Korean J. Chem. Eng., **17**(2), 230-236 (2000)

Drug Release from Nanoparticles of Poly(DL-lactide-co-glycolide)

Jae-Woon Nah†, Young-Il Jeong* and Jae-Joon Koh**

Department of Polymer Science and Engineering, Sunchon National University, Chonnam 540-742, Korea
*Department of Pharmaceutics and Pharmacokinetics, Kyoto Pharmaceutical University,
Yamashina-Ku, Kyoto 607-8414, Japan
**Department of Internal Medicine, Seoul Municipal Boramae Hospital,
Seoul National University, Seoul 156-707, Korea
(*Received 17 November 1999 • accepted 13 January 2000*)

Abstract–Nanoparticles of poly(DL-lactide-co-glycolide) (PLGA) were prepared by dialysis method without surfactant. The size of PLGA nanoparticles prepared from dimethylacetamide (DMAc), dimethylformamide (DMF), and dimethylsulfoxide (DMSO) as an initial solvent was smaller than that of acetone or 1,4-dioxane. Selected initial solvent used to dissolve the copolymer significantly affects the size of nanoparticles. Also, the size of PLGA nanoparticles was changed according to the copolymer composition. It was shown that PLGA nanoparticles have spherical shapes from the results of scanning electron microscope (SEM) and transmission electron microscope (TEM) observations. From these results was shown the potential that the PLGA nanoparticles could be formed successively by dialysis method without surfactant. The drug-loading contents were also dependent on the copolymer composition and initial feeding amount of the drug. The greater lactide ratio on the copolymer composition led to higher drug loading contents. Also, the higher the initial feeding amount of drug, the higher the drug loading contents. Clonazepam (CNZ) was used as a model drug. CNZ was slowly released in higher lactide ratio in the copolymer composition and in the higher drug loading contents.

Key words: Poly(DL-lactide-co-glycolide), Nanoparticles, Dialysis Method, Clonazepam, Controlled Drug Release

INTRODUCTION

Recently, nanoparticles have been widely used in biomedical and biotechnological applications, especially in drug delivery systems [Davis et al., 1981, 1993; Alleman et al., 1993]. Since their particle size ranges from 10 nm to 1,000 nm, nanoparticles are mainly employed in intravenous (i.v.) injection of drugs for drug targeting issues [Kreuter, 1991]. The potential of targeted drug delivery to a specific site in the body would be great benefit in the therapy of several disease states such as anticancer treatment, gene therapy, viral disease, and bacterial infections [Couvreur et al., 1991, 1992; Leroux et al., 1996]. Therefore, the application of nanoparticles for drug targeting in vivo has attracted considerable interest in order to achieve these objectives. On the other hand, the body distribution of nanoparticles after i.v. injection is greatly influenced by their interaction with the biological environment and their physicochemical properties such as particle size, surface charge, morphology, and hydrophilicity… Among them, the effect of nanoparticle size has been shown to be of primary importance [Davis, 1981; Seijo et al., 1990]. Administered particles of several micrometers in diameter mainly accumulate in the lung capillaries [Illum et al., 1982; Yoshioka et al., 1981], and submicron particles are rapidly cleared by the reticuloendothelial system (RES) [Dunn et al., 1994; Illum et al., 1986; Muller et al., 1992]. Such applications of nanoparticles on the drug targeting to the specific body sites have the advantage of avoiding any surgery, which can always be the source of infection. Also, nanoparticles have received much attention in nonparenteral drug delivery systems such as oral, pulmonary, nasal or ophthalmic delivery of drugs. In spite of the these advantages, production of nanoparticles has been limited due to the difficulties and complexities of the preparation method. So, novel preparation methods are needed for development of effective nanoparticulate drug delivery vehicles.

Although various polymers are possible for making nanoparticles for a drug delivery system in vitro, polymeric materials used to prepare microspheres or nanoparticles for injection into the human body are significantly limited to a few kinds of polymers due to the approval required of the Food and Drug Administration (FDA) of the USA. Among them, poly(L-lactide) or poly (DL-lactide) (PLA), poly(glycolide) (PGA), and their copolymers such as poly(DL-lactide-co-glycolide) (PLGA) are some of the most widely used biodegradable polymers for making micro- or nanoparticles for controlled drug delivery systems. The preparation method of nanoparticles is a critical problem for small-sized particles [Gref et al., 1996; Juliene et al., 1992; Venier-Julienne et al., 1996]. The emulsion solvent evaporation method is a most widely employed for preparation of nanoparticles or microspheres using PLGA [Ciftci et al., 1996; Jeffery et al., 1991; Scholes et al., 1993; Venier-Julienne et al., 1996] at present. In these methods, serious amounts of surfactants are required to stabilize the dispersed particles. Especially, poly(vinyl alcohol) (PVA) as a stabilizing agent is most frequently used to make micro- or nanoparticles. PVA has some problems in that it remains at the surface of particles and then is difficult to remove. Other surfactants

