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Effects of crystalline microstructure on drug release behavior of poly(ε-caprolactone) microspheres

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Abstract

This study investigates the release behavior of papaverine from poly(ε-caprolactone) (PCL) microparticles prepared by the oil/water solvent evaporation method. Microparticles were characterized in terms of crystalline morphology, size, drug loading, and encapsulation efficiency by using differential scanning calorimetry (DSC), small angle X-ray scattering (SAXS), scanning electron microscopy (SEM), and UV spectrometry. The release behavior of papaverine was governed by the microstructure of PCL microparticles, suggesting that the environment for diffusion changes according to processing conditions such as polymer solution concentration, thermal history, and polymer molecular weight. As the PCL solution concentration increased, the drug release behavior showed a more sustained pattern. This result indicates that the size of the PCL microparticles is a determining factor for drug release. And when higher PCL molecular weight is used for preparation of microparticles, it led to a rapid release. Furthermore, a more delayed pattern of drug release profile was obtained in the sample prepared with higher thermal treatment. These results suggest that the crystalline microstructure of PCL microparticles plays an important role in its drug release behavior.

Keywords: Biodegradable polymer; Poly(ε-caprolactone); Microparticles; Diffusion-controlled release; Crystalline microstructure

1. Introduction

The past 3 decades have witnessed the emergence of controlled release technology as an important field of pharmaceutical dosage forms. Since biodegradable polymers such as polycaprolactone (PCL), polylactide (PLA), and poly(lactide-co-glycolide) (PLGA) [1–9] have a long history of use in the biomedical field, many investigators have studied biodegradable polymers as a carrier for controlled release dosage forms. Recently, drug-loaded microparticles have been utilized for clinical applications of the controlled release of bioactive materials [10–19] because they have several advantages of improving the therapeutic effect, prolonging the biological activity, controlling the drug release rate, and decreasing the administration frequency.

Now it is important to gain insight into the mechanism of drug release in order to develop new dosage forms using these polymers. Three mechanisms have been identified for controlling drug release from bio-

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degradable polymers [20]: Fickian diffusion through the polymer matrix, diffusion through pores in the matrix, and drug liberation by polymer erosion. However, predicting precise drug release profiles is still difficult because these profiles are governed by various properties of the polymer, drug, and carrier system [21]. Namely, polymer-dependent factors include molecular weight, molecular weight distribution, and crystallinity. Important drug-dependent parameters are solubility of the drug in biological fluids, molecular weight, and possible polymer–drug interactions. Carrier system-dependent factors comprise the types of microparticle (microsphere vs. microcapsule), drug loading, physical state of the drug in the polymer matrix (dissolved vs. dispersed), particle size and particle size distribution, and porosity and internal structure of the microparticles [22].

Oral delivery is by far the easiest and most convenient way for drug delivery, especially when repeated or routine administration is necessary [23]. Therefore, various types of particulate systems such as biodegradable microspheres have been proposed as potential delivery vehicles to protect drugs in the gastrointestinal tract. The system we propose in this work is an oral delivery form of microparticles capable of obtaining a delayed release of drugs at the colonic region. We describe here the effects of the PCL microstructure as well as the processing conditions on the drug release behavior, focusing especially on the crystalline microstructure of PCL microparticles. Although it is recognized that the rate of drug release from polymer microparticles seems to be significantly affected by the microstructure of microparticles, there has been no systematic investigation on its effects. We have studied the release behavior of papaverine-loaded PCL microparticles prepared by the solvent evaporation method, focusing on the relationship between the crystalline microstructure of the polymer matrices and the drug release profiles. We found that poly(ε-caprolactone) gave microparticle matrices of differing microstructure depending on the processing conditions such as polymer solution concentration, polymer molecular weight, and thermal history. The present paper firstly describes the physical morphology and physicochemical properties of the microparticles such as particle size, drug loading, and efficiency of entrapment as well as the crystalline microstructure of PCL microparticles. We also describe the effect of PCL solution concentration, PCL molecular weight, and thermal history on drug release behavior. Especially, for the purpose of investigating the effect of crystalline microstructure of PCL microparticles on the drug release behavior effectively, we attempted to change the microstructure of PCL microparticles by using an annealing method.

