ELSEVIER

International Journal of Pharmaceutics 239 (2002) 93–101

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

A local delivery system for fentanyl based on biodegradable poly(L-lactide-*co*-glycolide) oligomer

Sun-Ah Seo [a], Hak Soo Choi [b], Gilson Khang [a], John M. Rhee [a], Hai Bang Lee [b,*]

[a] *Department of Polymer Science and Technology, Chonbuk National University, Dukjin Dong, Chonju 561-756, South Korea*
[b] *Biomaterials Laboratory, Korea Research Institutes of Chemical Technology, PO Box 107, Yusung, Taejon 305-606, South Korea*

Received 21 November 2001; received in revised form 15 January 2002; accepted 15 February 2002

Abstract

To obtain a sustained fentanyl delivery with effective and precise control, fentanyl loaded wafer was fabricated using poly(L-lactide-co-glycolide) (PLGA) oligomer by direct compression method. XRD and DSC analysis indicated the presence of crystalline drug in the wafers. The release of fentanyl from PLGA wafer was determined to be primarily diffusion controlled, but swelling and erosion also contributed to the release process. In vitro release studies showed that different release patterns and rates could be achieved by simply modifying factors in the preparation conditions. The wafer degradation profiles were also investigated to understand the drug release mechanism. Gravimetric studies of mass loss of wafers during the incubation revealed that the weight loss increased apparently after 4 days. These results indicate that the polymer degradation was contributed to drug release followed by diffusion. From the results, this constant localized release system can potentially provide anesthesia for a longer period than injection or topical administration.

Keywords: Fentanyl; Poly(L-lactide-co-glycolide); Local delivery; Gravimetric study

1. Introduction

Biodegradable polymers produce biocompatible, toxicologically safe by-products that are further eliminated by the normal metabolic pathways. It would deliver the drug at a continuous rate and reduce the administration difficulties (Maria et al., 2000). Poly(D,L-lactide-co-glycolide) (PLGA) has been widely used as carriers in controlled-release delivery systems due to its biodegradability and relatively good biocompatibility (Jalil and Nixon, 1990; Wu, 1995; Khang et al., 1999, 2000). The FDA has also approved it for drug delivery use, so it has been used for the study of a controlled release system over the past decade. The direct compression method has the following advantages: (1) it can control the release rates even for low molecular weight drugs; (2) it is applicable for unstable drugs such as peptides because no heating (Chandy and Sharma, 1992;

* Corresponding author. Tel.: +82-42-860-7220; fax: +82-42-861-4151.
E-mail address: hblee@krict.re.kr (H.B. Lee).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00074-1

Tateshita et al., 1993) or contact with organic solvent (Bakai et al., 1990; Sansdrap and Moes, 1993), such as the chlorinated solvent often used in the preparation of microspheres is required (Chuo et al., 1996); (3) the release rates can be controlled easily by appropriate selection of polymer species and formulation. The drug-release pattern from PLGA wafer is biphasic, combination of diffusion and biodegradation (Lewis, 1990). Initially, drug is released via diffusion through the polymer matrix as well as through the porous voids of the polymer structure, but biodegradation of PLGA continuously changes the drug-release pattern. The second process involves bulk erosion: the polymer matrix uptakes water and the polymer chains are degraded small enough to be soluble, and drug is released during the dissolution of the PLGA matrix. The initial burst is caused by release of drug that embedded in a superficial region of the wafer (Arshady, 1990; Lewis, 1990).