†To whom correspondence should be addressed.
E-mail: jwnah@sunchon.ac.kr

such as Span series or Tween series, poly(ethylne oxide) (PEO), and poloxamer [PEO-poly(propylene oxide) (PPO) block copolymer] are also used to make and stabilize particles [Sjostrom et al., 1993a, b]. Also, some disadvantages of these methods are the difficulty and necessity of removal of solvent and surfactant due to their toxicity and its solvent properties for polymer used, low particle yield, too many steps for preparation, and necessity for use of a great deal of surfactant for the preparation of small-sized spherical particles [Sjostrom et al., 1993a, b, 1995; Witschi and Doelker, 1997]. Recently, a dialysis method was developed for the simple preparation of drug carriers such as liposomes and polymeric micelles [Lasic, 1992; Kwon et al., 1995; Nah et al., 1998; Jeong et al., 1998; Cho et al., 1995]. The dialysis method is an acceptable simple and effective preparation method for small and narrow size distributed nanoparticles using block, graft copolymers and other amphiphilic materials [Lasic, 1992; Kwon et al., 1995; Nah et al., 1998; Jeong et al., 1998; Cho et al., 1995]. The application of the dialysis method for the preparation of nanoparticles using PLGA which is not amphiphilic material has not reported in detail until the present.

For this study, we prepared PLGA nanoparticles by dialysis method without surfactant, and studied the possibility of nanoparticles as drug carriers by using clonazepam (CNZ) as a model drug. CNZ is an anticonvulsant benzodiazepine which has considerable hydrophobic character [Solubility : < 14.66 μg/ml in PBS (pH 7.5, 37 °C), <18.40 μg/ml in PBS (pH 7.5, 25 °C)] [Mura et al., 1990] and, especially, has high interaction with proteins in vivo [White, 1995]. The drug loading contents, loading efficiency, changes of particle size, and physicochemical properties of PLGA nanoparticles after the drug is entrapped into the nanoparticles are investigated against various solvents and copolymer composition.

EXPERIMENTAL

1. Materials

PLGA and CNZ were purchased from Sigma Chem., Co. Ltd. USA. Molecular weight of PLGA (85/15), (75/25), and (50/50) was 48,400, 47,500, and 40,100 daltons from our gel permeation chromatography (GPC) measurements as described below. Various solvents, i.e., tetrahydrofuran (THF), dimethylformamide (DMF), dimethylsulfoxide (DMSO), dimethylacetamide (DMAc) and acetone, as a reagent grade were used without further purification.

2. Gel Permeation Chromatography (GPC) Measurement

M.W. of PLGA was measured from Waters LC system coupled with a Waters 410 Differential Refractometer using Waters Styragel™ HR1, HR2 and HR4 column at a flow rate of 1 ml/min. THF was used as an eluent. Average M.W. was evaluated by polystyrene as a standard [Shin et al., 1998; Zhang et al., 1996].

3. Preparation of PLGA Nanoparticles and Drug Loading Procedure

Preparation of PLGA nanoparticles was carried out by dialysis method without surfactant. Briefly, 20 mg of PLGA was dissolved in 10 ml of various solvents and solubilized entirely. The solution was introduced into a dialysis tube (molecular cutoff 12,000 g/mol) and dialyzed against 1.0 L×3 of distilled water for 3 hr and then distilled water exchanged at intervals of 3-4 hr during 24 hr. The resultant solution was used for analysis or freeze-dried.

Drug loading procedure was carried out as follows: 20 mg of PLGA was dissolved in 10 ml of various solvents, and subsequently 10-20 mg of clonazepam was added. The solution was stirred at room temperature and solubilized entirely. The solution was dialyzed using a dialysis tube (molecular cutoff 12,000 g/mol) against 1.0 L×3 of distilled water for 3 hr. The distilled water was exchanged at intervals of 3-4 hr during 24 hr. The solution was then used for analysis or freeze-dried.

For measurement of drug-loading content, freeze-dried samples of CNZ-loaded PLGA nanoparticles were suspended into methanol and vigorously stirred for 3 hr and sonicated for 15 min. Resulting solution was centrifuged with 12,000×g for 20 min and supernatant was taken for measurement of drug concentration using Ultraviolet (UV) spectrophotometer (Shimadzu UV-1201) at 310 nm.

