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Preparation and Characterization of Poly(lactide-*co*-glycolide) Microspheres for the Sustained Release of AZT

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Abstract : Biodegradable microspheres were prepared with poly(L-lactide-*co*-glycolide) (PLGA, 75 : 25 by mole ratio) by an oil/oil solvent evaporation method for the sustained release of anti-AIDS virus agent, AZT. The microspheres of relatively narrow size distribution (7.6± 3.8 μm) were obtained by controlling the fabrication conditions. The shape of microspheres prepared was smooth and spherical. The efficiency of AZT loading into the PLGA microsphere was over 93% compared to that below 15% for microspheres by a conventional water/oil/water method. The effects of preparation conditions on the morphology and *in vitro* AZT release pattern were investigated. *In vitro* release studies showed that different release pattern and release rates could be achieved by simply modifying factors in the fabrication conditions such as the type and amount of surfactant, initial amount of loaded drug, the temperature of solvent evaporation, and so on. PLGA microspheres prepared by 5% of initial drug loading, 1.0% (w/w) of surfactant concentration, and 25 °C of solvent evaporation temperature were free from initial burst effect and a near-zero order sustained release was observed. Possible mechanisms of the near-zero order sustained release for our system have been proposed.

Introduction

AZT (Zidovudine, or azidothymidine) is a strong inhibitor of reverse transcriptase, and is known as effective in the treatment of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Although orally administered AZT is rapidly absorbed from intestinal mucosa, it loses considerable potency during its first pass (40%) and then is rapidly eliminated from the body with a half-life of 1 hr. In addition, orally administered AZT often shows strong side effects on bone marrow resulting to develop leukopenia, which may be attributable to an excessive plasma level of AZT immediately after administration.[1,2] Therefore, an adequate zero -order release system is desired to decrease the high daily dose of AZT (5 ~ 10 mg/kg, every 4 hr), to maintain an expected anti-viral effect, which can be time-dependent, to reduce the strong side effects, and to improve patient compliance.[3-5]

The development of a sustained-release device and formulations of AZT would be beneficial in comparison with the recent intermittent dose regimens. Recently, we prepared Karaya gum matrix containing AZT and penetration enhancers for the transdermal delivery of AZT using iontophoresis in

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our previous studies.[6,7] We evaluated the feasibility of delivering AZT through skin to a sufficient level with clinical activity. The other way, biodegradable sustained-release microspheres have been developed for the numerous bioactive reagents.[8] One of the most significant candidates for biodegradable polymeric controlled release systems is the poly(L-lactide-co-glycolide) (PLGA) due to its controllable biodegradability and relatively good biocompatibility.[9~12] However, it is not easy to design a suitable formulation for the required periods because the drug release is affected by many factors such as polymer molecular weight, monomer composition, size of the microspheres and drug particle, solvent evaporation methods and temperature and so on. These parameters maybe determine the properties of the resulting biodegradable products, which govern the release patterns of the drug.[13~16]

The conventional solvent evaporation method for preparing the biodegradable microspheres was first described by Beck *et al.* for progesterone-loaded PLGA microspheres to prolong contraceptive steroid hormone.[17] However, the loading efficiency of water-soluble drugs such as AZT into PLGA microspheres is relatively low when a conventional oil-in-water (O/W) or water-in-oil-in-water (W/O/W) emulsion system is used for the solvent evaporation process, since such drugs can be readily diffused into the aqueous outer surface of the emulsion system.[18~22]

The aims of this study were (1) to develop and characterize biodegradable drug delivery system that provides the near zero-order rates over 30 days, (2) to prepare AZT-loaded PLGA microspheres with in around 10 μm sizes by means of oil-in-oil (O/O) solvent evaporation method for the increase of drug loading efficiency compared with W/O/W methods, (3) to investigate the effects of the solvent and continuous phase, initial drug loading, the effects of evaporation temperature and so on, and (4) to observe the *in vitro* release pattern of AZT and the morphology of the microspheres.