2. Materials and methods

2.1. Materials

Poly(ε-caprolactone) (number-average molecular weight = 10 000, 40 000, 80 000) and sodium dodecyle sulfate (SDS) were supplied by Aldrich Chemical. Papaverine (6,7-dimethoxy-1-veratrylisoquinoline) hydrochloride was obtained from Sigma. Papaverine-free base was prepared by increasing the pH of the solution above 10. Dichloromethane was purchased from J.T. Baker. All other chemicals were of reagent grade.

2.2. Preparation of microparticles

The PCL microparticles were prepared by the O/W emulsion solvent evaporation method [24]. This technique is commonly used for microencapsulation of water-insoluble drugs with hydrophobic polymers [25]. Drug (0.1–0.28 g) and PCL (0.4–1.12 g) were first dissolved in dichloromethane (20 ml). This solution was poured into 200 ml of purified water containing 0.5% (w/v) SDS. The dichloromethane was removed by stirring at 1000 rpm in room temperature for 1 h. When evaporation was complete, the microparticles were collected by filtration on a filter paper, washed three times in double-distilled water, and air-dried overnight at room temperature. Each formulation was prepared at least twice, and the resulting batches were combined for characterization studies.

2.3. Characterization of microparticles

Mean particle sizes, surface morphology, and the inner structure of the microparticles were observed on the scanning electron microscope (SEM). The micro-

particles were fixed on supports with carbon tape and coated with platinum under an argon atmosphere using a platinum sputter module in a high-vacuum evaporator. The samples were, then, observed with a Hitachi S-4200 SEM at 8 kV. To observe the cross-section of microparticles, particles dispersed in water were frozen and then sectioned using a cryogenic ultramicrotome system (RMC, MT-7000). The thermal properties of microparticles were determined by differential scanning calorimetry (DSC) measurements (TA Instrument, 2910). All the experiments were performed from 0 to 180 °C at a scanning rate of 10 °C/min. The instrument was calibrated using indium as the standard, and all the samples used were less than 10 mg. The wide angle X-ray scattering (WAXS) and small angle X-ray scattering (SAXS) experiment was conducted to investigate the crystalline structure of microparticles by using a synchrotron X-ray radiation source (4C1 beam-line, wavelength: 1.598 Å) at the Pohang Accelerator Laboratory. WAXS profiles were collected in the range of $2\theta = 10–40°$. The system is equipped with a Si(111) double crystal monochromator and a cylindrical mirror. The scattering intensity was corrected by background scattering. The amount of papaverine encapsulated per weight of microparticles was determined by UV spectrophotometry (UV-2401 PC, SHIMADZU). Each sample was assayed in triplicate.

2.4. Drug release experiment

The drug-loaded microparticles (25 mg) were suspended in 100 ml phosphate buffer solution (pH 7.4, 0.01 M). All the experiments were carried out in a 37 °C water bath at a frequency of 40 strokes/min for 1 week. The release medium (5 ml) was withdrawn periodically and replaced with an equivalent volume of fresh buffer. Drug concentrations were directly analyzed by using UV spectrophotometer at 237.8 nm. All the release experiments were conducted in duplicate or triplicate.