4. Scanning Electron Microscope (SEM) Observation

The morphology of the nanoparticles was observed by using an SEM (JEOL, JSM-5400, Japan). One drop of the nanoparticle suspension was placed on a graphite surface. After freeze-drying, the sample was coated with gold/palladium by using an Ion Sputter (JEOL, JFC-1100). Coating was provided at 20 mA for 4 min. Observation was performed at 25 kV.

5. Transmission Electron Microscope (TEM) Observation

One drop of nanoparticle suspension containing 0.01% of phosphotungstic acid was placed on a carbon film coated on a copper grid for TEM. Observation was done at 80 kV in a JEOL, JEM-2000 FXII, Japan.

6. Photon Correlation Spectroscopy (PCS) Measurements

PCS was measured with a Zetasizer 3000 (Malvern instruments, England) with He-Ne laser beam at a wavelength of 633 nm at 25 °C (scattering angle of 90°). A nanoparticle solution prepared by dialysis method was used for particle size measurement (concentration: 0.1 wt%) and measured without filtering.

7. X-Ray Diffractometer (XRD) Measurement

X-ray powder diffractograms were obtained with a Rigaku D /Max-1200 (Rigaku) using Ni-filtered CuKα radiation (35 kV, 15 mA).

8. *In Vitro* Release Studies

The release experiment in vitro was carried out as in a previous report [Nah et al., 1998; Peracchia et al., 1997]. First, 5 mg of CNZ loaded PLGA nanoparticles was suspended in 2 ml phosphate buffered saline (PBS, 0.1 M, pH 7.4) by sonication for 30 sec at 15 watts by using a bar type sonicator (Ultrasonic homogenizer, UH-50, SMT Co. Ltd., Japan) and then put into a dialysis tube (MWCO: 12,000). The dialysis tube was placed into a 100 ml bottle with 50 ml PBS and the medium was stirred at 100 rpm at 37 °C. Whole-media change method on the drug release study was used for prevention of saturation of the drug. At specific time intervals, the whole medium (50 ml) was taken and replaced with the same volume of fresh PBS (50 ml). The concentration of the released CNZ into PBS was determined by UV spectrophotometer (Shimadzu UV-1201) at 310 nm. Drug loading contents and loading efficiency were calculated as in the following equation: drug loading contents=[(weight of remaining

drug in the nanoparticles)/(weight of remaining drug in the nanoparticles+polymer weight)]×100; loading efficiency=[(amount of remaining drug in the nanoparticles)/(initial amount of drug)] ×100.

RESULTS AND DISSCUSSION

The PLGA nanoparticles were prepared by dialysis technique without any other surfactant. These surfactant-free PLGA nanoparticles were characterized by analysis of photon correlation spectroscopy, scanning electron microscope, and transmission electron microscope, etc. We have investigated the effect of the used initial solvents, copolymer composition, and drug loading contents on the change of the size of nanoparticles and their physicochemical properties.

DMAc as an initial solvent for preparation was used to make nanoparticles of PLGA (50/50) nanoparticles by dialysis without addition of any other surfactant. Milky-like suspension was observed after the end of the dialysis procedure of PLGA (50/50) against distilled water. To know whether or not nanoparticles were formed, particle size was analyzed by using photon correlation spectroscopy and their morphology was observed by TEM. Particle size distribution (a) and TEM photographs (b) of PLGA (50 /50) are shown in Fig. 1. Particle size of PLGA nanoparticles was 200.4±133.0 nm in number average. These results have shown that nanoparticles of PLGA were successfully prepared by dialysis procedure without surfactant and their morphologies have been observed as spherical shapes.

To evaluate the effect of initial solvent, various water-miscible solvents were used in the preparation of PLGA nanoparticles. All of the solvents used to dissolve the polymers resulted in a milky-like suspension without any other precipitate. Table 1 shows the particle size of the PLGA nanoparticles against used solvent. In these results, a significant difference in particle size among DMAc, DMSO, and DMF was not found. Particle sizes of PLGA nanoparticles prepared from acetone and 1,4-dioxane were relatively larger than that of other solvents. Also, PLGA nanoparticles prepared from DMAc, DMSO, and DMF as a used initial solvent were relatively more transparent than those prepared from ace-

Table 1. Effects of various initial solvent on the particle size distribution of PLGA (50/50) nanoparticles

Solvent	Particle size distribution (nm)		
	Intensity average	Volume average	Number average
DMSO	236±94	267±187	210±117
DMF	267±59	270±75.7	263±73
DMAc	242±102	240±122	200±133
1,4-Dioxane	514±83	488±63	483±57
Acetone	503±249	564±286	580±123