Experimental

Materials. AZT as shown in Figure 1 for it's chemical structure was purchased from Sigma

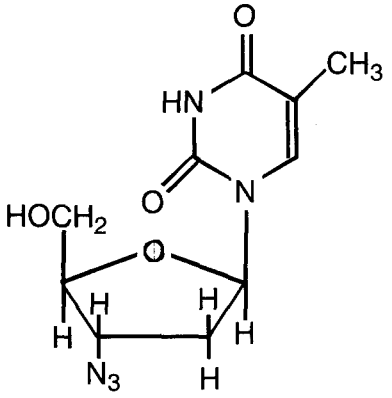


Figure 1. Chemical Structure of AZT.

Chem. Co. (St. Louis, USA). Monomers as L-lactide and glycolide were supplied by Boehringer-Ingelheim, Germany. Stannous 2-ethylhexanoate as a catalyst was purchased from Wako Chemical Co., Japan. Emulsifiers as sorbitan monooleate (Span 80, Duksan Pharm. Co. Ltd., Korea) and soybean lecithin (Sigma Chem. Co., USA), continuous phases such as light mineral oil (Sigma Chem. Co., USA), soybean oil (Aldrich Chem. Co., USA), and cotton seed oil (Sigma Chem. Co., USA), and solvents such as acetonitrile (Duksan Pharm. Co. Ltd., Korea), and methylene chloride (MC, Jin Chem. Pharm. Co. Ltd., Korea) were used as received. All other chemicals were reagent grade.

Polymerization and Characterization of PLGA. A 30g mixture of the L-lactide (75 mole%) and glycolide (25 mole%) was preheated in a polymerization reactor at 60°C for 2 h to remove water trace. Stannous 2-ethylhexanoate (150 ppm) in toluene was added to polymerization reactor. Dry nitrogen gas was flushed through whole processing. After adding the catalyst, the copolymerization reaction was carried out 165°C for 1 h. The light brownish PLGA polymer obtained was purified by dissolving in MC, followed by slow precipitation in excess methanol. The polymer was dried in vacuo at room temperature for 7 days and kept until use.

To characterize synthesized PLGA, gel permeation chromatography (GPC) was performed. Measurement was carried out on a Waters Chromatograph 200 Series equipped with Styragel®

columns with 10^5, 10^4, 10^3 and 500Å of pore size, respectively. Tetrahydrofuran (THF, Aldrich Chem. Co., USA) was used as an eluent solvent. The temperature, the flow rate, and the injection volume were 30°C, 1 mL/min, and 15 μL, respectively. A series of polystyrene monodisperse standards were used to calibrate the molecular weight (M_w) of sample. The molecular weight of synthesized polymer was 70,000 g/mole with 3.0 hrs reaction time.

Preparation of AZT-loaded PLGA Microspheres. Microspheres were prepared using a solvent evaporation methods with O/O and conventional W/O/W systems. Briefly, for O/O method, 200 mg PLGA was dissolved in 10 mL acetonitrile. AZT (10, 50, and 200 mg each) was added to this PLGA solutions. This organic phase obtained was emulsified in 100 mL continuous phase containing 1 wt% Span 80. The mixtures were stirred at 250 rpm and stayed at different temperatures (10, 25, and 50°C) to evaporate the acetonitrile. After overnight, the resulting microspheres prepared from nine different conditions were washed three times in n-hexane to remove excessive surfactant on the surface and light mineral oil. The resulting AZT-loaded microspheres were dried at -70°C for 24 hrs by using freeze drier (model FDU-540, EYELA®, Japan), and then stored in vacuum oven. The variables and conditions to prepare microspheres are listed in Tables I and II. The schematic diagram of the process of this work is shown in Figure 2.

SEM Observation. Microspheres were observed by scanning electron microscopy (SEM) (model S-2250N, Hitachi Co., Ltd., Japan) in order to examine the morphology and to calculate the size and size distribution of the microspheres. Samples for SEM were mounted on metal stub with double-sided tape and coated with platinum for 30 sec under argon atmosphere. The size and size distributions of microspheres were determined according to a reference scale.

