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Evaluation of *In Vitro* Release Profiles of Fentanyl-Loaded PLGA Oligomer Microspheres

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Abstract : In order to the development of the delivery device of long-acting local anesthetics for postoperative analgesia and control of chronic pain of cancer patient, fentanyl-loaded poly(L-lactide-*co*-glycolide) (PLGA, molecular weight, 5,000 g/mole; 50 : 50 mole ratio by lactide to glycolide) microspheres (FMS) were studied. FMS were prepared by an emulsion solvent-evaporation method. The influence of several preparation parameters such as initial drug loading, PLGA concentration, emulsifier concentration, oil phase volume, and fabrication temperature has been investigated on the fentanyl release profiles. Generally, the drug showed the biphasic release patterns, with an initial diffusion followed by a lag period before the onset of the degradation phase, but there was no lag time in our system. Fentanyl was slowly released from FMS over 10 days *in vitro* with a quasi-zero order property. The release rate increased with increasing drug loading as well as decreasing polymer concentration with relatively small initial burst effect. From the results, FMS may be a good formulation to deliver the anesthetic for the treatment of chronic pain.

Keywords : fentanyl, poly(L-lactide-*co*-glycolide), microspheres, solvent-evaporation method.

Introduction

Fentanyl is a potent synthetic opiate commonly used for surgical analgesia and sedation.[1,2] It is approximately 100 times more potent than morphine, has a rapid onset (1-2 min), and short duration of action (30-60 min).[3] Because of its potency and quick onset, even a very small dose of fentanyl can lead to sudden death.[4-7] Moreover, the expected concentration range of fentanyl is very low. A concentration of 1-3 ng fentanyl per mL of plasma is effective therapeutic range for analgesia and toxicity is reached between 3 and 5 ng/mL when fentanyl is abused. Therefore, the detection of lower levels of the compound from analgesic doses is important.[8-10] In addition, intravenous administration of fentanyl results in a relatively short half-life, about 3.7 h in plasma and simple parenteral administrations of fentanyl may not be fully effective, so frequent injections and continuous infusions are required to ensure adequate plasma levels.[7] However, these methods have the disadvantage of potentially causing irreversible damage to nerve or surrounding tissues due to fluctuations in concentration and high levels of anesthetic. Additionally, anesthetic delivered in the form of pulse instead of zero-order kinetics may aggravate adverse reactions due to over-dosage.[11,12] Therefore, a sustained release system is needed to prolong the action of local anesthetic as well as to avoid the inconvenience of patients and to maintain constant therapeutic levels.[13] The development of long-acting local anesthetics is also needed for postoperative analgesia and control of chronic pain of cancer patients.[6,7,14]

Generally, 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.[15-27] Poly(D,L-lactide-*co*-glycolide) (PLGA) has been widely used as carriers in controlled-release delivery systems and in tissue engineering area[28-30] due to its biodegradability and relatively good biocompatibility. The Food and Drug Administration 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 drug-release pattern from PLGA microspheres is biphasic, combination of diffusion and biodegradation.[31] 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

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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 is loosely bound to the surface or embedded in a superficial region of the microspheres.[32,33]

We developed the sustained release devices for fentanyl-loaded PLGA with various manufacturing conditions. The aims of this study were 1) the possibility of the fentanyl-loaded microspheres (FMS) for local anesthesia with the precise and effective control of fentanyl administration, 2) the development of a rapid and sensitive analytic method for fentanyl in the concentration range of 0.5 - 50 ng/mL by using gas chromatography (GC) with a nitrogen-phosphorous detector (NPD) based on the previous studies,[8-10,26,27] and 3) the effects of the preparation conditions such as initial drug/polymer loading ratio, polymer concentration, emulsifier concentration, solvent volume, and so on on morphology and release profiles using PLGA oligomer.

