ELSEVIER

International Journal of Pharmaceutics 181 (1999) 235–242

international journal of pharmaceutics

Clonazepam release from bioerodible hydrogels based on semi-interpenetrating polymer networks composed of poly(ε-caprolactone) and poly(ethylene glycol) macromer

Chong-Su Cho [a,*], Soo-Yeon Han [b], Jeong-Hun Ha [b], Sung-Ho Kim [b], Dong-Yoon Lim [c]

[a] *Division of Biological Resources and Materials Engineering, College of Agriculture and Life Sciences, Seoul National University, 103 Serdoon-dong, Kwonsun-ku, Suwon 441-744, South Korea*

[b] *College of Pharmacy, Chosun University, Kwangju 501-759, South Korea*

[c] *Department of Pharmacology, College of Medicine, Chosun University, Kwangju 501-759, South Korea*

Received 1 August 1998; received in revised form 12 January 1999; accepted 21 January 1999

Abstract

Poly(ethylene glycol)(PEG) macromers terminated with acrylate groups and semi-interpenetrating polymer networks (SIPNs) composed of poly(ε-caprolactone)(PCL) and PEG macromer were synthesized to obtain a bioerodible hydrogel. Polymerization of PEG macromer resulted in the formation of cross-linked gels due to the multifunctionality of macromer. Glass transition temperature (T_g) and melting temperature (T_m) of PEG networks and PCL in the SIPNs were inner-shifted, indicating an interpenetration of PCL and PEG chains. Water content in the SIPNs increased with increasing PEG weight fraction due to the hydrophilicity of PEG. The amount of clonazepam (CNZ) released from the SIPNs increased with higher content in the SIPNs, lower drug loading, lower concentration of PEG macromer during the SIPNs preparation, and higher molecular weight of PEG. In particular, a combination with low PEG content and low CNZ solubility in water led to long-term constant release from these matrices in vitro and in vivo.

Keywords: Clonazepam release; Bioerodible hydrogel; Poly(ethylene glycol) macromer; Semi-interpenetrating polymer network

1. Introduction

The delivery of bioactive agents from polymeric materials has attracted the considerable attention. The trend in drug delivery technology has been toward biodegradable polymer excipients requiring no follow-up surgical removal once the drug supply is depleted. The advantages of biodegradable polymers have been described (Heller, 1984; Baker, 1987; Hsieh, 1988).

* Corresponding author.

0378-5173/99/$ - see front matter
PII: S0378-5173(99)00031-9

Clonazepam (CNZ), a chlorinated derivative of nitrazepam, is an anticonvulsant benzodiazepine widely used in the treatment of epilepsy. It is also effective in the management of some types of neuralgia (Kulkarni et al., 1966). Considering effective treatment of epilepsy and pharmacological features of CNZ such as low daily dosage, need for prolonged administrations and high first pass metabolism (Domb et al., 1988), long-term delivery of CNZ using a biodegradable system may be desirable.

In this study, we have investigated CNZ release from bioerodible poly(ε-caprolactone)(PCL)/poly(ethyleneglycol)(PEG) semi-interpenetrating polymer networks (SIPNs) in vitro and in vivo to evaluate the possibility of CNZ implantable delivery system. PCL is one of biodegradable polyesters with non-toxicity. But the PCL has very slow biodegradation (Pitt et al., 1977). The biodegradability of PCL can be enhanced by copolymerization (Jenkins, 1977), or blends (Koleske, 1978) with a variety of other polymers. We incorporated PEG known to be non-immunogenic (Merill and Salzman, 1983) into PCL chains to enhance the biodegradability of PCL (Cho et al., 1996). We also examined biocompatibility of SIPNs pellets.

It may be expected to achieve easy administration, maintenance of effective CNZ level in blood, prevention of side effects as blood levels oscillate, high therapeutic efficacy.