Determination of Loading Efficiency of AZT. Microspheres of each formulation (40 mg) were dissolved in MC and AZT was extracted from these microspheres with 10 mL of phosphate buffered saline (PBS, pH 7.4). AZT in the aqueous layer was analyzed by high performance

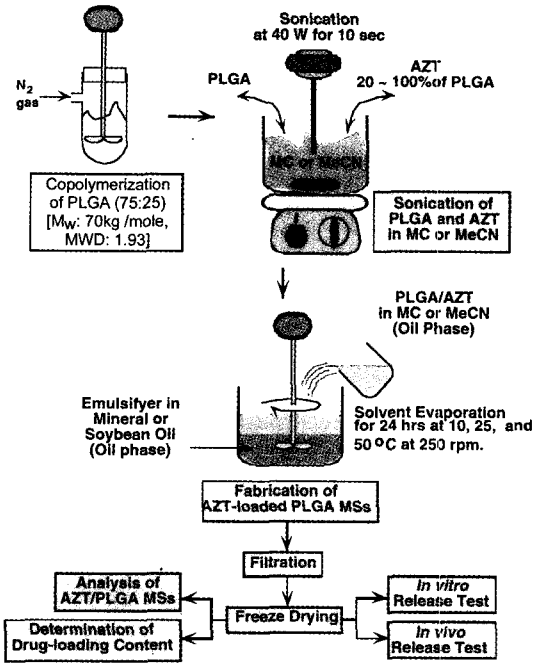


Figure 2. Schematic diagram of experimental processing.

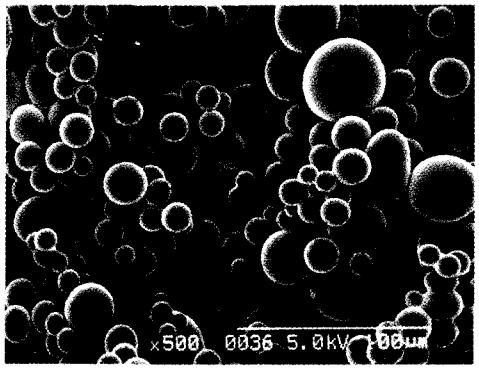
liquid chromatography (HPLC). The HPLC system was consisted of a Ranin solvent delivery pump (Dynamax, model SD-200, USA) and an UV detector (model UV-1, USA). The detection wave length was set at 265 nm, the separation was by using a reversed phase column (μBondapak C_{18}: 3.9× 300 mm, Waters Co. Ltd., USA), and a flow rate of the mobile phase was 1.0 mL/min. The mobile phase consisted of 85% of 0.1% acetic acid and 15% of acetonitrile or 75% of 0.1% acetic acid and 25% of acetonitrile.[23]

***In Vitro* Release Study.** The microspheres (20 mg) were suspended in 5 mL of PBS (pH 7.4) and the resulting suspension was stored in vials. The vials were placed in a shaking bath kept at 37 °C, 20 rpm. The release medium was periodically taken out from the vials with pipette and same volume of the fresh was replaced. The amount of AZT released was determined by HPLC. Release profiles were calculated in terms of cumulative release % with incubation time.