Experimental

Materials. Fentanyl base was purchased from McFarland Smith (Edinburgh, UK) and papaverine hydrochloride (internal standard (IS)) from Sigma Chem. Co., Ltd. (Steinheim, Germany) (Figure 1). PLGA (Resomer® RG 502; PLGA 50 : 50 mole ratio by lactide to glycolide; molecular weight, 5,000 g/mole) was obtained from Boelinger Ingelheim (GmbH, Germany). Poly(vinyl alcohol) (PVA; molecular weight, 30,000-70,000 g/mole), ethyl acetate (EA), *n*-butyl chloride, toluene, and methanol were purchased from Sigma Chem. Co., Ltd. (St. Louis, MO, USA). 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.

Fabrication Method of FMS. The conventional oil-in-water (O/W) emulsion method was applied to fabricate FMS since fentanyl is a slightly water-soluble drug. EA was used as an O phase because of its relatively low toxicity, ease of removal, and excellent ability to dissolve the polymer.[3] We encapsulated fentanyl in PLGA microspheres using an O/W method that was followed by solvent evaporation. PLGA and fentanyl were dissolved in EA and then emulsified by dropping in W phase containing 3% PVA as an emulsifier. EA was removed at 35 °C by evaporation, and monolithic microspheres containing fentanyl were obtained. As the solvent was removed, the emulsifier continued to maintain the oil droplets in their spherical configuration and prevented from aggregating them until the solvent was completely removed, and the microspheres were hardened as discrete particles. Finally, the hardened FMS were washed, centrifuged, and freeze-dried at 70 °C using a freeze drier (FDU-540, EYELA®, Japan). More detailed procedures were explained in previous studies.[8-10,26,27] The preparation conditions such as initial drug loading ratio, polymer concentration, emulsifier concentration and solvent volume of FMS are listed in Tables I - IV, respectively.

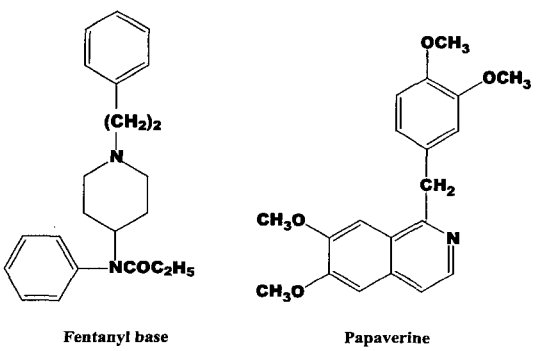

Figure 1. Chemical structures of fentanyl base and papaverine (IS).

Morphology Observation. Scanning electron microscopy (SEM, S-2250N, Hitachi, Japan) was used to study FMS size and size distribution as well as to reveal the surface quality and porosity of microspheres. 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 500 K, UK) under argon atmosphere. Cross sectional morphology of microspheres was obtained by embedding the microspheres in an aqueous solution containing 30% gelatin and 5% glycerin. The size distributions of microspheres were determined according to a reference scale.

X-ray Analysis. Powder X-ray diffraction patterns from X-ray diffractometer (XRD, D/MAX III, Rigaku, Japan) were obtained to study physical states of drug, polymer, physical mixture of drug/polymer (10/90), and drug-loaded microspheres (batch A2). The test was carried out at 40 kV and over a 2θ range of 0 - 40°. Physical mixture was made of grinding drug and polymer materials.

***In Vitro* Drug Release Test.** The fentanyl release from the microspheres was established by suspending 20 mg of FMS (n = 3) in 50 mL of phosphate-buffered saline (PBS, pH 7.4). The sample tubes were incubated at 37 °C with continuous shaking. The tubes were centrifuged, and the supernatant was withdrawn at a scheduled time period. 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 12,000 rpm. The solution was injected into the GC system via splitless mode.

Quantitative Analysis of Fentanyl. The amount of fentanyl

released from the FMS was determined by GC analysis as follows.[8-10] 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% phenylmethylsiloxane 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 2 min. The oven temperature was programmed to 270 °C at 30 °C/min, held 10 min, then to 280 °C at 5 °C/min, and held 7 min (overall run time 25 min). The temperatures 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.