We have been investigating various approaches to deliver the anesthetics with zero-order release profiles. In our previous papers, we have discussed about fabrication of fentanyl delivery system, especially microspheres using PLGA (Choi et al., 2001) as well as the analysis method of fentanyl (Choi et al., 2002). In this system, they released anesthetics over 15 days with first-order release pattern although the optimum release period, we expected, is about 10 days. Based on the above observation, PLGA oligomer wafers were employed to develop anesthetic delivery system within 10 days with zero-order release pattern. In this study, we demonstrated the possibility of precise and effective control of fentanyl administration with the PLGA local devices. In order to understand the drug release mechanism from wafer, the effects of the preparation conditions on morphology and release profiles were investigated. The pattern of the drug release depends on various factors: initial drug/polymer loading ratio, wafer thickness, and additive content. The water-soluble polymer hydroxypropyl methylcellulose (HPMC) was used in order to adjust the water permeability of PLGA and so attempt to achieve a zero order release of fentanyl over long period. The physical characteristics were studied using X-ray analysis and differential scanning calorimeter (DSC) analysis. Also, the in vitro release pattern of fentanyl and the morphology of wafer have been investigated by means of gas chromatography with a nitrogen-phosphorous detector (GC-NPD) and scanning electron microscope (SEM), respectively.

2. Materials and methods

2.1. Materials

Fentanyl base was purchased from McFarland Smith (Edinburgh, UK). PLGA 50:50 (PLGA-5005, molecular weight: 5000 g/mol) was obtained from Wako Chem. Co., Ltd. (Osaka, Japan). The matrix binder HPMC was purchased from Sigma Chem. Co., Ltd. (St. Louis, MO). The extraction solvent such as toluene, *n*-butyl chloride, and methanol were also obtained from Sigma Chem. Co., Ltd. (St. Louis, MO). Water was obtained by a Milli-Q purification system from Millipore (Molsheim, France). All other chemicals were of analytical grade and used with distilled purification.

2.2. Preparation of wafer

Fentanyl loaded PLGA wafers (5.0×0.9 mm^2 base) were prepared by direct compressing the drug–polymer mixture using a manual press (MH-50Y, Masada, Japan). Before the preparation of the wafer, to decrease the crystallinity of fentanyl and to make homogeneous and minuted powder, we used freezer-mill (SPEX 6700, Metuchen, USA) freezing with liquid nitrogen. PLGA and drug mixture was obtained homogeneous powder and put into 5 mm diameter mold then pressed (MH-50Y, Japan) under 40 kgf/cm^2 at room temperature. And these wafers were stored in refrigerator until analyzed morphology and in vitro release. The variables and conditions of wafer formulations are listed in Table 1. The effects of initial drug loading ratio, different thickness of wafers, and additive content on the fentanyl release profile have been investigated.

2.3. X-ray analysis

Powder X-ray diffraction (XRD, D/MAXIII, Rigaku, Japan) patterns were obtained to study physical state of drug, polymer, and drug–polymer mixture. The test was carried out at 40 kV of voltage and 25 mA of current. The scanning rate was 4°/min over a 2θ range of 1.5–40°. Physical mixture was made of grinding drug and polymer material.

2.4. DSC analysis

DSC was used to determine the glass transition temperature (T_g) and melting point (T_m) of fentanyl loaded PLGA wafer. The wafer samples for thermal analysis were equilibrated with PBS for 11 days at 37 °C, subsequently, dried, weighed, and finally analyzed with DSC. Thermal analysis was performed using a Metler TA 4000 system with a DSC equipped with a computerized data station (Mettler DSC, AC, USA). All samples were heated at scanning rate of 10°C/min between 30 and 270 °C.

2.5. In vitro release test

The fentanyl release from the wafers was established by emerging the wafers into 40 ml of phosphate-buffered saline (PBS, pH 7.4). The sample tubes were incubated at 37 °C under continuous shaking. The release medium (200 μl) was periodically taken out from the tube with pipette and same volume of the fresh medium was replaced. To extract fentanyl from the buffer solution, 10 μl of IS (1 μg/ml) was added to 200 μl of sample in a centrifuge tube. The aqueous phase was extracted with 600 μl of 5% isopropyl alcohol in *n*-butyl chloride. The tube was vortex-mixed and the upper organic phase was transferred to a second centrifuge tube. The sample was evaporated in a vacuum concentration system (Spinvac, Hanil, Korea) at 40 °C. Extraction residue was reconstituted in 50 μl toluene, sonicated, and centrifuged at 12000 rpm. The solution was injected into the GC system via splitless mode.