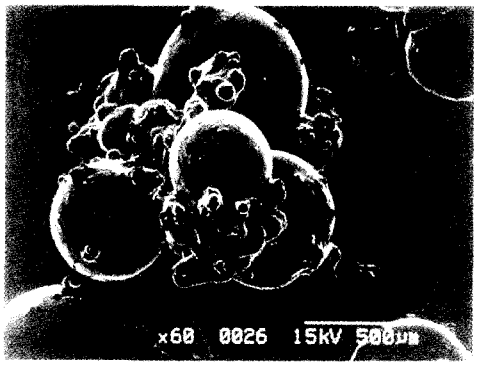
Results and Discussion

Effect of the Formulation Conditions on

the Morphology of Microspheres. In order to establish the pre-condition of the O/O solvent evaporation method to prepare microspheres using AZT and PLGA, two types of solvents (acetonitrile and methylenechloride), two types of emulsifiers (Span 80 and soybean lecithin), and three types of continuous phases (mineral oil, soybean oil, and cotton seed oil) were combined. Table I lists the formulation conditions of PLGA (20 w/v% of solvent) microspheres with various emulsifiers, solvents and continuous phases at 25 °C. From the observation of morphologies of AZT-loaded microspheres, it was found that the microspheres having best morphology was obtained using acetonitrile, 1% of Span 80 and mineral oil as solvent, emulsifier, and continuous phase, respectively, as shown in Figure 3(a). Based on this result, the formulation No. 1 may be chosen as the optimal O/O system for the manufacturing AZT/PLGA microspheres. In case of No. 3, however, almost of microspheres were clustered in addition to the size distributions were relatively broad (Figure 3(b)). It is important to control all the processing and formulation factors because these factors influence the stability of the emulsion state and eventually the particle size, surface morphology and release pattern of the microspheres.[9~25] That is to say, it may be suggested that the emulsion condition of No.1 environment may be the most stable state among the combinations used in this study. So, further in this study, the O/O condition using acetonitrile, Span 80 and mineral oil was fixed and then another factors as



(a)



(b)

Figure 3. SEM microphotographs of AZT-loaded PLGA microspheres prepared at different emulsifiers and continuous phases. (a) Formulation No. 1 and (b) Formulation No. 3.

Table I. Formulation Conditions of PLGA Microspheres with Various Emulsifiers and Continuous Phases [a]

No.[b]	AZT (mg)	Solvent (mL)	Emulsifier (g)	Continuous Phase (mL)	Morphology of Microspheres
1	25	MeCN; 5	Span 80; 0.4	Light mineral oil; 40	Excellent
2	25	MeCN; 5	Soybean lecithin; 0.4	Light mineral oil; 40	Good
3	25	MeCN; 5	Span 80; 0.4	Soybean oil; 40	Bad[c]
4	25	MeCN; 5	Soybean lecithin; 0.4	Soybean oil; 40	Bad[c]
5	25	MeCN; 5	Span 80; 0.4	Cotton seed oil; 40	Bad[c]
6	25	MC; 5	Span 80; 0.4	Soybean oil; 40	Good
7	25	MC; 5	Span 80; 0.4	Soybean oil; 40	Bad[c]
8	25	MC; 5	PVA; 0.4	Water; 40	Excellent

[a] PLGA, 20 w/v% in solvent; solvent evaporation temperature, 25 °C

[b] No. 1~7 : O/O method and No. 8 : W/O/W method.

[c] bad means that the PLGA microspheres were aggregated or did not formed.

Table II. Characteristics of Prepared PLGA Microspheres (n = 3)

Formulation Code	Amount of PLGA (mg)	Temp (°C)	Initial Loading(mg)		Loading Efficiency (%)	Average Microsphere Size(μm)
			Theoretical	Actual		
A	200	25	200	207.8	103.9± 5.08	7.6± 3.8 μm
B	200	50	200	245.2	122.6± 52.81	Aggregated
C	200	25	50	46.75	93.5± 50.64	5.8± 3.2 μm
D	200	50	50	49.95	99.9± 51.89	Aggregated
E	200	25	10	9.45	94.5± 59.70	6.2± 4.2 μm
F	200	50	10	10.03	100.3± 58.70	Aggregated
G	200	10	200	209.4	104.7± 58.87	Do not formed
H	200	10	50	49.95	99.9± 51.14	Aggregated
I	200	10	10	11.5	115.1± 55.83	Aggregated

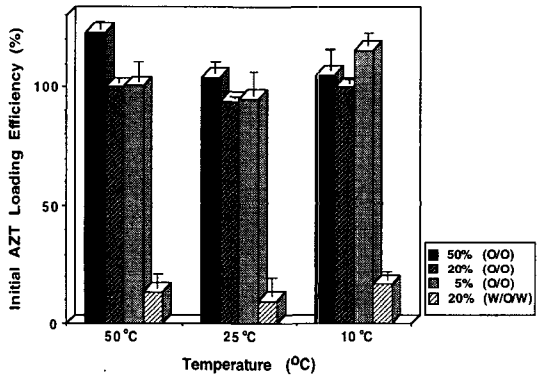


Figure 4. Comparison of initial AZT loading efficiency of the PLGA microspheres prepared by O/O and W/O/W methods.