Determination of Drug Content. The encapsulation efficiency of microspheres was measured by dissolving 10 mg of FMS in 10 mL of methylene chloride and reprecipitating the polymer with a known volume of methyl alcohol. After filtration, the solution was injected in GC system operated according to above described method. Fentanyl content in FMS was calculated as the ratio of actual-to-theoretical drug content in the microspheres.

Results and Discussion

Morphology of FMS. The morphology of microspheres depends on the rate of polymer precipitation and solvent removal at the interface.[19,23] The surface is generally smooth when the polymer precipitates slowly due to slow removal of the organic solvent. Moreover, the presence of PVA in W phase enhances the stability of the emulsion for the O/W system. As shown in Figure 2(a), FMS from various preparation conditions had a spherical shape and smooth surface with few pores. From the cross-sectional images of the

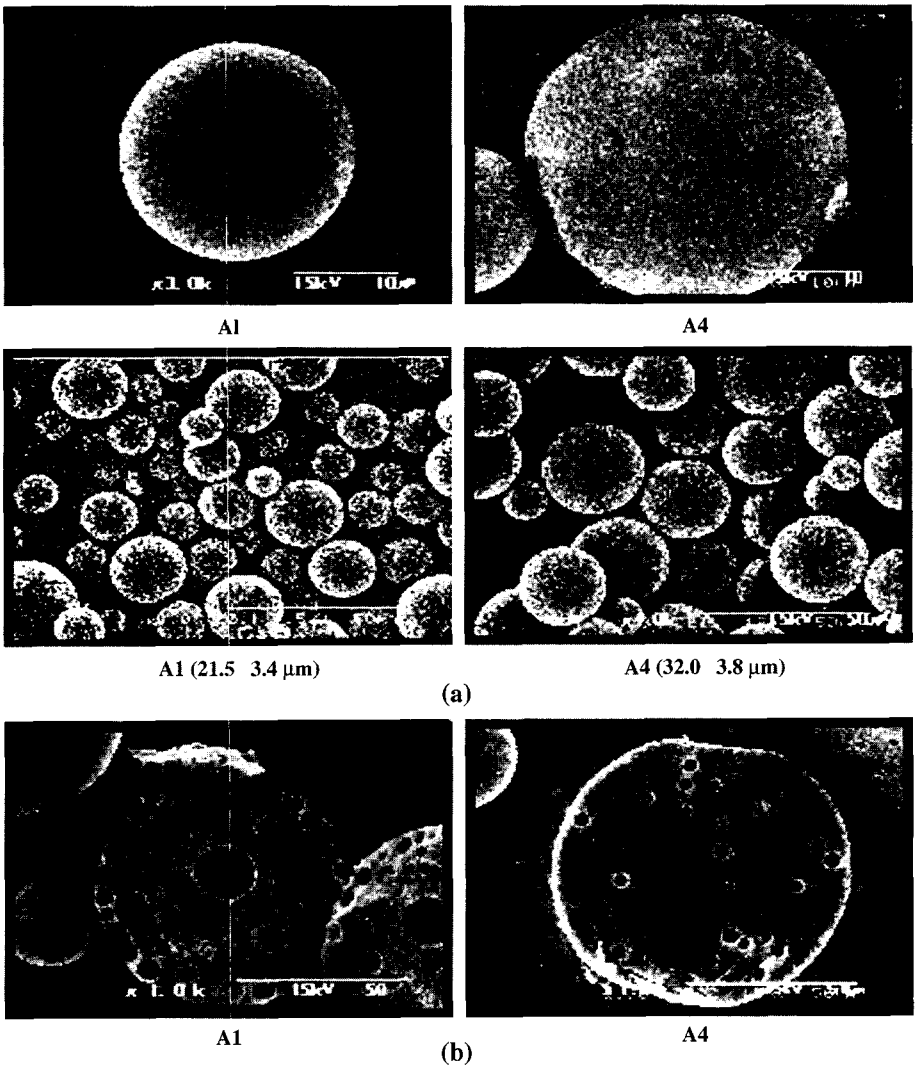

Figure 2. SEM morphology of FMS. (a) surface (upper; original magnification × 3,000 and lower; original magnification × 1,000) and (b) cross-section.