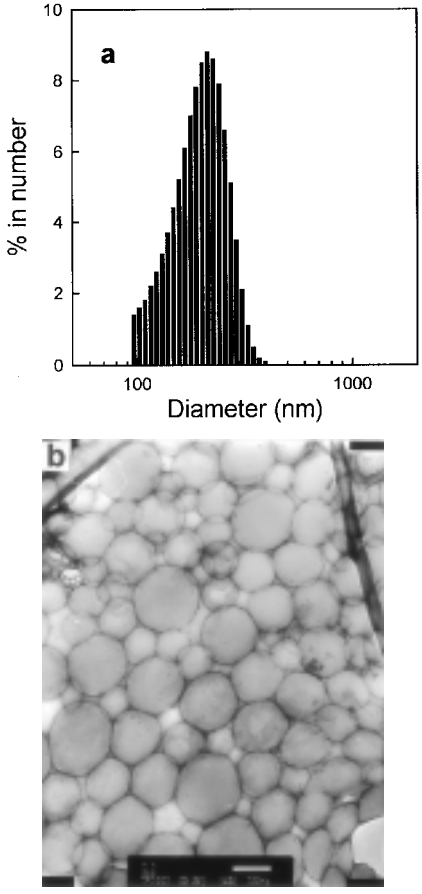

Fig. 1. Particle size distribution (a) and transmission electron microphotograph (b) of PLGA (50/50) nanoparticles prepared from DMAc as an initial used solvent.

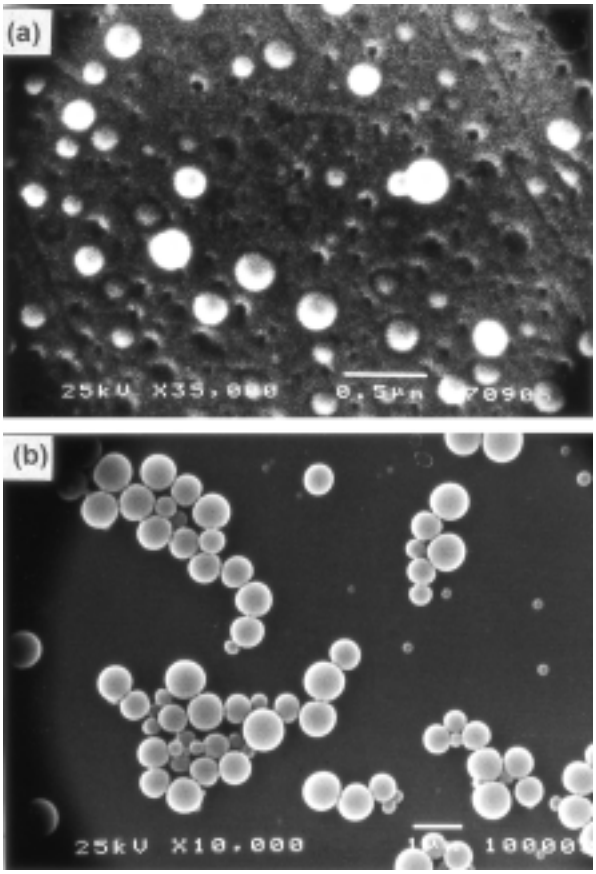

Fig. 2. SEM of PLGA (50/50) nanoparticles prepared from DMAc (a) and acetone (b) as an initial solvent.

Table 2. Particle size distribution of PLGA nanoparticles against lactide/glycolide ratio

PLGA copolymer ratio	Particle size distribution (nm)		
	Intensity average (% in area)	Volume average (% in area)	Number average (% in area)
85/15	382±89 (61.8%)	355±97 (90.1%)	344±90 (71.2%)
	220±89 (38.2%)	227±50 (9.9%)	220±65 (28.8%)
75/25	267±92	305±96	296±120
50/50	242±102	240±122	200±133

Table 3. Effects of polymer concentration in the initial solvent on the particle size distribution of PLGA (50/50) nanoparticles

Polymer (mg)	Initial solvent (ml)	Polymer conc. in the solvent (wt%)	Particle size distribution (nm)		
			Intensity average	Volume average	Number average
20	5	0.4	276±124	374±258	264±174
20	10	0.2	242±102	240±122	200±133
20	20	0.1	227±24	227±25	225±25
20	30	0.067	215±73	210±81	192±85
20	40	0.05	195±72	186±80	166±86