3. Results and discussion

3.1. Physicochemical identification of PCL microparticles

The first part of this work attempted to optimize the particle preparation process [26] to produce PCL microparticles. With this in mind, several parameters were evaluated: the drug to polymer ratio, the PCL solution concentration, and the PCL molecular weight. The experimental conditions and corresponding values for each sample are summarized in Table 1. At a high drug to polymer ratio (1:4), encapsulation efficiency is very low. This means that the quantity of polymer present was insufficient to cover the drug completely. Therefore, the subsequent samples were prepared at 1:9 fixed ratio. Three different polymer concentrations were studied to investigate the effect of PCL solution concentration (2.5, 5, and 7 wt.%). The viscosity of PCL solutions depends directly on polymer concentration, organic solvent, and temperature [27]. Accordingly, an increase in the polymer concentration produced a significant increase in the viscosity, thus

Table 1
Effect of the processing variables on the size, drug loading, and encapsulation efficiency of PCL microparticles produced by the emulsion evaporation method

Sample	Drug/ polymer ratio	PCL solution concentration (wt.%)	PCL Mw (K)	Particle size (μm)	Drug loading (%)	Encapsulation efficiency (%)
P1/4	1:4	7.0	40	39.1 ± 3.1	1.9	9.6
P1/9	1:9	7.0	40	38.8 ± 2.8	4.1	41.0
P1/19	1:19	7.0	40	38.2 ± 3.3	2.0	39.2
P2.5	1:9	2.5	40	21.3 ± 3.3	1.1	11.0
P5	1:9	5.0	40	29.5 ± 4.5	2.9	28.5
P7	1:9	7.0	40	38.8 ± 2.8	4.1	41.0
P10K	1:9	7.0	10	25.5 ± 5.5	4.8	48.1
P40K	1:9	7.0	40	38.8 ± 2.8	4.1	41.0
P80K	1:9	7.0	80	40.8 ± 1.8	3.3	32.6

leading to an increase of the emulsion droplet size and, finally, to a higher PCL microparticle size. Moreover, the high concentration of polymer in the emulsion droplets led to an enhancement of the encapsulation efficiency, because the high viscosity of the organic phase tends to restrict partitioning of the drug into the external aqueous phase [28]. The other group is related to molecular weight of PCL. The more molecular weight of PCL increased, the greater was the size of particles. However, changes in particle size caused by molecular weight were smaller than that caused by polymer concentration. As shown in Fig. 1a and b, the PCL microparticles containing papaverine presented a smooth surface without apparent porosity. Moreover, no drug crystals were observed on the surface of the microparticles. And the SEM micrograph of the cross-section of the microparticles (Fig. 1c) revealed a compact structure without cavities or internal porosity.

3.2. Characterization of the physical state of drugs in PCL microparticles

In order to identify the mechanism of sustained drug release, we first characterized the solubility and physical state of the drug within the microparticles (Fig. 2). A sharp and large melting peak ($\Delta H = 128$ J/g) was observed for papaverine at 148 °C, corresponding to its melting transition point. However, the melting peak was absent on the DSC thermogram of PCL microparticles containing papaverine, indicating that the drug was dispersed in the microparticles as an amorphous form [29]. This phenomenon was further confirmed by X-ray diffraction patterns. The crystal peak of papaverine is clearly observed by X-ray data shown in Fig. 3. However, the diffraction patterns of the PCL microparticles containing papaverine were similar to that of PCL microparticles not containing papaverine. These PCL microparticles did not contain any peaks associated with the crystals of the drug, suggesting that the drug was amorphous in the poly-