2. Experimental

2.1. Materials

PEGs with molecular weights 1000 and 7500 (Wako Pure Chem. Inc.), PEG with molecular weight 20 000 (Sigma Chem. Co.), PCL with molecular weight 40 000 (Aldrich Chem. Co.), CNZ(Roche, Korea), 2-hydroxy isobutyl phenol (Kansai Paint Co. LTD.) and acryloyl chloride (Janssen of Reagent Chimica) were used. All PEGs were α,ω-dihydroxy, and purified by azeotropic distillation from benzene solution. All other chemicals used were of reagent grade and were used without further purification.

2.2. Synthesis

2.2.1. Synthesis of PEG macromer

PEG macromer was prepared by the previously reported method (Cho et al., 1996). Briefly, a total 12 g (3.2 mM) of the PEG 7.5 K dissolved in 150 ml of benzene was heated to 80°C. A total of 0.89 ml (3.2 mM) of triethylamine and 0.61 ml (7.5 mM) of acryloyl chloride were added and the reaction mixture was stirred for 3 h at 80°C. After filtering triethylamine·HCl, the macromer was obtained by pouring the filtrate into *n*-hexane. Then, it was dried at 40°C under vacuum overnight.

2.2.2. Synthesis of PCL/PEG semi-IPNs

The semi-IPNs were prepared by a simultaneous IPNs method. Briefly, 10 μl of the initiator solution (100 mg of 2-hydroxy isobutyl phenol dissolved in 1 ml of *N*-vinylpyrrolidinone) was added to the methylene chloride solution of PCL and PEG macromer as the method previously reported (Kim et al., 1995). And, the mixture solution was irradiated for 5 min using a low-intensity LWUV lamp (Toshiba Chemical Lamp FL 20 LB: wave range 300–400 nm, maximum intensity 360 nm), and the solvent was then evaporated to dryness at 4°C. The solid was further dried at 40°C under vacuum. Then, the prepared semi-IPNs were repeatedly washed to remove unreacted PEG macromer with cold water for 2 days and lyophilized.

2.3. Measurements

2.3.1. Differential scanning calorimetry (DSC)

The glass transition temperature (T_g) and melting temperature (T_m) were measured with a Mettler DSC-30 differential scanning calorimeter. The measurement were carried out in the range of −100–100°C under nitrogen at a scanning rate of 10°C/min.

2.3.2. Water content

Purified and dried SIPNs discs (7 mm in diameter and 2 mm in thickness) without drug were incubated in distilled water at 37°C. At preset time intervals, hydrated samples were weighed after blotting the surface water with filter paper.

Water contents were calculated as $(W_s - W_d)/W_s \times 100\%$, where W_s and W_d are wet weight and dry weight of the SIPNs, respectively.

2.3.3. In vitro degradation

The dried polymer discs were equilibrated in phosphate buffered saline (PBS) solution (0.1 M, pH 7.4) which contained 0.02 wt.% sodium azide to inhibit bacterial growth at pH 7.4 and incubated at 37°C. Weight loss was monitored gravimetrically at various intervals of time.

2.3.4. CNZ loading

The desired amount of CNZ sieived through 150 μm pores was dispersed in a methylene chloride solution of PCL and PEG macromer. The dispersed solution was then exposed to the LWUV lamp for 5 min and the solution was then evaporated to dryness at 4°C. The solid residue was further dried at room temperature under vacuum for 1 day. CNZ loading content was calculated as $(W_{CNZ}/(W_{polymer}W_{CNZ}) \times 100)$.

2.3.5. In vitro release

Dried polymer discs of CNZ loaded polymer were introduced into a vial containing 10 ml of PBS (0.1 M, pH 7.4). The contents were mixed in a shaker and the temperature was maintained at 37°C. At pre-determined time intervals, aliquots of 10 ml of the aqueous solution were withdrawn and another 10 ml of PBS was put into the vial. The concentration of CNZ released was estimated at 306 nm using a Shimadzu UV-1201 spectrophotometer. The cumulative amount of CNZ released from the SIPNs was determined from the appropriate calibration curves. The data represents mean $\pm$ S.D. from three independent experiments.