2.6. Quantitative analysis of fentanyl

The amount of fentanyl released from the wafer was determined by GC analysis as described before (Choi et al., 2001). Chromatography was performed on a Hewlett–Packard 6890 GC, equipped with an autosampler (HP 7683) and a NPD. High purity helium was used as the carrier gas at a constant pressure of 25 psi. A HP-5 5% phenyl methyl siloxane capillary column (60 m × 0.32 mm I.D. and 0.25 μm film thickness) was used. The initial oven temperature was 150 °C for 1 min. The oven temperature was programmed to 270 °C at 30 °C/min, held 2 min, then to 280 °C at 5 °C/min, and held 9 min (overall run time 18

Table 1
Preparation conditions of fentanyl-loaded PLGA wafers ($n = 3$)

Batch	Drug ratio (%)	HPMC (%)	Weight (mg)	Thickness (mm)
FW1	3	–	20	0.90
FW2	5	–	20	0.90
FW3[a]	10	–	20	0.90
FW4	20	–	20	0.90
FW5	10	–	10	0.45
FW6	10	–	50	2.25
FW7	10	–	100	4.50
FW8	10	2	20	0.90
FW9	10	5	20	0.90
FW10	10	10	20	0.90

[a] Control.

min). The temperature of the injector and the detector were maintained at 285 and 310 °C, respectively. Flow rates were 2.0 ml/min for the helium gas, 60 ml/min for air, and 3.0 ml/min for hydrogen. Deactivating all glassware, including disposable culture tubes and the injection port liner, with a 5% solution of dimethyldichlorosilane and vapor of hexamehyldisilazane (HMDS) were necessary to avoid adsorption of the drug onto the glassware and optimize recovery.

2.7. Water uptake and mass loss of wafer

In order to study degradation and weight loss of wafer, incubation was performed in the same conditions as those used for the drug release studies. At different time intervals, the samples were collected and dried for at least 24 h at room temperature under vacuum. The weight loss was determined gravimetrically (Hausberger and DeLuca, 1995). Water uptake was calculated according to the difference between the wet weight (W_w) and the dry weight (W_d) and expressed by the relationship:

$$\text{Water uptake } (\%) = (W_w - W_d) \times 100/W_d$$

The mass loss was expressed as:

$$\text{Mass loss } (\%) = (W_o - W_d) \times 100/W_o$$

where W_o is the implant weight determined initially. The dried wafer samples were further used for morphological characterization.

2.8. SEM observation

In order to examine the surface and cross-sectional morphology, microphotographs of the wafers were observed with SEM (S-2250N, Hitachi, Japan) operated at 15 kV. Before SEM observation, all samples were mounted on metal stubs and coated with a thin layer of platinum by means of a plasma sputtering apparatus (Emscope, SC 500K, UK) under argon atmosphere. To obtain a cross-sectional morphology, the fractured samples were prepared by submerging the wafers into liquid nitrogen. The observations were studied before and after in vitro test.

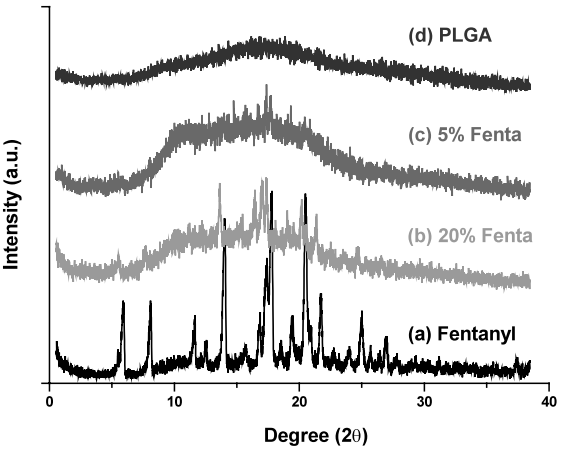

Fig. 1. Powder X-ray diffraction patterns of (a) fentanyl, (b) FW4 (after 10 days), (c) FW2 (after 10 days) and (d) PLGA.