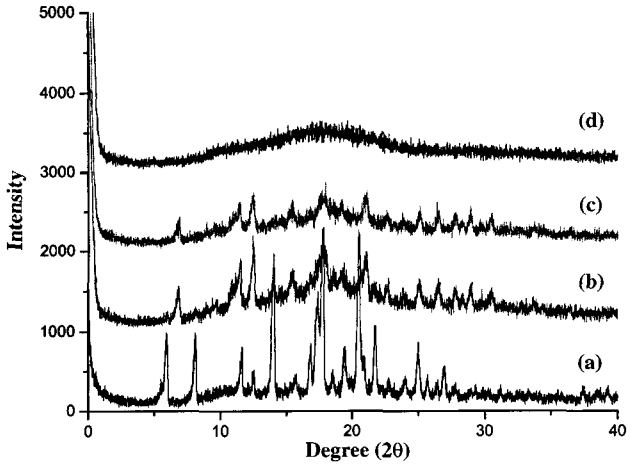

Figure 3. X-ray diffraction patterns of (a) fentanyl base, (b) physical mixture of fentanyl/PLGA (10/90), (c) 10% drug-loaded microspheres, and (d) PLGA.

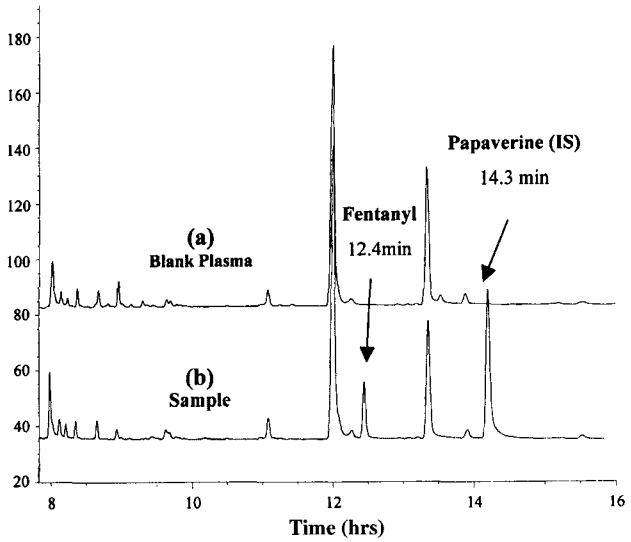

Figure 4. Chromatograms of (a) blank plasma sample and (b) extracted standard sample containing 10 ng/mL fentanyl.

microspheres, it is clear that internal phase of the microspheres are porous and the hollow core (Figure 2(b)). In our case, although the FMS have a hollow internal structure, FMS was fabricated with a uniform size distribution.

As shown in Figure 3, fentanyl showed several peaks corresponding to the crystalline form while PLGA is mainly amorphous with a poor crystalline part. On the other hand, samples of microspheres (A2) and fentanyl/PLGA mixture after dissolution in EA and evaporation (containing the same quantities of fentanyl and PLGA) showed a halo pattern in which diffraction peaks of drug decreased indicating that fentanyl in both preparations was in the amorphous state. These results proved that fentanyl and PLGA, after dissolution in EA, produced after solvent evaporation of the solvent a solid dispersion in which the drug was dispersed in a molecular state.[9,10]

Quantitative Analysis of Fentanyl. There have been lots of efforts for minimizing the complex and time-consuming steps to extract fentanyl.[2,4,5] We obtained an optimal condition of extraction solvents and instrumentals for fentanyl. From this condition, fentanyl was well separated on the GC chromatogram with a run time of 12.4 min as shown in Figure 4. The standard curve of fentanyl was linear over the concentration range from 0.5 to 50 ng/mL. The average slopes of the standard curves and the average correlation coefficient were 1.6608 and 0.9997, respectively. The limit of detection and limit of quantitation were 0.1 and 0.5 ng/mL, respectively.