tone and 1,4-dioxane. These results indicated that selected initial solvent used to dissolve the copolymer significantly affects the sizes of nanoparticles. These phenomena could be explained as that differences of solubility and miscibility between polymer and solvent, or water and solvent would affect the sizes of nanoparticles. Fig. 2 shows photographs of SEM of PLGA nanoparticles prepared from DMAc (a) and acetone (b), respectively. As shown, PLGA nanoparticles prepared by dialysis method have good spherical shapes in both cases. As expected, however, particle sizes between DMAc (a) and acetone (b) were differently observed. PLGA (50/50) nanoparticles prepared from DMAc and acetone ranged from about 100-400 nm and 400-1,000 nm, respectively, which is almost a similar result with the particle size analysis. Table 2 shows particle sizes of PLGA prepared against copolymer composition DMAc. PLGA nanoparticles have different particle sizes against copolymer composition. The more lactide ratio in copolymer composition resulted in increased particle sizes. These results may be regarded as the methylene moiety in the lactide segments being relatively more hydrophobic than the proton moiety of glycolide segments. The effect of the amount of initial solvent on the particle sizes of PLGA nanoparticles prepared from DMAc as an initial solvent is shown in Table 3. As shown in Table 3, particle sizes of PLGA were slightly decreased with an increased amount of solvent, although differences in particle sizes were not significantly large. When a small amount of initial solvents was used, PLGA might have less aggregated to nanoparticles than that of a greater amount of used initial solvents.

As a hydrophobic model drug, CNZ was used to evaluate the drug loading capacity of PLGA nanoparticles prepared by dialysis method without surfactant. Generally, in self-assembling nanoparticulate systems such as core-shell type nanoparticles [Gref et al., 1994] and polymeric micelles [Kwon et al., 1995] using block or graft copolymer, the entrapment process of hydrophobic drugs into the particles is thought to be a hydrophobic interaction between drug and hydrophobic segment of polymeric chains. At present, although the mechanism of nanoparticle formation of PLGA without surfactant is not fully understood, PLGA nanoparticles might be formed by hydrophobic aggregation through hydrophobic interaction between each polymeric chain. Additionally, they would be stabilized solely by the presence of charged groups at the surface of the PLGA nanoparticles [Govender et al., 1999]. Also, the drug loading process into the nanoparticles might be hydrophobic interaction.

Table 4 shows the effect of copolymer composition and initial drug feeding amount on the drug loading contents, loading efficiency and particle sizes of PLGA nanoparticles. The sizes of PLGA nanoparticles against copolymer composition were slightly increased after drug loading. Also, the particle sizes of PLGA (50/50) nanoparticles against drug loading contents were slightly increased with higher drug loading than that of lower drug loading. Drug loading contents were dependent on the copolymer composition, i.e., the higher the lactide ratio, the higher the drug loading contents and loading efficiency. Also, drug loading contents were increased according to the increased amount of initial drug feeding whereas loading efficiency was relatively decreased. It was also reported by our previous reports [Jeong et al., 1998; Nah et al., 1998] and other researches [Shin et al., 1998] that increased hydrophobic segments in the polymer chain induced increased drug loading contents. X-ray powder diffractometer was employed to confirm the characteristics of CNZ-loaded PLGA nanoparticles. Fig. 3 shows the X-ray diffraction scans of CNZ-loaded PLGA nanoparticles and the corresponding physical blend. It can be observed that the X-ray diffraction peak characteristics of CNZ, which were visible in the pattern obtained for the physical blend, disappeared in those correspond-

Table 4. Effects of drug loading contents on the particle size distribution of PLGA nanoparticles

PLGA	Initial polymer weight (mg)	Initial drug weight (mg)	Drug loading contents (wt%)	Particle size distribution (nm)		
				Intensity average	Volume average	Number average
85/15	20	20	11.7	454±176	471±220	421±187
75/25	20	20	10.6	281±146	355±218	277±126
50/50	20	20	10.5	297±120	337±173	289±190
50/50	20	40	15.7	276±119	344±203	297±203