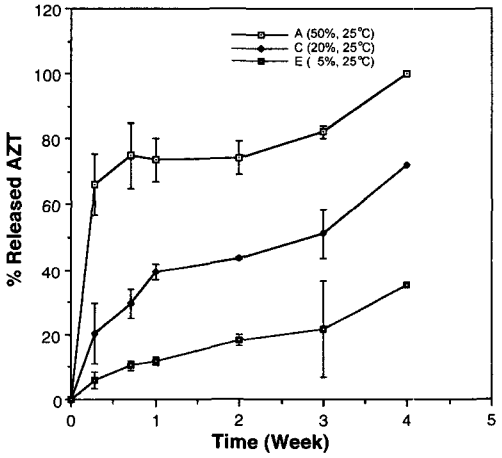


Figure 5. AZT release behavior from PLGA microspheres of different initial drug loading at 25°C. Batch A (50% of AZT), C (20% of AZT) and E (5% of AZT). n = 3.

initial loading and manufacturing temperature were varied to investigated to optimize PLGA microspheres conditions.

Efficiency of AZT Loading. As listed in Table II and shown in Figure 4, the loading efficiency of AZT in each sample of the microspheres prepared by the O/O nonsolvent-induced coacervation method was more than 93% compared to that below 15% for microspheres by conventional W/O/W method.[18~22] In an O/O solvent evaporation method, the high drug encapsulation efficiency of the microspheres may be attributed to the employment of an oil continuous phase in preparing the emulsion. Consequently, this prevents the partition or diffusion of the highly water-soluble AZT drug into the outer continuous phase. This O/O method was successful for higher drug loading efficiency because of the absence of the aqueous phase responsible for the high amount of the drug loss.[9~12]

Effect of Initial Loading on *In Vitro* Release. The influence of drug loading on the release profiles at 25°C is shown in Figure 5. It was found that AZT initial loading has a significant effect on release pattern. The release rate was in the order of 50, 20 and 5% for the initial loading. In the case of the lower initial loading (5 and 20%), the molecules of AZT were finely dispersed in the PLGA matrix with no contacts between

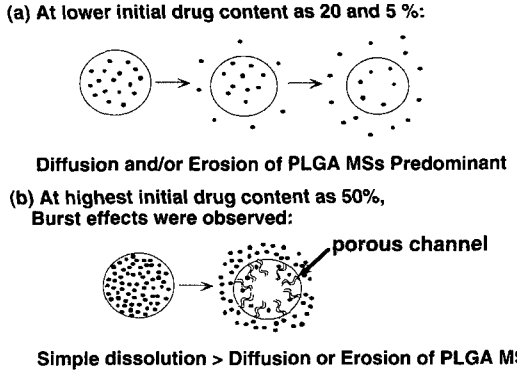


Figure 6. Schematic diagrams depicting the release mechanisms at high and low initial AZT content from PLGA microspheres.

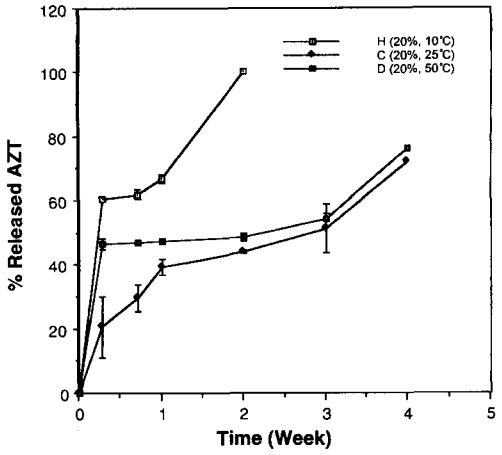


Figure 7. AZT release behavior from PLGA microspheres prepared at different fabrication temperatures with 20% of initial drug loading content. Batch H (10°C of fabrication temp.), C (25°C of fabrication temp.) and D (50°C of fabrication temp.). n = 3.