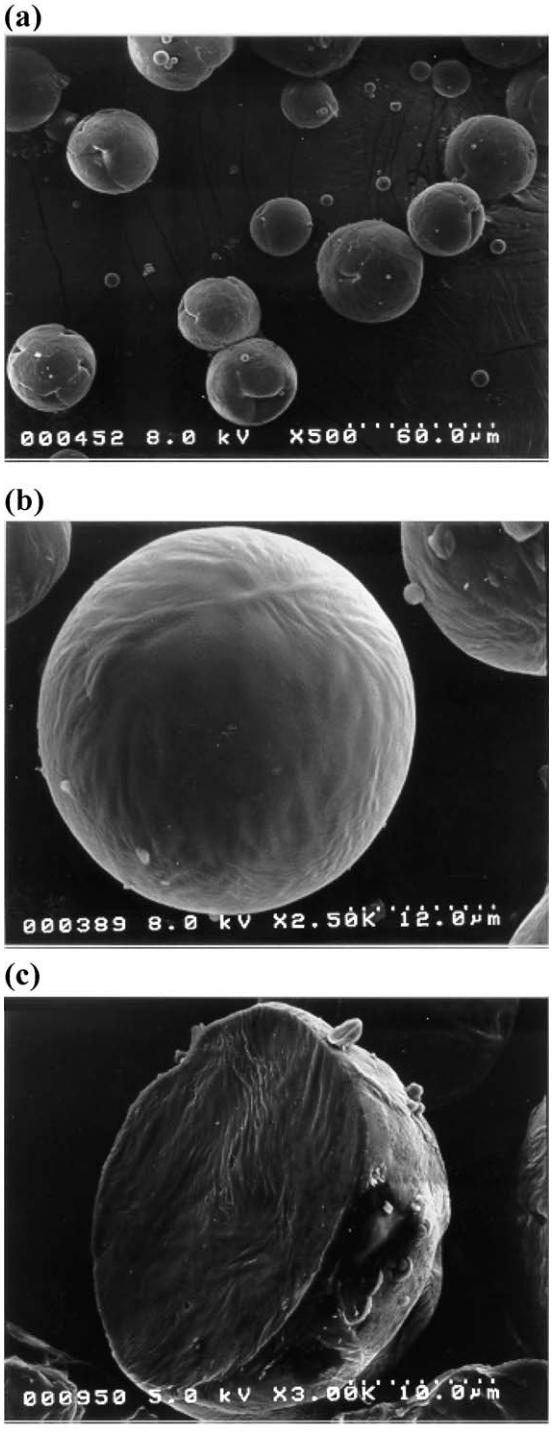

Fig. 1. SEM micrographs of (a), (b) PCL microparticles containing drug prepared by emulsion evaporation technique and (c) cross-section of a PCL microparticles. The microparticles were fixed on support with carbon tape and coated with platinum. In order to observe cross-section of microparticles, the microparticles dispersed in water were frozen and then sectioned using a cryogenic ultramicrotome.

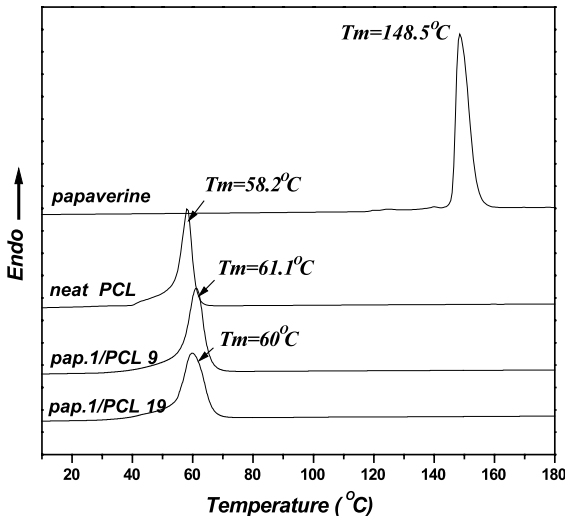

Fig. 2. DSC thermograms obtained from papaverine, neat PCL microparticles, and PCL microparticles containing papaverine. The neat PCL is a microparticle non-containing drug. The pap.1/PCL 9 and pap.1/PCL 19 are microparticles containing drug in an amount of 4.1 and 2.0 wt.%, respectively.

mer matrix. This result corresponds to DSC thermograms (Fig. 2). It is also estimated that the drug is dispersed in the amorphous region of the polymer matrix, not in the crystalline region. In addition, Ha et al. [30] have shown that the degradation of PCL was very slow in an aqueous medium because of its semi-crystallinity and hydrophobicity. This indicates that the drug release was a result of diffusion, and not of the degradation of the polymer. It follows that the diffusion through the polymer is the only possible mechanism of drug release.