2.3.6. In vivo release

Four male rabbits, initial weights $2.5 \sim 2.8$ kg were housed and free access to food and water. After animals were anesthetized with thiopental-sodium (40 mg/kg) intraperitoneally. CNZ pellets ($95 \sim 105$ mg) were implanted and tissues were suctured.

Blood samples (2 ml each) were collected through ear vein to determine the released CNZ concentrations. Samples were instantly centrifuged at 3000 rpm to separate plasma for 15 min. Deproteinization agents (Cadmium sulfate 1.5 ml, 1 N sodium hydroxide solution 0.5 ml, and distilled water 5 ml) were added to plasma 0.5 ml. The plasma was again centrifuged at 3000 rpm for 15 min. Plasma concentration of CNZ was estimated by a UV spectrophotometer.

2.3.7. In vivo biocompatability tests

At 45 days after pellet implantation, some tissues of implantation sites were taken and examined microscopically to confirm whether morphological and pathological changes were induced or not.

3. Results and discussion

Water-soluble PEG macromer forms a cross-linked three-dimensional networks upon free-radical polymerization. These PEG macromers underwent rapid photo-polymerization, even in the presence of oxygen. But UV curing time was 5 min to achieve complete three-dimensionally cross-linked network. Oxygen is usually an inhibiter of free-radical polymerization. But when PEG is end-capped with hydrophobic polymerizable units, it forms micellar structure in water. This micellar structure raises the effective condensation of double bond within the micelle, which leads to an increased rate of the propagation reaction and a decrease in the termination rate in a free-radical polymerization. Those results in a high polymerization rate and rapid gelation. After photo-polymerization, the gel content of 1.0, 7.5 and 20 K PEG macromers were 78.1, 94.0 and 88.8 wt.%, respectively (concentration of PEG macromer: 30 wt.%: w/w). Non-cross-linked PCL chains were interpenetrated into the cross-linked three-dimensional networks of PEG. It is expected that PEG macromer forms a gel network and PCL chains entangled through the gel network resulting in semi-IPNs (SIPNs). The SIPNs films were mechanically strong enough to handle and getting stronger with increasing PCL content due to the relative hydrophobicity of PCL. Physical properties were improved by independently cross-linking

a hydrophobic network within the cross-linked hydrophilic network (Eschbach and Huang, 1993). This mixture of two independently cross-linked polymers that cannot be physically separated is an interpenetrating polymer networks (IPNs). If only one of the two polymers is cross-linked, the product is called a semi-interpenetrating polymer networks (SIPNs). It is usually made by polymerizing or cross-linking one component in the presence of the other. In this system, PEG macromer is cross-linked in the presence of PCL by UV. In order to make a SIPNs, two polymers should dissolve in the same solvent. In this system, methylene chloride was used as the cosolvent of two polymers.

Results of DSC studies were shown in Table 1. These results showed that the glass transition temperature (T_g) and melting point (T_m) of PCL for the SIPNs (PEG/PCL: 70/30) occurs at -43.0 and 55.8°C, respectively. Those can be compared with the T_g and T_m of the parent component PCL that occurs at -52.0 and 64.6°C. This displacement of 9.0 and 8.8°C shows that the formation of the interpenetrating PCL and PEG chains or existence of some degree of miscibility (Olabisi et al., 1979). The T_g of PCL in the SIPNs were also inner-shifted. Also the more inner-shift in T_g of PCL was observed with increasing PEG weight fraction in the SIPNs. T_m of PCL in the SIPNs were also inner-shifted with an increase of PEG in the SIPNs as shown in the Table 1. Furthermore, T_m of cross-linked PEG in the SIPNs were decreased with an increase of PCL content in the SIPNs, whereas T_m of cross-linked PEG itself was at 51.8°C.

Table 1
Thermral properties of PEG/PCL SIPNs measured by DSC[a]

Polymer	T_g (°C)		T_m (°C)	
	PEG	PCL	PEG	PCL
Cross-linked PEG	−14	–	51.8	–
PEG/PCL (70/30)	N.M.[b]	−43	47.8	55.8
PEG/PCL (50/50)	N.M.[b]	−48	45.9	56.1
PEG/PCL (30/70)	N.M.[b]	−50	N.M.[b]	53.1
PCL	–	−52	–	64.6

[a] M.W. of PEG: 7500.
[b] N.M.: non-measurable.