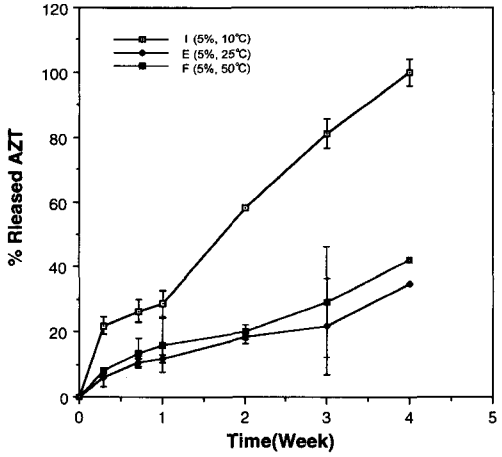
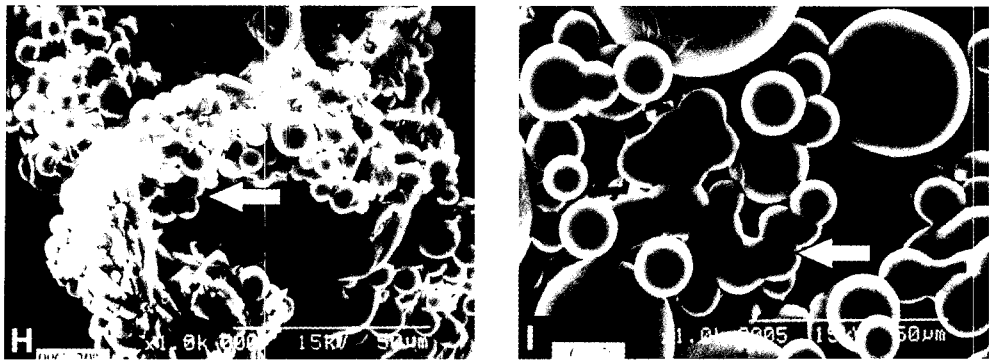


Figure 8. AZT release behavior from PLGA microspheres prepared at different fabrication temperature with 5% of initial drug loading content. Batch I (10°C of fabrication temp.), E (25°C of fabrication temp.) and F (50°C of fabrication temp.). n = 3.

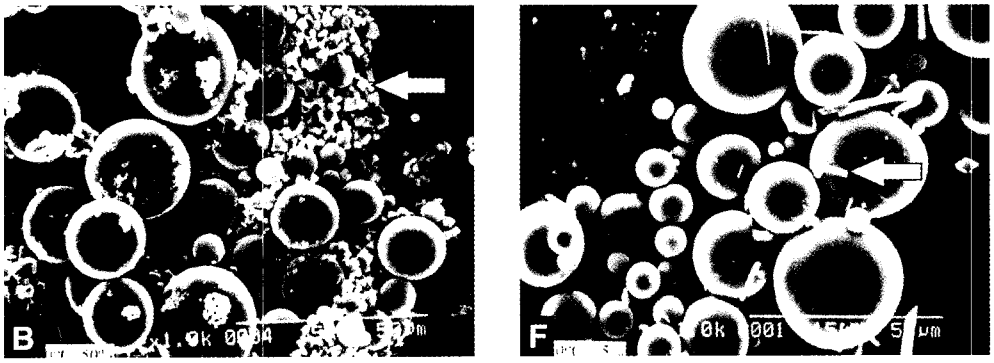
each other. Thus, the diffusion of AZT and/or the erosion of PLGA is predominant rather than simple dissolution of AZT, shown as schematic diagrams in Figure 6(a). In the considerably higher initial loading as 50%, however, all the AZT entrapped in the PLGA forms like a network which the majority of the drug in contact each other, consequently, easily can make porous channel and AZT is relatively faster released due to simple dissolution and diffusion (Figure 6(b)).

Effect of Fabrication Temperature on *In Vitro* Release. The influence of fabrication temperature on the release profiles is shown in Figures 7 and 8. Figure 7 shows the effect of fabrication temperature (10, 25 and 50°C) on AZT release profile of the microspheres with 20% of the initial drug loading. In the case of 10°C of fabrication temperature, the initial burst of over 60% release within 1 day was observed. Also the initial burst was observed in the case of 50°C. In Figure 8, it was observed the effect of fabrication temperatures of the microspheres with 5% of initial drug loading. Although the initial burst was not observed in the microspheres of the 5% of initial AZT loading, the release profile was in the order of 10, 50 and 25°C of fabrication temperatures. In summary, we observed the severe initial burst phenomena on the 10 and 50°C of fabrication temperature. To correlate between the release profiles of AZT and the morphology of PLGA microspheres, SEM microphotographs were taken in Figures 9, 10, and 11 for 10, 50 and 25 °C of fabrication temperature, respectively. Almost of PLGA microspheres were aggregated at 10°C of fabrication temperature with 20% of initial drug content (arrow in Figure 9(a)), and some portions of PLGA were stick together at 10 °C with 5% of initial drug content (arrow in Figure 9(b)). Also, in Figure 10, many of AZT crystals were observed between PLGA microspheres at 50°C of fabrica-