3. Results and discussion

3.1. Physical characteristics of FWs

To evaluate the crystallinity and miscibility of fentanyl and PLGA, XRD and DSC analysis were employed. As shown in Fig. 1, the XRD patterns show the decrease of fentanyl crystals at the after 10 days of the release study. Fentanyl showed several peaks corresponding to the crystalline form while PLGA is mainly amorphous with a poor crystalline part. In contrast, samples of wafer showed a halo pattern in which diffraction peaks of the drug decreased indicating that fentanyl in both preparations was in the amorphous state. These results proved that the drug was dispersed in a molecular state of PLGA. In these graphs, more fentanyl remained in FW4 (20% fentanyl loading) than FW2 (5% fentanyl loading), and this result coincides with Fig. 3. On the other hand, the crystals of copolymers were appeared after drug release. The original PLGA was an amorphous copolymer with a glass transition temperature (T_g) at 49.0 °C. However, after degrading in release medium for 10 days, a T_m could be detected and the T_g was found to move to a higher temperature, though the thermograms are not presented. This means that the PLGA copolymer degraded to acid forms and changed from an amorphous state to a crystalline state.

3.2. Morphology observation

Fig. 2 shows SEM microphotographs of the surface and cross-sectional morphology of 0, 1 and 4 days after in vitro release test for 10% fentanyl-loaded PLGA wafer. The SEM micrographs indicate that wafer degradation occurs in a heterogeneous manner, i.e. degradation proceeds more rapidly in the center than at the surface. This is manifested by the rapid progression in pore formation within the wafer in comparison to the relatively intact surface crust. This behavior has recently been demonstrated for a variety of polyester microspheres (Li et al., 1990). This results in the enhanced autocatalytic hydrolysis in the central region of the matrix, while the surface region, which is in contact with the bulk medium, degrades at a slower rate.

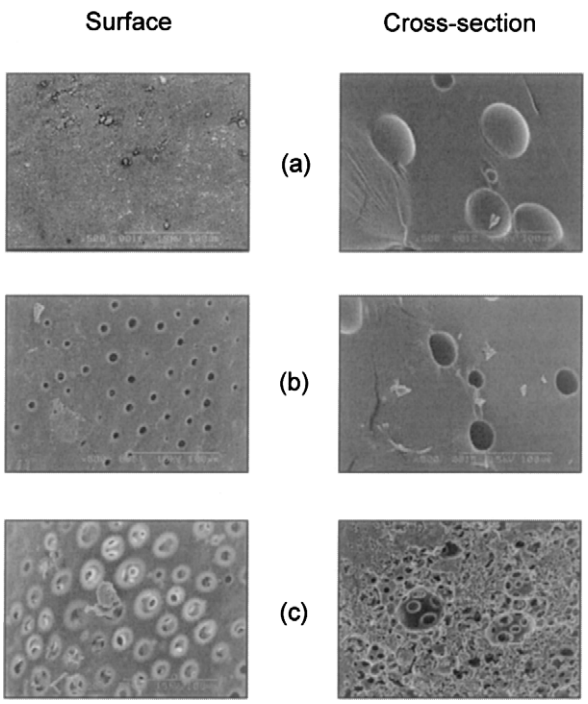

Fig. 2. SEM micrographs of wafer FW3: (a) before in vitro, (b) after 1 day and (c) after 4 days release (original magnifications × 500).