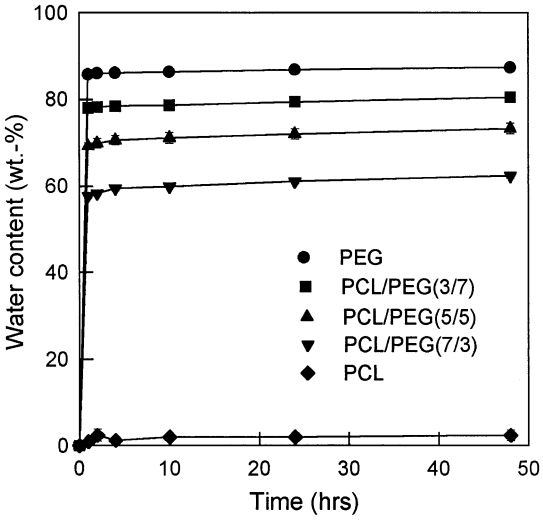

Fig. 1. Water content of PCL/PEG SIPNS against weight fraction of PEG in distilled water at 37°C (conc. of PEG macomer: 20 wt.%). Each point represents the mean ± S.D. of at least three experiments.

Water content of PCL/PEG SIPNs versus an incubation time according to the weight fraction of PEG in distilled water at 37°C is shown in Fig. 1. These results showed that water contents were dependent on the weight fraction of PEG in the SIPNs and increased with increasing PEG weight fraction due to the hydrophilicity of PEG. Also, the water contents rapidly increased with incorporation of PEG in the SIPNs, whereas PCL itself got little water-uptake with time. SIPNs composed of PCL and PEG macromer are expected to have different degrees of matrix hydration depending on the nature, cross-link density and amounts of PEG. Water content of PEG network against an incubation time according to the molecular weight (M.W.) of PEG is shown in Fig. 2. These results show that the water content of the PEG network is dependent on the M.W. of PEG. The higher M.W. of PEG, the larger the water content. It is probable that the higher is the molecular weight of PEG macromer, the lower is the cross-linking density of the PEG network and thus the higher water content. Also, the water

content was dependent on the concentration of PEG macromer in the SIPNs preparation. The higher concentration of the PEG macromer, the smaller the water content. It is probable that higher concentration of PEG macromer in the SIPNs preparation causes the higher cross-linking density.

Total released of CNZ from the PCL/PEG SIPNs against weight fraction of PEG is shown in Fig. 3. These results indicate that the release of CNZ from the SIPNs increased with incorporation of PEG in the SIPNs. As shown in Fig. 3, the larger the amounts of PEG, the faster the release of the drug due to more hydration of the SIPNs.

Release rate of CNZ from PCL/PEG (60/40: w/w) SIPNs is shown in Fig. 4. It was found that the release rate increased with incorporation of PEG in the SIPNs. Also, the larger the amounts of PEG, the more increased of release rate. The release rates for PCL and PCL/PEG SIPNs after initial rapid release fluctuated around 200 and 300 μg/day/kg for 250 days, respectively. Therefore, the increase of the release rate was attributed to the increase of hydrophilicity of PEG in the SIPNs. Accordingly, the penetration of water molecule within SIPNs was easier and made the transport of the drug to the surrounding aqueous medium faster.