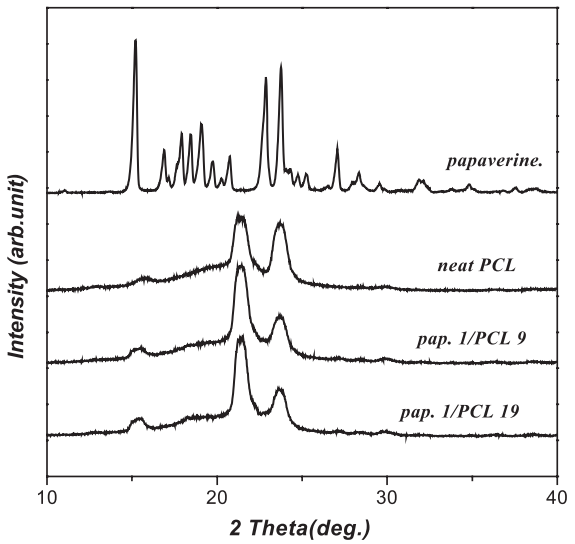

Fig. 3. X-ray diffraction patterns obtained from papaverine, neat PCL microparticles, and PCL microparticles containing papaverine. The neat PCL is a microparticle non-containing drug. The pap.1/PCL 9 and pap.1/PCL 19 are microparticles containing drug in an amount of 4.1 and 2.0 wt.%, respectively.

3.3. Characterization of the crystalline microstructure of PCL microparticles

The main part of this work concentrates on the crystalline microstructure of PCL microparticles because it is expected that the behavior of drug release depends on the crystalline microstructure [31]. The crystalline microstructure of polymers is known to be influenced by the processing condition such as solution concentration and thermal history [32,33]. And the molecular weight of polymers has effect on the microstructure of crystal such as crystallinity and long period length. Therefore, the following three factors were selected to test the validity of these expectations: PCL solution concentration, PCL molecular weight, and thermal history.

Papaverine-containing microparticles were prepared using different concentrations of PCL solution (2.5, 5, and 7 wt.%) to investigate the eventual modifications such as the crystalline microstructure and particles size. The crystallinity of the samples is shown in Table 2. Regardless of PCL concentrations, three samples had almost the same crystallinity (from

Table 2
Crystallinity and long period length of PCL microparticles with a different process condition

Sample	Crystallinity (%)[a]	Long period length (nm)[b]
P2.5	65.3	13.7
P5	67.6	13.6
P7	69.7	13.5
P10K	77.0	12.4
P40K	69.7	13.5
P80K	63.0	15.0
P725I (25 °C annealed)	69.7	13.5
P740I (40 °C annealed)	71.7	14.0
P750I (50 °C annealed)	69.6	15.4

[a] Calculated from the DSC.
[b] Estimated from the Lorentz-corrected SAXS profiles.

65% to 70%), which means that they have the same ratio of crystalline phase to amorphous phase. Moreover, as shown in the SAXS data, long period length of three samples was also same. This means that the total length of lamellar and amorphous phase is the same, which indicates that the increasing values of polymer concentration produce the same crystalline microstructure, resulting only in an increase in the particle size (Table 1).

As shown in Table 2, in the case of samples with different PCL molecular weights, there was a significant difference in crystallinity. Namely, as the molecular weight increased, the crystallinity was considerably reduced (from 77% to 63%), thus the parts of the crystalline region decreased significantly. However, SAXS data show that long period length increases (Table 2). These results mean that the higher the molecular weight is, the greater the amorphous phase increases, which results in a coarse crystalline microstructure.