Effect of Initial Drug Loading. To determine an optimal drug loading, various ratios of initial drug loading to the polymer matrix, 5, 10, 20, and 30%, were investigated as listed in Table I. A decrease in encapsulation efficiency attributed to the increase in the ratio of initial drug loading to the polymer. The influence of drug loading on the release profiles of fentanyl is shown in Figure 5. In our system, as the drug loading increased, the mean size of microspheres increased. Because fentanyl crystals increased with the presence of crystalline fentanyl within the matrix with increasing drug loading.[34] The release rate of A1 (5% drug loading) was faster than that of A4 (30% drug loading) because the mean size of A1 FMS (21.5 mm) was smaller than that of A4 (32.0 mm) resulting in the larger surface area. In ordinary case, the release rate of lower initial drug content was slower than that of higher ones because the crystals of drug in low initial drug loading were finely dispersed in the PLGA matrix whereas higher initial drug loading

Table I. The Preparation Conditions and Characteristics of FMS (10 wt/v% Polymer, 3 wt/v% Emulsifier, and 5 mL EA): Effect of Initial Drug Loading Ratio

Batch	Initial Drug Ratio (wt%)	FMS Mean Size ± SD (μm)	Encapsulation Efficiency (%)	Yield (%)
A1	5	21.5 ± 3.4	98.5	64.2
A2*	10	27.5 ± 4.3	97.5	64.7
A3	20	30.0 ± 3.9	96.4	70.1
A4	30	32.0 ± 3.8	92.2	61.9

*Control.

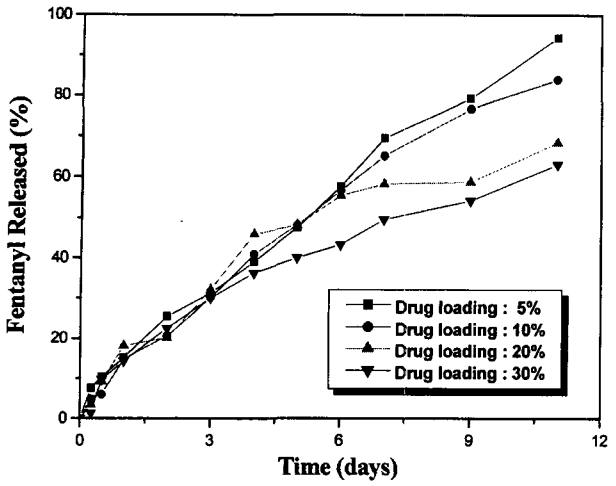

Figure 5. Effect of initial drug loading ratio on the fentanyl release pattern (n = 3, 10 wt/v% polymer, 3 wt/v% emulsifier, and 5 mL EA).

entrapped drugs in the polymer matrix like network and drugs were released by simple dissolution and diffusion.[17,19,36-40]

However, in our system, it can be explained that the effect of the size of FMS was predominant than that of the initial drug loading content.

Effect of Polymer Concentration. As shown in Table II, the PLGA concentrations in O phase (5 mL EA, fixed) were varied with 5, 10, 20, and 30 wt/v%. The larger size of microspheres was obtained from a higher PLGA concentration than a lower one. Also lower solvent volume came to the same result. It can be explained that the increasing PLGA concentration resulted in high solution viscosity and thus increasing microsphere size.[17,19] The cumulative drug release profiles of FMS from different PLGA concentrations are shown in Figure 6. Whatever the types of FMS, no burst effect has been observed and all FMS exhibited a S-shaped release pattern. The slowest release rate was observed in B3 (30% PLGA). It can be explained that microspheres fabricated at high concentration of PLGA were observed a lower density, fully porous internal structure, larger mean size and broader size distribution (Table II).[9,10]

Table III. The Preparation Conditions and Characteristics of FMS (10% Drug Loading, 10 wt/v% Polymer, and 5 mL EA): Effect of Emulsifier Concentration

Batch	Emulsifier Concentration (wt/v%)	FMS Mean Size ± SD (μm)	Encapsulation Efficiency (%)	Yield (%)
C1	PVA 0.5	32.5 ± 5.6	88.6	45.5
C2	PVA 1	26.0 ± 4.3	82.3	66.0
A2*	PVA 3	27.5 ± 4.3	97.5	64.7
C3	PVA 5	20.8 ± 2.8	78.8	63.8

*Control.