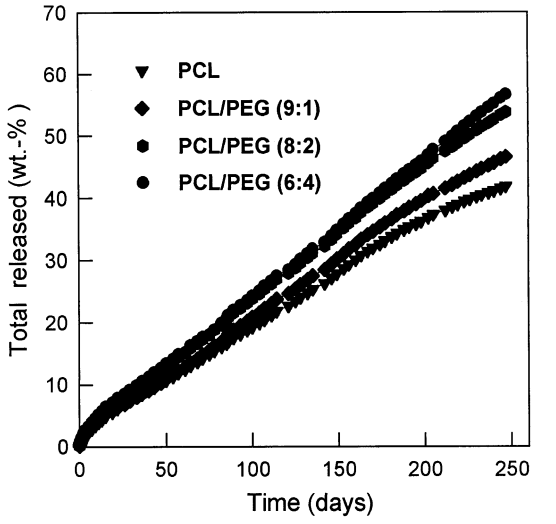

Fig. 3. Total released of CNZ from the PCL/PEG SIPNs against weight fraction of PEG in PBS buffer at 37°C. Each point represents the mean ± S.D. of at least three experiments.

Total released of CNZ from the PCL/PEG SIPNs (80/20: w/w) against concentration of PEG macromer in the SIPNs preparation is shown in Fig. 5. These results indicate that the lower concentration of PEG macromer in the SIPNs lead to a fast drug release than higher concentration of PEG macromer. An increase of concentration of

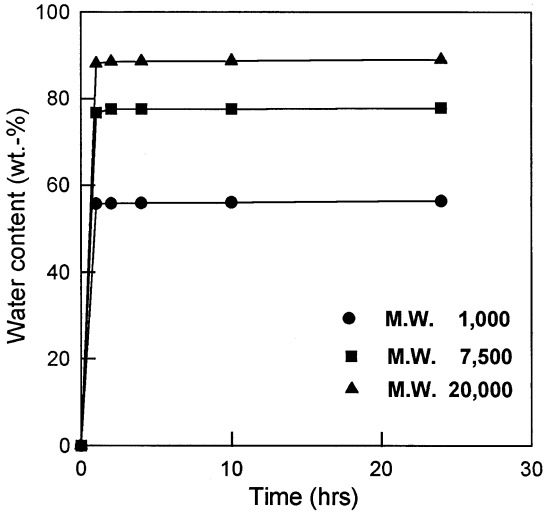

Fig. 2. Water content of PEG network against molecular weight of PEG in distilled water at 37°C. Each point represents the mean ± S.D. of at least three experiments.

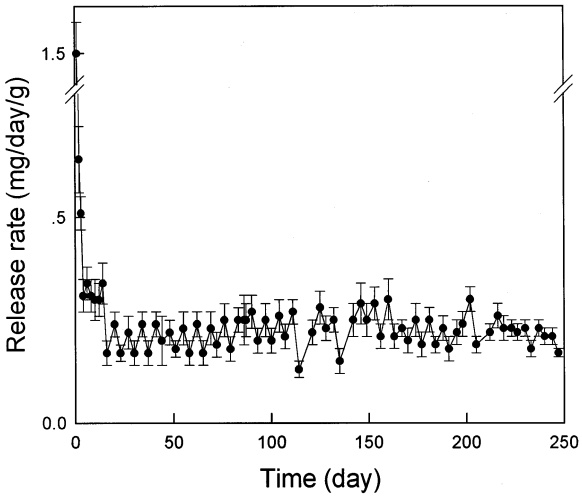

Fig. 4. Release rate of CNZ from PCL/PEG (60/40: w/w) SIPNs in PBS buffer at 37°C. Each point represents the mean ± S.D. of at least three experiments.

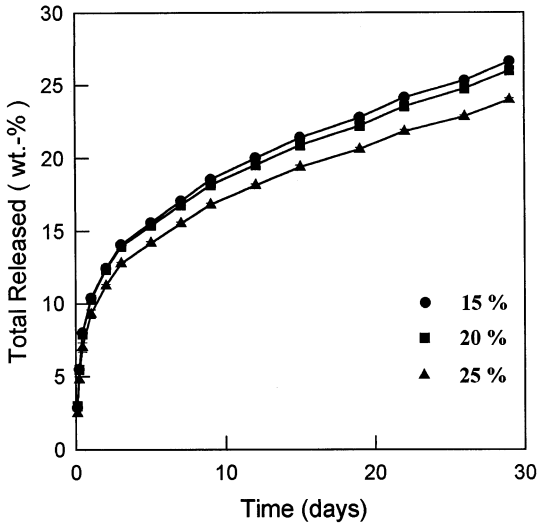

Fig. 5. Total released of CNZ from the PCL/PEG (60/40: w/w) SIPNs against concentration of PEG macromer during the SIPNs preparation (M.W. of PEG: 7500 and drug loading: 5 wt.%). Each point represents the mean ± S.D. of at least three experiments.