It is well known that semi-crystalline polymers often show different crystalline structures after different thermal histories [34–36]. In order to study the effect of thermal history on the crystalline microstructure, the samples prepared under same condition were annealed at different temperatures (25, 40, 50 °C). Fig. 4a shows DSC thermograms of the PCL microparticles prepared by different thermal treatments. When the annealing temperature is 25 °C, a considerable portion of melting peak below 50 °C is shown, which might be attributed to the small crystals and imperfection of crystallites. Whereas the annealing temperature is increased, the melting peaks below annealing temperature mostly disappeared, and the position of the maximum melting peak increased slightly. These results are closely related to the recrystallization of some microcrystals and the degree of crystal perfection. Therefore, as the annealing temperature is increased, larger and more perfect cystallites are formed. The crystalline structure of the PCL microparticles was further characterized by small angle X-ray scattering (SAXS). Fig. 4b shows the SAXS spectra of the PCL microparticles prepared by different thermal treatments. When the annealing temperature is increased, long period length is changed slightly. When the annealing temperature is 25 and 40 °C, the difference of long period length was as small as about 0.5 nm. However, in the case of 25

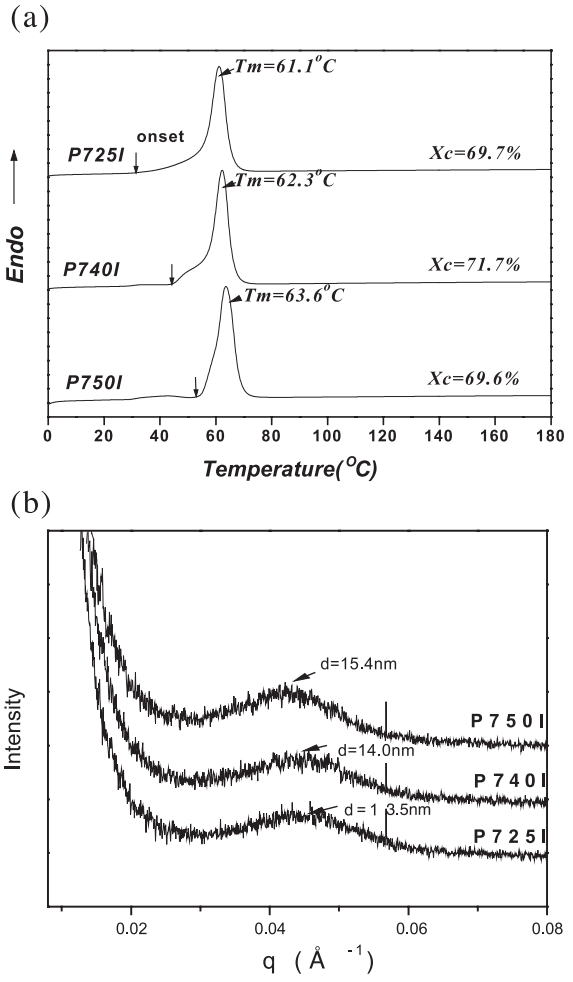

Fig. 4. DSC thermograms (a) and SAXS spectra (b) obtained from PCL microparticles prepared with a different thermal history. The PCL microparticles (P7) were annealed at different temperatures (25, 40, 50 °C) for 3 days.

and 50 °C, there was a clear distinction between the long spacing values of the samples: a difference of about 2 nm. Although long period length of crystalline structure is measured in this experiment, it should be assumed that long period length is identical to the size of crystallites due to the similar crystallinity of the samples (Table 2). These results suggest that the size of lamellae becomes larger with increasing annealing temperature, especially, in the case of 50 °C. Since glass transition temperature (T_g) of the PCL is remarkably low as −60 °C, polymer chains are easy to move under thermal treatment conditions.