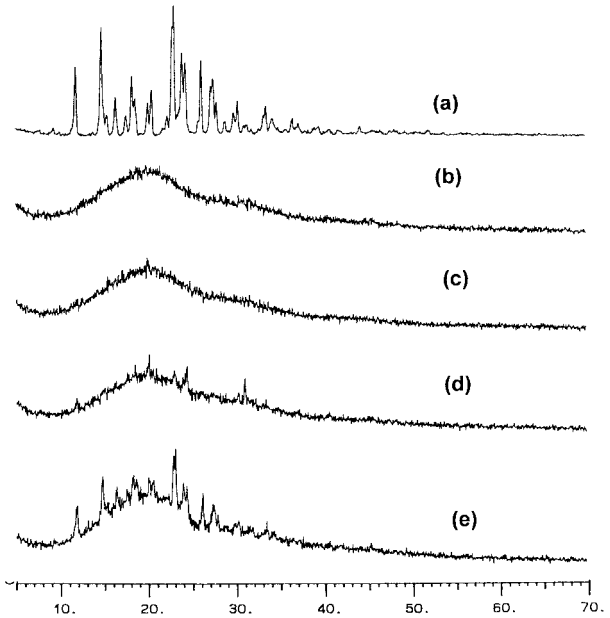

Fig. 3. X-ray diffractometer patterns of PLGA (50/50) nanoparticles. CNZ (a), PLGA nanoparticles themselves (b), CNZ loaded PLGA nanoparticles (drug loading contents: 10.5 wt%) (c), CNZ loaded PLGA nanoparticles (drug loading contents: 15.7 wt%) (d), and physical mixture of CNZ /PLGA nanoparticles (weight ratio of CNZ/polymer=1/ 10) (e).

ing to CNZ-entrapped nanoparticles. The results indicated that CNZ exists in molecular dispersion in the polymeric nanoparticles.

Fig. 4 shows the CNZ release from PLGA nanoparticles against copolymer composition (a) and drug loading contents of PLGA (50/50) (b). Generally, the drug release rate from the nanoparticles is relatively faster than that of other microsphere systems because of the high surface area. As shown in Fig. 4 (a), it was found that the higher the lactide ratio, the slower the drug release. These results may be due to the large particle size and more hydrophobic interaction between hydrophobic lactide segments and drug than that of glycolide segments. Also, the more hydrophobic lactide of polymer could lead to the stronger hydrophobic interaction [Gref et al., 1994; Jeong et al., 1998; Nah et al., 1998; Peracchia et al., 1997]. CNZ release from PLGA (50/50) nanoparticles against drug loading contents is shown in Fig. 4(b). These results indicate that the more the drug loading content, the slower the drug release. These phenomena were reported by several authors [Gref et al., 1994; Jeong et al., 1998; Nah et al., 1998; Peracchia et al., 1997]. Langer et al. [Gref et al., 1994; Peracchia et al., 1997] reported that hydrophobic drug existed as a crystallization state inside the nanoparticles and a phase separation occurs at higher loading contents of drug in the nanoparticles, leading to the crystallization of part of the drug [Gref et al., 1994; Peracchia et al., 1997]. Hydrophobic drug loaded into nanoparticles is slowly released at higher drug loading contents, which is different from hydrophilic water-soluble drugs. Our results indicate that CNZ was slowly released from the nanoparticles with higher drug loading contents. At low drug loading, CNZ is relatively present as a molecular dispersion inside the nanoparticles

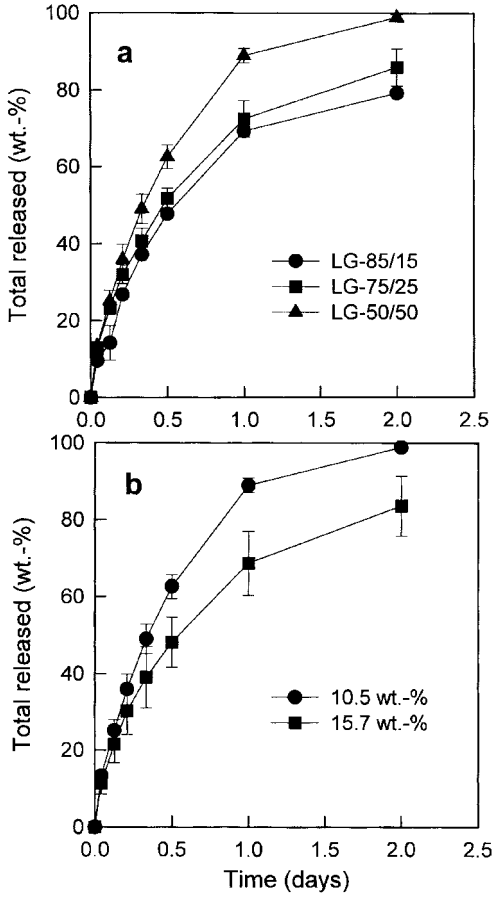

Fig. 4. CNZ release from PLGA nanoparticles against copolymer composition [drug loading contents: 11.7 wt% for PLGA (85/15), 10.6 wt% for PLGA (75/25) and 10.5 wt% for PLGA (50/50)] (a) and drug loading contents (b).