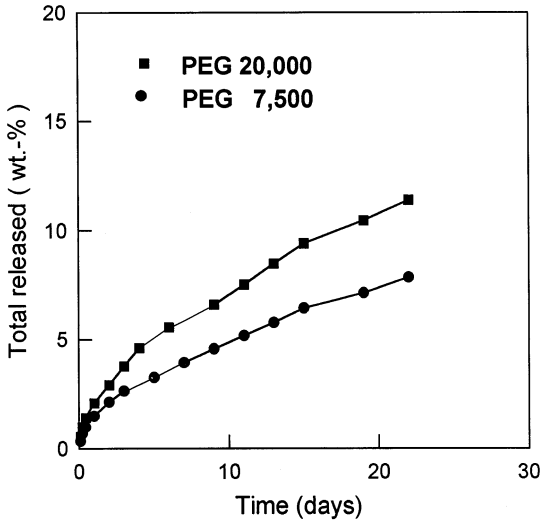

Fig. 6. Total released of CNZ from the PCL/PEG (80/20: w/w) SIPNs against M.W. of PEG (conc. of PEG macromer: 20 wt.% and drug loading: 5 wt.%). Each point represents the mean ± S.D. of at least three experiments.

the PEG macromer in the SIPNs preparation causes the increased probability that diacrylate unit of the PEG is the site of a cross-linking bridge. Therefore, the higher concentration of PEG macromer leads to the formation of the higher cross-link density of PEG network than lower one of it. Accordingly, the penetration of water molecules within SIPNs prepared by lower concentration of PEG macromer was easier and made the drug release to the surrounding aqueous medium faster.

Total released of CNZ from the PCL/PEG (80/20: w/w) SIPNs against M.W. of PEG macromer is shown in Fig. 6. As shown in the figure, the higher molecular weight of PEG macromer, the faster released. This was attributed to the increase of water content with the higher molecular weight of PEG macromer than lower one as shown in Fig. 4.

Fig. 7 shows total released of CNZ from the PCL/PEG SIPNs (70/30: w/w) against drug loading. As shown in the figure, the smaller the amounts of loaded CNZ, the faster CNZ release due to the relatively hydrophobic drug properties. Therefore, as the drug becomes more hydrophobic by increasing the amounts of drugs, hydrophobic interaction is stronger and drug release is slower.

Fig. 8 shows degradation profiles for PCL/PEG SIPNs against incubation time according to the

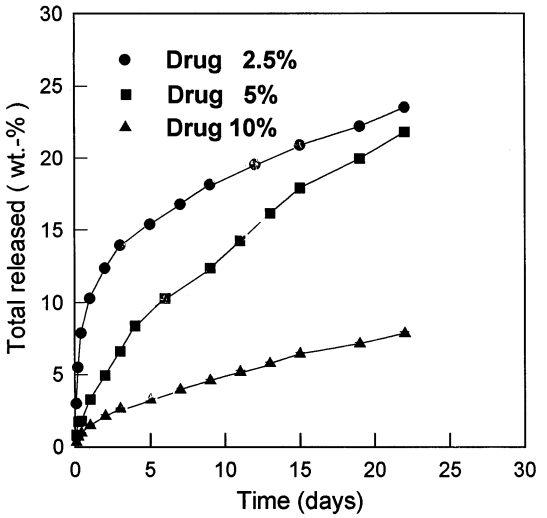

Fig. 7. Total released of CNZ from the PCL/PEG (60/40: w/w) SIPNs with different drug loading (M.W. of PEG: 7500 and conc. of PEG macromer: 20 wt.%). Each point represents the mean ± S.D. of at least three experiments.