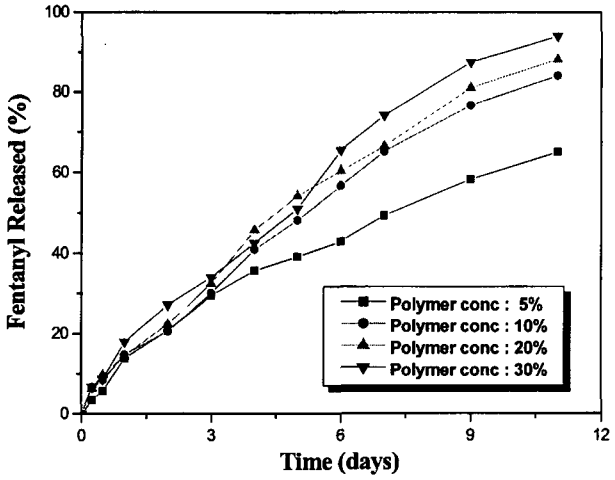

Figure 6. Effect of polymer concentration on the fentanyl release pattern (n = 3, 10% drug loading, 3 wt/v% emulsifier, and 5 mL EA).

Effect of Emulsifier Concentration. The influence of emulsifier concentration on the FMS characteristics was studied in Table III. The presence of emulsifier in the external phase stabilizes microspheres against coalescence, resulting in smaller and more stable microspheres in water phase. Lower emulsifier concentration showed faster release pattern. This phenomenon may arise from two factors. First, a higher emulsifier concentration increases the viscosity of the external phase and results in an increased difficulty for

Table II. The Preparation Conditions and Characteristics of FMS (10% Drug Loading, 3 wt/v% Emulsifier, and 5 mL EA): Effect of Polymer Concentration

Batch	Polymer Conc. (wt/v%)	FMS Mean Size ± SD (μm)	Encapsulation Efficiency (%)	Yield (%)
B1	PVA 0.5	32.5 ± 5.6	88.6	45.5
A2	PVA 1	26.0 ± 4.3	82.3	66.0
B2*	PVA 3	27.5 ± 4.3	97.5	64.7
B3	PVA 5	20.8 ± 2.8	78.8	63.8

*Control.

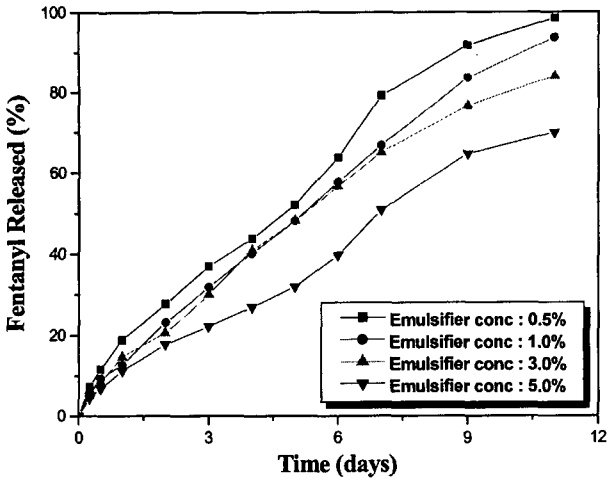

Figure 7. Effect of emulsifier concentration on the fentanyl release pattern (n = 3, 10% drug loading, 10 wt/v% polymer, and 5 mL EA).