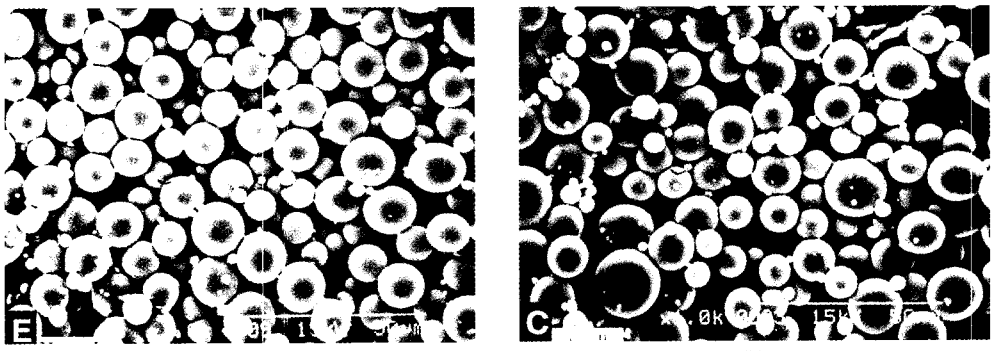
(a) (b)

Figure 9. SEM microphotographs of AZT-loaded PLGA microspheres at 10 °C of fabrication temperature with (a) 20% of initial drug loading (Batch H) and (b) 5% of initial drug loading (Batch I).



(a) (b)

Figure 10. SEM microphotographs of AZT-loaded PLGA microspheres at 50 °C of fabrication temperature with (a) 50% of initial drug loading (Batch B) and (b) 5% of initial drug loading (Batch F).



(a) (b)

Figure 11. SEM microphotographs of AZT-loaded PLGA microspheres at 25 °C of fabrication temperature with (a) 5% of initial drug loading (Batch E) and 20% of initial drug loading (Batch C).

tion temperature with 50 (arrow in (a)) and 5% of initial drug content (arrow in (b)). In contrast, the shapes of PLGA microspheres at 25°C of fabrication temperature with 5 and 20% of initial drug

content were spherical and had nonporous surface structures with relatively narrow size distributions and without any aggregation and AZT crystallines. From these results, it can be suggested that the fabrication temperature plays a very important role for good morphology, desirable release patterns of drug, and inter-correlation between morphology and release patterns. It may be explained that the velocity of evaporation of acetonitirile in PLGA microspheres can be the best at 25°C. PLGA microspheres fabricated at 10°C tend to aggregate due to low evaporation rate during the formation of microspheres, whereas AZT crystallines tend to exclude out PLGA microspheres along the evaporating acetonitrile due to high evaporation rate at 50°C of fabrication temperature.

Conclusions

Biodegradable controlled AZT releasing microspheres were manufactured and their *in vitro* release patterns were investigated. The higher efficiency of initial drug loading from an O/O solvent evaporation method was observed compared to that from conventional W/O/W method probably due to highly water-soluble AZT. The best combination of a solvent, an emulsifier, and a continuous phase appeared acetonitrile, Span 80, and mineral oil, respectively. Also, the fabrication temperature plays an important role for the morphology of PLGA microspheres as well as *in vitro* release pattern of AZT. This study demonstrated that the morphology of PLGA microspheres as well as *in vitro* release pattern of AZT can be improved by optimizing the fabrication conditions of the microspheres. The AZT-loaded PLGA microspheres with 5% of initial drug loading, 25°C of fabrication temperature, acetonitrile as a solvent, 1% of Span 80 as an emulsifier, and mineral oil as continuous phase (Formulation No. E) appears to be a promising near zero-order release device for the reduction of the strong side effects and the improvement of the patient compliance. Studies on the animal experiment are in progress.

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