3.3. Effect of initial drug loading ratio

To determine an optimal drug loading, various ratios of initial drug loading to the polymer matrix, 3, 5, 10 and 20%, were investigated (Table 2). For these formulations, a higher drug loading resulted in a higher initial burst (Fig. 3). Fentanyl-loaded PLGA wafers produced from direct compression with a drug loading of 20% (FW4) had an initial burst of 10.7%. This initial burst could be reduced to 2.4% by lowering the drug loading to 3% (FW1). Analysis by scanning electron microscopy revealed that FW4 had more drugs on the surface than FW1 (results are not shown). The increasing of drug on the surface may account for the increase in diffusion of the drug from the surface during the initial release phase. The lower loading can avoid the occurrence of the initial burst, which can be related to the reduced probability that a drug particle is situated on or near the surface of the device. Thus, a lower loading will be the best choice if a constant and a lower release-rate is desirable. In addition to the initial burst, drug loading also affected the erosion-controlled release phase. As shown in Fig. 3, FW4 had a daily release rate of about 5% whereas FW1 had a daily release rate of about 10%. In order to obtain continuous release wafer, continuous erosion of the polymer by hydrolysis to generate new pores for continuous diffusion of drug from wafer is required. That is, the diffusional phase and erosion-controlled release phase must overlap (Cleland and Jones, 1996). In this case, the degradation of PLGA oligomer matrix is compensating the drug release followed by initial dissolution and diffusion.

3.4. Effect of wafer thickness

The influence of wafer thickness (0.45, 0.9, 2.25 and 4.5 mm) on the release profiles of drug from 10% fentanyl-loaded PLGA wafers is shown in Fig. 4. It can be seen that the thickness of wafers has a significant effect on the release pattern. The amounts of after 11 days release for the wafer thickness 0.45, 0.9, 2.25 and 4.5 mm were about 0.988 (98.8%), 1.51 (75.3%), 3.07 (61.4%), and 4.43 mg (44.3%) with zero-order release, respec-

Table 2
Water uptake and mass loss of fentanyl-loaded PLGA wafers ($n = 3$)

Batch	W_o (mg)	Time (day)	W_w (mg)	W_d (mg)	Water uptake (%)	Mass loss (%)
FW3	20.0	1	22.8	19.3	11.8	2.5
		2	24.5	19.1	28.3	4.5
		4	25.6	18.9	35.5	5.5
		6	25.9	17.6	47.2	9.0
		10	–	11.9	–	41
FW6	50.0	1	56.5	48.6	16.5	1.8
		2	59.7	48.1	24.1	3.6
		4	63.3	47.2	34.1	5.6
		6	63.9	44.9	42.3	10
		10	–	31.3	–	35
FW7	100	1	111	97.7	13.6	2.3
		2	115	96.3	19.4	3.7
		4	122	95.2	28.2	4.8
		6	126	89.1	41.4	10.9
		10	–	63.8	–	36.2
FW9[a]	20.0	1	23.9	19.3	22.6	2.5
		2	25.2	19.1	30.6	3.5
		4	25.6	19.1	34.0	4.5
		6	26.1	17.6	48.3	12
		10	–	13.6	–	32

W_o, original weight; W_w, wet weight; W_d, dried weight
[a] 20-mm wafer containing 5% HPMC.

tively. That is to say, the increase of wafer thickness significantly decreased initial burst and could be reached more close zero-order release pattern. It can be explained that this wafer acts like simple 'depot'. The thinner wafer, the faster the simple dissolution and diffusion due to small volume of depot of drug also could be happened. From these results, the desirable duration of fentanyl release can be obtained by the controlling of the wafer thickness.

3.5. Effect of additive content

Efforts have been made in order to reduce the extent of the initial burst and release rate, including adding HPMC (content with 2, 5 and 10%) into the PLGA matrix as a matrix binder. HPMC is well known binder to cause a slower release when added to a porous matrix (Li et al., 1990). In the present study, it seems that using over 10% of HPMC in the matrix does not provide any positive effect on slowing down drug release (results are not shown). As shown in Fig. 5, it has been widely recognized that the in vitro release of fentanyl from biodegradable wafers without additive (FW3) showed typical biphasic release kinetics, a slow diffusion release followed by a little faster erosion-mediated release (Cleland and