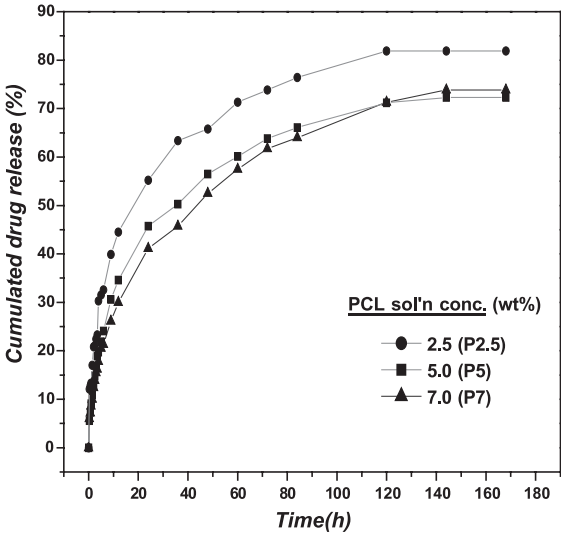

Fig. 5. In vitro drug release profiles from PCL microparticles prepared with a different PCL solution concentration for 1 week.

Therefore, such a flexible mobility of polymer chains enables the lamellae to grow larger. This speculation is consistently supported by the DSC results as shown in Fig. 4a.

3.4. In vitro drug release behavior of PCL microparticles

In addition to the identification of the drug release mechanism, we also studied the relation of the crystalline microstructure with the drug release behavior. The release behavior of papaverine from PCL microparticles is illustrated in Figs. 5–7, which indicate the sustained release pattern for 1 week. At the initial stage, the burst effect related to the drug entrapped near the surface of the microparticles [37] was remarkably small, and it was followed by a very slow release stage. Such a small initial burst is an especially interesting phenomenon, which is probably due to the low permeability of water in PCL [5]. To be released, preferentially, the diffusion path must be filled up by water [38,39]. In other words, the hydrophobic property of PCL causes the delay of water penetration, thus the diffusion of the drug through the amorphous region into the release medium was retarded, which results in a small burst effect. At the later stage, the drug was released more slowly, whose rate is determined not by the erosion of polymer but by the diffusion of the drug through the amorphous region of polymer matrix. This fact can be explained as follows. Although PCL is a biodegradable polymer, biodegradation of the PCL is considerably slower than that of other degradable polymers. Therefore, since PCL is hardly degraded during the diffusion process, the diffusion through the polymer is the only possible mechanism of drug release.

The in vitro release profiles shown in Fig. 5 indicate the differences between PCL microparticle formulations caused by the polymer solution concentration. When PCL concentration was 2.5%, the rate of drug release was the fastest. As the PCL concentration increased, the drug release behavior showed a more sustained pattern. This result could be explained by the crystalline microstructure and particle size of these samples. Namely, crystallinity and long period length of three samples (2.5%, 5%, 7%) are the same. Therefore, they have the same internal microstructure, and the only difference is in the particle size (Table 1). As the PCL concentration increases, the particle size increases, which results in a decrease in the total surface area of microparticles. Such a surface area decrease directly reduces contact with water. Therefore, the drug release is sustained in a more progressive manner.

Fig. 6 shows a drug release from PCL microparticles prepared with a different molecular weight.

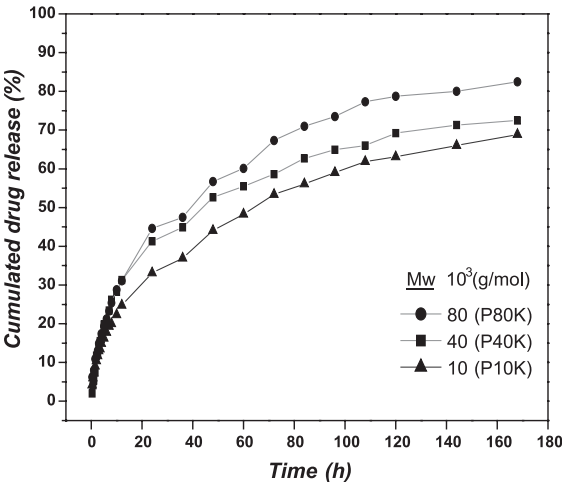

Fig. 6. In vitro drug release profiles from PCL microparticles prepared with a different molecular weight for 1 week.