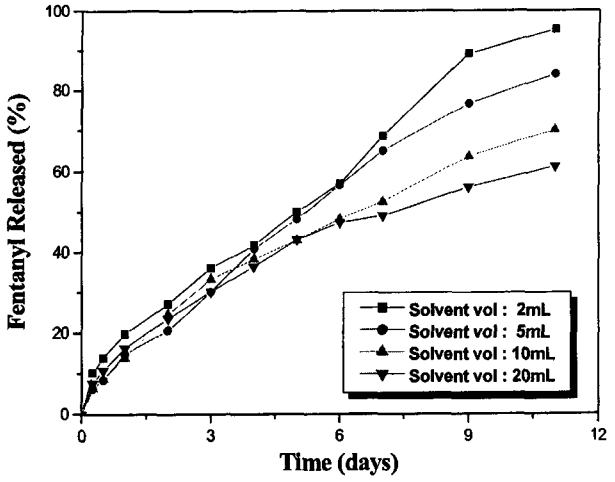

Figure 8. Effect of solvent volume on the fentanyl release pattern (n = 3, 10% drug loading, 10 wt/v% polymer, and 3 wt/v% emulsifier).

the drug in the oil phase to diffuse out. Second, a higher emulsifier yields a more stable emulsion which hinders the mass transfer of drug with surroundings, Consequently, drug is distributed more evenly within the interior of the microspheres, an shown in Figure 7.[32,33,35]

Effect of Solvent Volume. The influence of solvent volume at a constant W phase volume (100 mL) on the FMS characteristics was studied in Table IV. The O phase volume had a significant effect on the size of FMS: the FMS fabricated in 2 mL of EA were larger (D1, 40.7± 7.2 μm) than those fabricated in 20 mL of EA (D3, 25.4± 3.4 μm). The viscosity of the O phase decreased with decreasing solvent volume, as only the volume of water were fixed. Moreover, it could be observed that the FMS from a high solvent volume showed denser inner structure and further long release profiles than those from a low volume (Figure 8).

Conclusions

Biodegradable microspheres for controlled analgesics release were manufactured and their release patterns were investigated successfully. To obtain the sustained fentanyl delivery with effective and precise control, FMS were fabricated using the conventional O/W emulsion solvent-evaporation

Table III. The Preparation Conditions and Characteristics of FMS (10% Drug Loading, 10 wt/v% Polymer, and 5 mL EA): Effect of Emulsifier Concentration

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C2	PVA 1	26.0 ± 4.3	82.3	66.0
A2*	PVA 3	27.5 ± 4.3	97.5	64.7
C3	PVA 5	20.8 ± 2.8	78.8	63.8

*Control.

Table IV. The Preparation Conditions and Characteristics of FMS (10% Drug Loading, 10 wt/v% Polymer, and 3 wt/v% PVA): Effect of Solvent Volume

Batch	Solvent Volume (mL)	FMS Mean Size ± SD (μm)	Encapsulation Efficiency (%)	Yield (%)
D1	EA 2	40.7 ± 2.8	84.6	67.4
A2*	EA 5	27.5 ± 4.3	97.5	64.7
D2	EA 10	26.5 ± 7.7	71.4	61.5
D3	EA 20	25.4 ± 1.4	72.2	61.4

*Control.

method. From X-ray diffraction and SEM results, it was appeared that experimental conditions used in this work allowed the formation of microspheres having a matrix structure in which fentanyl was uniformly dispersed in molecular state. From the results of preparation conditions, the encapsulation efficiencies of drug were between 57.0 and 99.1%, depending on the particular formulation. The total microspheres recovered amount varied between 55.8 and 70.1%. It was observed that the encapsulation efficiency decreased as a function of the increase in initial drug loading amount (Table I). As PLGA concentration increased from 5 to 30%, encapsulation efficiency increased from 89.1 to 99.1% (Table II). The increased viscosity in the O phase caused by the increased PLGA concentration will decrease the loss transport of fentanyl and contribute to the enhanced entrapment efficiencies.[41] Moreover, the O droplets containing fentanyl formed from the W phase were very small and the diffusion amount of fentanyl to the external phase during the solvent evaporation was relatively small, explaining the high encapsulation efficiency obtained. The pattern of drug release depends on various factors, such as initial drug loading ratio, polymer concentration, emulsifier concentration, and solvent volume in W phase. From the results, constant localized release system can potentially provide anesthesia for a longer period than injection or topical administration. Studies on the comparison with other molecular weight, the development of another devices and the animal experiments are in progress.

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