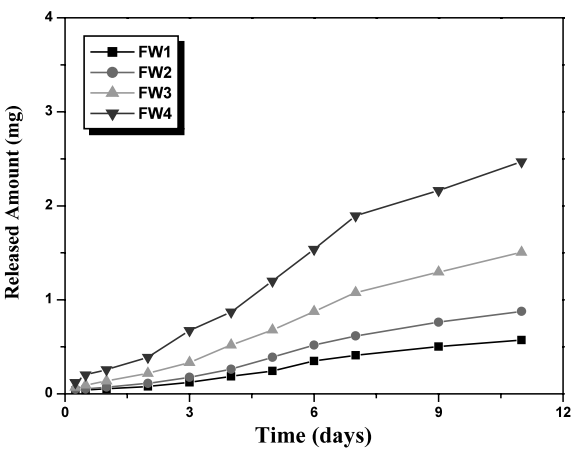

Fig. 3. Effect of initial drug loading ratio on the fentanyl release profiles (5.0×0.9 mm^2). Each point represents the mean of at least three runs.

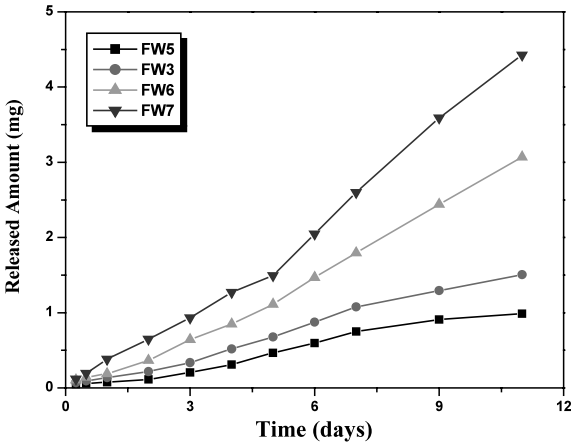

Fig. 4. Effect of wafer thickness on the fentanyl release profiles (10% drug content). Each point represents the mean of at least three runs.

Jones, 1996). However, the wafers containing HPMC as a wafer binder are shown decreased release rate after diffusion and dissolution. Among the wafers, FW9 (5% HPMC) was shown the slowest release rate of fentanyl with zero-order. FW8 and FW9 were shown similar release profiles slower than FW3. When HPMC was associated with PLGA, fentanyl entrapment efficiency was close of superior to the theoretical value. This is likely due, at least in part, to an impossible migration of drug into the aqueous

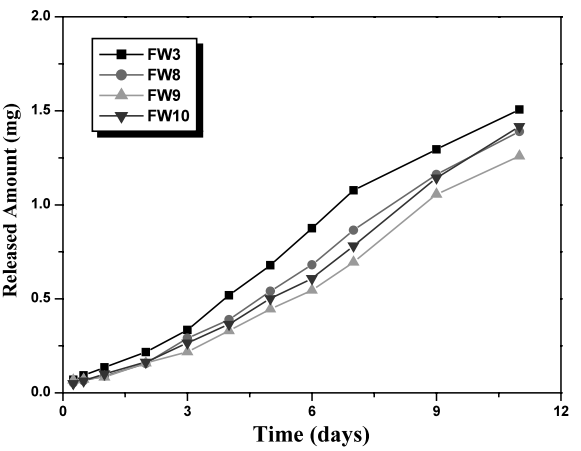

Fig. 5. Effect of HPMC concentration on the fentanyl release profiles (0.9 mm thickness). Each point represents the mean of at least three runs.