[Gref et al., 1994]. These results showed the same tendency as the results of XRD patterns in Fig. 3. At lower drug loading, XRD patterns were almost similar with the patterns of nanoparticles themselves. But, at higher drug loading, peaks of crystallized drug in the nanoparticles were slightly increased at higher drug loading. The crystallized drug should be dissolved more slowly and diffused into the outer aqueous phase than that of molecular dispersion. As shown in Fig. 6(a) and (b), CNZ release rate was observed mostly as a pseudo zero-order kinetics for 2 days. Also, because of differences in the diffusivity of drug molecules to the outer aqueous phase, drug-release kinetics is affected not only by drug loading contents but also by the size of nanoparticles and polymer degradation rates. For same drug loading contents, the drug release rates from large nanoparticles were slower than that of small-sized nanoparticles as reported elsewhere [Alleman et al., 1993; Leroux et al., 1996]. At present, a direct comparison of drug release rate with different size of nanoparticles could not be performed because of different drug loading. Further investigations on the drug release characteristics will be needed for similar-sized nanoparticles as a function of drug loading contents, the sizes of nanoparticles and polymer used. As a result, control of drug release kinetics can be achieved by optimizing the chemical nature of the used polymers, drug loading contents, and the sizes of nanoparticles.

CONCLUSIONS

PLGA nanoparticles were prepared by dialysis method without surfactant. The sizes of PLGA nanoparticles prepared from DMAc and acetone were 200.4±133.0 nm in number average. The sizes of PLGA nanoparticles prepared from DMAc, DMF, and DMSO as initial used solvents were smaller than that of acetone or 1,4-dioxane. Selected initial solvent used to dissolve the copolymer significantly affects the sizes of nanoparticles. Also, the sizes of PLGA nanoparticles were changed according to the copolymer composition. PLGA nanoparticles have spherical shapes from the SEM and TEM observations. From these results, it is possible that the PLGA nanoparticles could be formed by dialysis method without surfactant. The drug-loading contents were also dependent on the copolymer composition and initial feeding amount of drug. The greater the lactide ratio on the copolymer composition led to higher drug loading contents. Also, the higher the initial feeding amount of drug, the higher the drug loading contents. CNZ was slowly released in higher lactide ratio in the copolymer composition and in the higher drug loading contents. CNZ release rate was observed mostly as a pseudo zero-order kinetics for 2 days.

ACKNOWLEDGEMENT

This research was supported by a grant from Sunchon National University (1999).

REFERENCES

Alleman, E., Gurny, R. and Doelker, E., "Drug-Loaded Nanoparticles-Preparation Methods and Drug Targeting Issues," *Eur. J. Pharm. Biopharm.*, **39**, 173 (1993).

Cho, C. S., Na, J. W., Jeong, Y. I., Kim, S. H., Lee, Y. M. and Sung, Y. M., "Micelle Formation of the Hexablock Copolymer Consisting of Poly(γ-benzyl L-glutamate) as the Hydrophobic Part and Poly(ethylene oxide) as the Hydrophilic Part," *Polymer (Korea)*, **19**, 926 (1995).

Ciftci, K., Suheyla Kas, H., Atilla Hincal, A., Meral Ercan, T., Guven, O. and Ruacan, S., "In Vitro and In Vivo Evaluation of PLGA (50/50) Microspheres Containing 5-Fluorouracil Prepared by a Solvent Evaporation Method," *Int. J. Pharm.*, **131**, 73 (1996).

Couvreur, P., Fattal, E. and Andremont, A., "Liposomes and Nanoparticles in the Treatment of Intracellular Bacterial Infections," *Pharm. Res.*, **8**, 1079 (1991).

Couvreur, P., Fattal, E., Alphandary, H., Puisieux, F. and Andremont, A., "Intracellular Targeting of Antibiotics by Means of Biodegradable Nanoparticles," *J. Control. Release*, **19**, 259 (1992).

Davis, S. S., "Colloids as Drug-Delivery Systems," *Pharmaceut. Technol.*, **5**, 71 (1981).

Kreuter, J., "Nanoparticle-Based Drug Delivery Systems," *J. Control. Release*, **16**, 169 (1991).

Davis, S. S., Illum, L., Moghimi, S. M., Davies, M. C., Porter, C. J. H., Muir, I. S., Brindley, A., Christy, N. M., Norman, M. E., Williams, P. and Dunn, S. E., "Microspheres for Targeting Drugs to Specific Body Sites," *J. Control. Release*, **24**, 157 (1993).

Dunn, S. E., Brindley, A., Davis, S. S., Davies, M. C. and Illum, L., "Polystyrene-Poly(ethylene glycol) (PS-PEG 2000) Particles as Model Systems for Site Specific Drug Delivery. 2. The Effect of PEG Surface Density on the In Vitro Cell Characterization and In Vivo Biodistribution," *Pharm. Res.*, **11**, 1016 (1994).