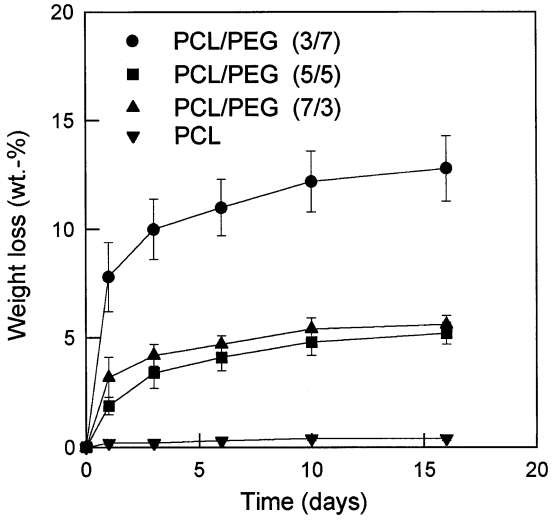

Fig. 8. Degradation of the PCL/PEG SIPNs in vitro against weight fraction of PEG. Each point represents the mean ± S.D. of at least three experiments.

weight fraction of PEG in the SIPNs in PBS solution, pH 7.4 at 37°C. These results showed that the degradation rate for the SIPNs decreased with increasing of PCL weight fraction as the similar behaviors of the water content. These

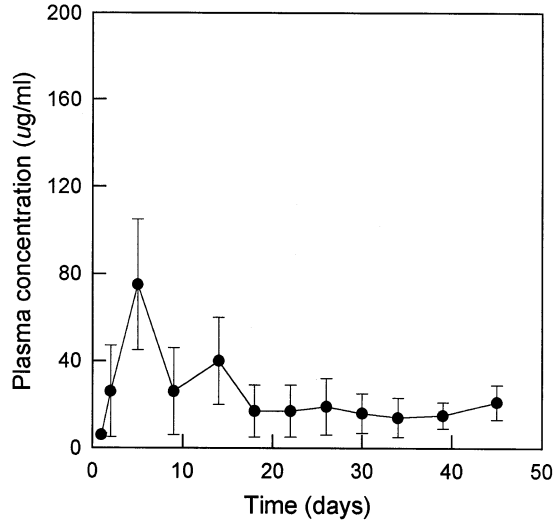

Fig. 9. Plasma concentration of CNZ after the s.c. implantation (conc. of PEG macromer: 20 wt.%, drug loading: 5 wt.%). Each point represents the mean ± S.D. of at least four experiments.

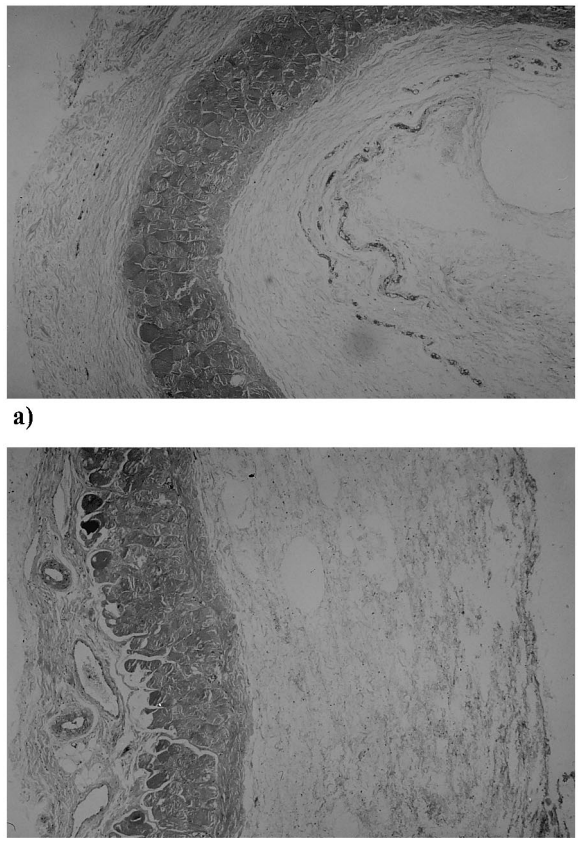

Fig. 10. Hematoxylin and eosin-stained sections of tissue at 45 days after subcutaneous implantation of CNZ-loaded PCL/PEG (60/40: w/w) SIPNs into rabbit; control (a) and CNZ-loaded SIPNs (b).