When using the highest PCL molecular weight (80 K), drug release occurred most rapidly. In this case, because the particle size slightly increased, the rate of drug release must be reduced apparently. However, the result indirectly indicates that another factor mainly acted on drug release besides a particle size effect. The difference in the internal microstructure mentioned previously could explain this release behavior. The rapid release is possibly related to a coarse crystalline microstructure, which could be understood as follows. As the molecular weight increases, the crystallinity is considerably reduced, and long period length increases (see Table 2). Based on these facts, when the molecular weight is large, the amorphous region will be wide open and form a coarse crystalline microstructure through which the drug will diffuse rapidly. Thus, it was found that the internal crystalline microstructure compared with particle size effect plays an important role in drug release.

Taking drug release behavior into consideration for these two factors, we attempted to interpret the drug release mechanism systematically. On the foundation of this study, we intended to change the internal microstructure of PCL microparticles artificially in order to obtain the more sustained release behavior. Thus, the samples with different thermal histories were prepared. Namely, the samples prepared under same processing condition were annealed at 25, 40, 50 °C for 4 days. Fig. 7 shows in vitro drug release profiles from these samples. The release profiles of the microparticles treated at 25 and 40 °C were very similar. However, when annealing temperature was 50 °C, it showed a relatively slower drug release. Such a delayed pattern is probably due to changes in the crystalline microstructure. As mentioned in Section 3.3 earlier, these samples have the same crystallinity and different long period length and crystal size. In the case of 25 and 40 °C, their release behaviors are similar because there is little difference, whereas in the case of 50 °C, long spacing increases about 2 nm as compared with 25 °C (Table 2). Therefore, the size of lamellae for the annealing sample at 50 °C is larger than the one for the annealing sample at 25 °C. Moreover, crystallites of PCL microparticles become more perfect with the increase in annealing temperature (Fig. 4a). Because lamellae plays the role of a barrier in the diffusion of the drug, the bigger size and the more perfectly shaped crystalline lamellae of PCL

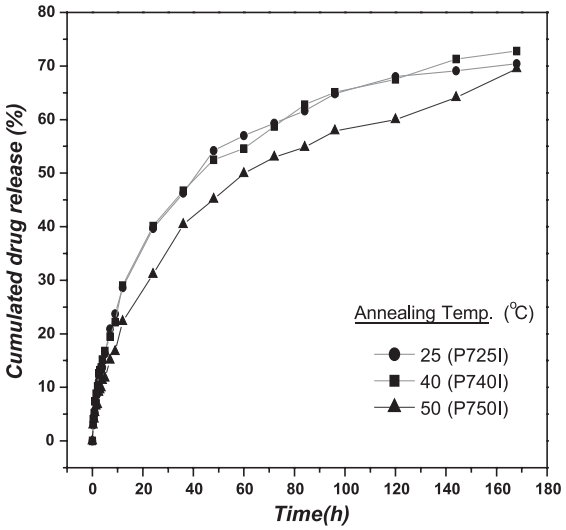

Fig. 7. In vitro drug release profiles from PCL microparticles prepared with a different thermal history for 1 week.

microparticles should be an effective obstacle for drug release, which offers a direct cause of sustained drug release [40–42].

4. Conclusions

The release of papaverine from PCL microparticles is controlled by drug diffusion through the amorphous region of the polymer matrix, not by polymer erosion. The environment for drug diffusion changes according to the processing conditions. The sample prepared with a higher PCL solution concentration showed a lower drug release rate, which is caused by the difference in the particle size without the effect of the microstructure, whereas in the case of a high molecular weight, the microstructure had a coarse internal structure, which resulted in a rapid release. This result implied that the drug release behavior is affected by the internal microstructure. Moreover, the internal crystalline microstructure of PCL microparticles was successfully controlled by applying different thermal histories without altering the overall crystallinity. The sample annealed at a higher temperature exhibited tendency to more sustained release pattern, indicating that the internal crystalline microstructure plays an important role in drug release behavior.

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