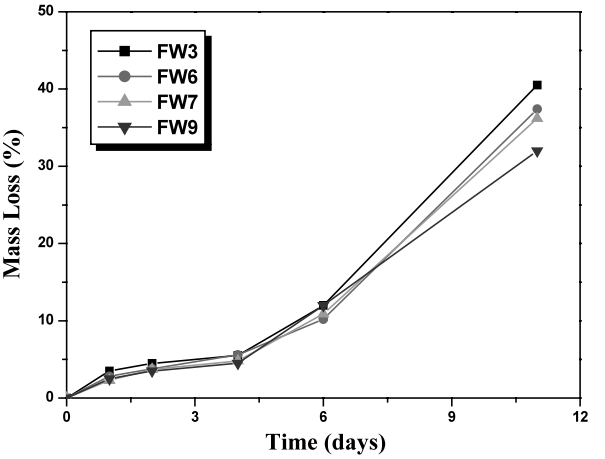

Fig. 6. Mass loss profiles of fentanyl-loaded PLGA wafers. Each point represents the mean of at least three runs.

phase by attached binder leading to control of drug/polymer ratio. From these results, it can be summarized that fentanyl release mechanism from PLGA wafer may be controlled by altering amount of additive.

3.6. Degradation and weight loss of wafer

The wafer degradation profiles were investigated to understand the drug release mechanism. The degradation of PLGA polymers is influenced by several physical and chemical factors, such as initial pH, ionic strength and temperature of the external bulk medium, molecular weight, crystallinity, exposure to gamma radiation and presence of drugs and other agents in the matrices (Kenley et al., 1987). In general, an erosion process involves three steps: the penetration of water into the device, the hydrolysis of ester bonds in the polymer main chain leading to water soluble degradation products, and the mass loss of polymer from the device through transport of cleavage products into the surrounding medium (Mathiowitz et al., 1993). Fig. 6 is a plot of the mass erosion profiles of the PLGA wafers with M_w of 5000. Low molecular weight PLGA shows no apparent lag phase. This indicates that the hydrolytic rate of the polymer chains is quite high leading to the rapid loss of the water-soluble oligomeric fragments. Fig. 6 shows that about

40% of the polymer mass is lost in 11 days. The weight loss during the first 4 days of incubation represents less than the amount of fentanyl left in the wafer at the same time. This can be explained by the fact that during the incubation an important amount of buffer penetrates deeply into the polymer matrix. Mass loss cannot be occurred during the swelling step after 4 days incubation so that they produce a weight increase, which is balanced by fentanyl loss.

4. Conclusion

Biodegradable PLGA wafers have been manufactured by means of direct compression and their in vitro release patterns have been investigated. The fentanyl-loaded PLGA wafers with appropriate factors of drug loading, thickness and additive content could be reached controlled zero-order release. This study has demonstrated that the release pattern of drug from wafer could be improved by optimizing the preparation conditions of the wafers.

Generally, the drug showed the biphasic release patterns, with an initial diffusion followed by a lag period before the onset of the degradation phase (Mathiowitz et al., 1993). However, these wafers showed zero-order release because the degradation of matrix promoted the drug release followed by a diffusion process. In vitro release studies showed that different release patterns and rates could be achieved by simply modifying factors in the preparation conditions. Gravimetric studies of mass loss of wafers during the incubation revealed that the weight loss increased apparently after 4 days and about 40% of mass loss was observed after 11 days fentanyl release. These results indicate that the polymer degradation was contributed to drug release followed by diffusion. In conclusion, this system showed very few initial burst and zero-order release profiles. The fentanyl-loaded PLGA wafers appear to be a promising analgesic delivery device for the treatment of chronic pain without second operation for the removal of the implants after releasing of drug.

Acknowledgements

This work was supported by KOSEF RO1-2001-00431 and KMOHW.

References

Arshady, R., 1990. Biodegradable microcapsular drug delivery systems: manufacturing methodology, release control and targeting prospects. J. Bioact. Compatible Polym. 5, 315–342.

Bakai, A., Pathak, Y.V., Benita, S., 1990. Polyacrylate (Eudragit retard) microspheres for oral controlled release of nifedipine. I. Formulation design and process optimization. Drug Dev. Ind. Pharm. 16, 2057–2075.

