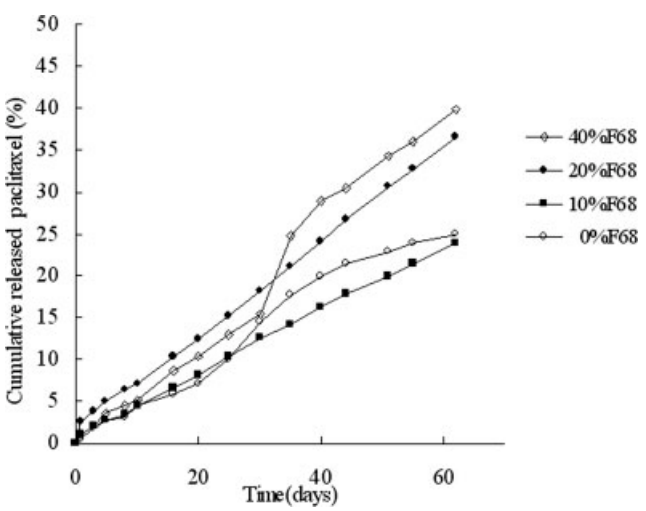

Figure 4 *In vitro* release of paclitaxel from PCL/F68 blend microspheres with various amounts of F68.

As mentioned earlier, the porosity of matrices of PCL/F68 blend microspheres was proportional to the amount of blended F68 initially. This result was also consistent with the observation from Figure 4, which showed that the release rate of paclitaxel from the PCL/F68 blend microspheres increased as the initial amount of blended F68 increased. This could be explained with percolation theory.[16,17] When the pores created in matrices were few and isolated, which was not favorable for generating the interconnected open pore structure. Once the porosity increased above the percolation threshold (the critical porosity), the interconnecting pathways in porous matrices would be formed easily and became more ''filled in'' by release medium, facilitating drug to diffuse and release from the matrices.

As shown in Figure 4, the microspheres blended with 10 and 20% of F68 resulted in a perfect constant release for the entire experimental period without any lag time. The constant release behaviors could be due to the porous structure in PCL matrices formed by F68. Drug release from the PCL matrices was a combined result of two processes: drug diffusion and matrix degradation.[18] As for the PCL degradation process, Pitt et al. concluded that the mechanism of PCL degradation is attributed to random hydrolytic chain scission of the ester linkages, which causes a decrease in molecular weight.[19] Ali et al. studied the mechanism of PCL degradation *in vitro* by means of GPC, DSC, and SEM, and they hypothesized that the hydroxyl radical is likely to be a significant cause of PCL degradation in implantable devices.[20] Therefore, we hypothesized that the existence of porous structure will accelerate the hydrolysis rates of PCL degradation, which allowed aqueous medium to diffuse into the interior portion of the microspheres. But PCL is a highly hydrophobic crystalline polymer that degrades very slowly *in vitro* in the absence of enzymes.[19,21,22] Chalwa and Amiji studied that the degradation of PCL (Mw = 14 800 Da) nanoparticles in PBS and they found that even after 140 days in PBS at 37°C, no significant reduction in molecular weight was observed in PCL nanoparticles.[21] Therefore, we thought that the degradation of PCL/F68 microspheres with molecular weight of 65,000 Da in the entire release experimental period could be neglected.

As mentioned earlier, the degradation of PCL/F68 microspheres in the entire release experimental period could be neglected. In other words, the drug diffusion process is the key factor to determine the drug release from the PCL/F68 blend microspheres. As for the drug diffusion process, a concentration difference (ΔC) in PCL matrices was the driving force to allow drug diffuse out from microspheres. ΔC would decrease as the drug concentration reduced during the release process. It was hypothesized that the porous structure allowed it to be feasible for release medium to diffuse into the drug located portion and dissolve the drug, which complemented the reduction of ΔC in PCL matrices during the release process and maintained drug constant release.

CONCLUSIONS

In this study, F68 was incorporated into PCL matrices as both a pore-forming agent and a drug releasing enhancer. Microporous PCL/F68 blend microspheres facilitate the paclitaxel release as desired. Faster and controlled release was achieved in comparison with the PCL microspheres. The amount of blended F68 initially affected the microsphere morphology and paclitaxel release. The porosity of surface of PCL/F68 blend microspheres and the release rate of paclitaxel from the PCL/F68 blend microspheres increased as the initial amount of F68 increased. Through this study, the developed microporous PCL/F68 blend microspheres could be used as a drug delivery system to enhance drug release and could facilitate controlled drug release in future systems.

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