results are attributed to the hydrophilic nature of PEG which increases the accessibility of water to the polymer matrix. Also, PCL has been known to degrade very slowly because of its hydrophobic structure which does not allow fast water penetration. PCL degradation by random hydrolytic chain scission of the ester linkages has documented by Pitt et al. (1981). But in this time, it is not clear whether the weight loss of the SIPNs comes from enhanced degradation of PCL by the PEG or hydrolysis of PEG-acrylate network. The PEG-acrylate network is known to be relatively stable.

Plasma concentration of CNZ after S.C. implantation ($n = 4$) is shown in Fig. 9. It was found

that the plasma concentration of CNZ was almostly constant for 45 days after initial burst effect.

Hematoxylin and eosin-stained sections of tissue at 45 days after subcutaneous implantations of CNZ sustained release dosage form is shown in Fig. 10. In comparison with normal control group (a), a little intercelluar edema, a few neutrophlic filtrates in the peritorium, and a little dilatation of vascular channels (b) were only observed.

References

Baker, R., 1987. Controlled release of biologically active materials. John Wiley and Sons, New York.

Cho, C.S., Ha, J.H., Kim, S.H., Han, S.Y., Kwon, J.K., Sung, Y.K., 1996. Tetracycline release from bioerodible hydrogels based on semi-interpenetrating polymer networks composed of poly(ε-caprolactone) and poly(ethylene glycol) macromer in vitro. J. Polym. Sci. 60, 161–167.

Domb, A., Ron, E., Langer, R., 1988. Polyanhydrides. II. One step polymerization using phosgene or diphosgene as coupling agents. Macromolecules 21, 1925–1929.

Eschbach, F.O., Huang, S.J., 1993. Interpenetrating polymer networks, Advances in chemistry series 239. Am. Chem. Soc., New York, 205.

Heller, J., 1984. Bioerodible systems. In: Langer, R., Wise, D.L. (Eds.), Medical applications of controlled release, vol. 1. CRC Press, Boca Raton, pp. 70–98.

Hsieh, D.S.T., 1988. Controlled release systems: Fabrication technology, vol. 2. CRC Press, Boca Raton.

Jenkins, V.F., 1977. Caprolactone and its polymers. Polym. Paint Colour J. 167, 626–627.

Kim, S.S., Lee, Y.M., Cho, C.S., 1995. Synthesis and properties of β-chitin and poly(ethylene glycol) macromer. Polymer 36, 4497–4501.

Koleske, J.V., 1978. Blends containing Poly(ε-caprolactone) and releated polymers. In: Paul, D.R., Newan, S. (Eds.), polymer biends, vol. 2. Academic Press, New York, pp. 369–389.

Kulkarni, R.K., Pani, K., Neuman, C.C., Leonard, F., 1966. Polylactic acid for surgical implants. Arch. Surg. 93, 839–843.

Merill, E.W., Salzman, E.W., 1983. ASAIO 6, 60.

Olabisi, O., Robeson, L.M., Shaw, M.T., 1979. Polymer–polymer miscibility, Academic Press, New York (chapter 3).

Pitt, C.G., Christensen, D., Jeffcoat, R., Kimmel, G.L., Schindler, A., Wall, M.E., Zweidinger, R.A., 1977. In: Gabelnick, L.H. (Ed.), Proceedings: Drug delivery system, DHEW Publication No. (NIH) 77:1238, Baltimore, MD, pp. 142–192.

Pitt, C.G., Gratzl, M.M., Kimmel, G.L., Surles, J., Schindler, A., 1981. Aliphatic polyesters. II The degradation of poly(DL-lactide), poly(ε-caprolactone), and their copolymers in vivo. Biomaterials 2, 215–220.