Chandy, T., Sharma, C.P., 1992. Chitosan beads and granules for oral sustained delivery of nifedipine: in vitro studies. Biomaterials 13, 949–952.

Choi, H.S., Shin, H.C., Khang, G., Rhee, J.M., Lee, H.B., 2001. Quantitative analysis of fentanyl in rat plasma by gas chromatography with nitrogen phosphorous detection. J. Chromatogr. B 765, 63–69.

Choi, H.S., Seo, S.A., Khang, G., Rhee, J.M., Lee, H.B., 2002. Preparation and characterization of fentanyl-loaded PLGA microspheres: in vitro release profiles, Int. J. Pharm. 234, 195–203.

Chuo, W.H., Tsai, T.R., Hsu, S.H., Cham, T.M., 1996. Preparation and in-vitro evaluation of nifedipine loaded albumin microspheres cross-linked by different glutaraldehyde concentrations. Int. J. Pharm. 144, 241–245.

Cleland, J.L., Jones, A.J.S., 1996. Stable formulations of recombinant human growth hormone and interferon-γ for microencapsulation in biodegradable microspheres. Pharm. Res. 13, 1464–1475.

Hausberger, A.G., DeLuca, P.P., 1995. Characterization of biodegradable poly(D,L-lactide-co-glycolide) polymers and microspheres. J. Pharm. Biomed. Anal. 13, 747–760.

Jalil, R., Nixon, J.R., 1990. Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. J. Microencapsul. 7, 297–325.

Kenley, R.A., Lee, M.O., Mahoney, T.R., Sanders, L.M., 1987. Poly(lactide-co-glycolide) decomposition kinetics in vivo and in vitro. Macromol. 20, 2398–2403.

Khang, G., Cho, J.C., Lee, J.W., Rhee, J.M., Lee, H.B., 1999. Preparation and characterization of Japanese encephalitis virus vaccine loaded PLGA microspheres for oral immunization. Bio-Med. Mater. Eng. 9, 49–59.

Khang, G., Lee, J.H., Lee, J.W., Cho, J.C., Lee, H.B., 2000. Preparation and characterization of PLGA microspheres for the sustained release of AZT. Korea Polymer J. 8, 80–88.

Lewis, D.H., 1990. Controlled release of bioactive agents from lactide/glycolide polymers. In: Chasin, M., Langer,

R. (Eds.), Biodegradable Polymers as Drug Delivery Systems. Marcel Dekker, New York, pp. 1–41.

Li, S., Garreau, H., Vert, M., 1990. Structure-property relationships in the case of the degradation of massive aliphatic poly-(alpha-hydroxy acids) in aqueous media, part II: degradation of lactide-glycolide copolymers PLA37.5GA25 and LA75GA25. J. Mater. Sci. Mater. Med. 1, 131–139.

Maria, J., Kamel, B., Oliver, Z., Dani, A., Piero, O., Frederic, H., Hans, P.M., Bruno, G., 2000. In vitro and in vivo evaluation of a somatostatin analogue released from PLGA microspheres. J. Controlled Rel. 67, 19–28.

Mathiowitz, E., Jacob, J., Pekarek, K., Chickering, D., 1993. Characterization of the erosion and intact zones in polyanhydrides using scanning electron microscopy. Macromolecules 26, 6756–6765.

Sansdrap, P., Moes, A.J., 1993. Influence of manufacturing parameters on the size characteristics and the release profiles of nifedipine from poly(D,l-lactide-co-glycolide) microspheres. Int. J. Pharm. 98, 157–163.

Tateshita, K., Sugawara, S., Imai, T., Otagiri, M., 1993. Preparation and evaluation of a controlled-release herbicide formulations based on polymeric microspheres. Bio. Pharm. Bull. 16, 420–424.

Wu, X.S., 1995. Synthesis and properties of biodegradable lactic/glycolic acid polymers, in: Wise et al. (Eds.), Encyclopedic Handbook of Biomaterials and Bioengineering. Marcel Dekker, New York, pp. 1015–1054.