Govender, T., Stolnik, S., Garnett, M. C., Illum, L. and Davis, S. S., "PLGA Nanoparticles Prepared by Nanoprecipitation: Drug Loading and Release Studies of a Water Soluble Drug," *J. Control. Release*, **57**, 171 (1999).

Gref, R., Minamitake, Y., Peracchia, M. T., Trubetskoy, V., Torchilin, V. and Langer, R., "Biodegradable Long-Circulating Polymeric Nanospheres," *Science*, **263**, 1600 (1994).

Illum, L., Davis, S. S., Wilson, C. G., Frier, M., Hardy, J. G. and Thomas, N. W., "Blood Clearance and Organ Deposition of Intravenously Administered Colloidal Particles: The Effects of Particle Size, Nature and Shape," *Int. J. Pharm.*, **12**, 135 (1982).

Illum, L., Hunneyball, I. M. and Davis, S. S., "The Effect of Hydrophilic Coatings on the Uptake of Colloidal Particles by the Liver and by Peritoneal Macrophages," *Int. J. Pharm.*, **29**, 53 (1986).

Jeffery, H., Davis, S. S. and O'Hagan, D. T., "The Preparation and Characterization of Poly(lactide-co-glycolide) Microparticles. I. Oil-in Water Emulsion Solvent Evaporation," *Int. J. Pharm.*, **77**, 169 (1991).

Jeong, Y. I., Cheon, J. B., Kim, S. H., Nah, J. W., Lee, Y. M., Sung, Y. K., Akaike, T. and Cho, C. S., "Clonazepam Release from Core-Shell Type Nanoparticles *in vitro*," *J. Control. Release*, **51**, 169 (1998).

Juliene, M. C., Alonso, M. J., Gomez Amoza, J. L. and Benoit, J. P., "Preparation of Poly(dl-lactide/glycolide) Nanoparticles of Controlled Particle Size Distribution: Application of Experimental Design," *Drug Dev. Ind. Pharm.*, **18**, 1063 (1992).

Kwon, G. S., Naito, M., Yokoyama, M., Okano, T., Sakurai, Y. and Kataoka, K., "Physical Entrapment of Adriamycin in AB Block Copolymer Micelles," *Pharm. Res.*, **12**, 192 (1995).

Lasic, D. D., "Mixed Micelles in Drug Delivery," *Nature*, **355**, 279 (1992).

Leroux, J. C., Allemann, E., Jaeghere, F. D., Doelker, E. and Gurny, R., "Biodegradable Nanoparticles-From Sustained Release Formulations to Improved Site Specific Drug Delivery," *J. Control. Release*, **39**, 339 (1996).

Muller, R. H., Wallis, K. H., Troster, S. D. and Kreuter, J., "In Vitro Characterization of Poly(methyl-methacrylate) Nanoparticles and Correlation to their In Vivo Fate," *J. Control. Release*, **20**, 237 (1992).

Mura, P., Liguori, A., Bramanti, G., Corti, P., Murratzu, C. and Celesti, L., "In Vitro Study of Clonazepam Diffusion Kinetics from Solutions or Hydrophilic Gel," *Pharm. Acta Helv.*, **65**, 298 (1990).

Nah, J. W., Jeong, Y. I. and Cho, C. S., "Clonazepam Release from Core-Shell Type Nanoparticles Composed of Poly(γ-benzyl L-glutamate) as the Hydrophobic Part and Poly(ethylene oxide) as the Hydrophilic Part," *J. Polym. Sci., Polym. Phys.*, **36**, 415 (1998).

Peracchia, M. T., Gref, R., Minamitake, Y., Domb, A., Lotan, N. and Langer, R., "PEG-Coated Nanospheres from Amphiphilic Diblock and Multiblock Copolymers: Investigation of their Drug Encapsulation and Release Characteristics," *J. Control. Release*, **46**, 223 (1997).

Scholes, P. D., Coombes, A. G. A., Illum, L., Davis, S. S., Vert, M.

and Davies, M. C., "The Preparation of Sub-200 nm Poly(lactide-co-glycolide) Microspheres for Site-Specific Drug Delivery," *J. Control. Release*, **25**, 145 (1993).

Seijo, B., Fattal, E., Roblot-Treupel, L. and Couvreur, P., "Design of Nanoparticles of Less than 50 nm Diameter: Preparation, Characterization and Drug Loading," *Int. J. Pharm.*, **62**, 1 (1990).

Shin, I. G., Kim, S. Y., Lee, Y. M., Cho, C. S. and Sung, Y. K., "Methoxy Poly(ethylene glycol)/-Caprolactone Amphiphilic Block Copolymeric Micelle Containing Indomethacin. I. Preparation and Characterization," *J. Control. Release*, **51**, 1 (1998).

Sjostrom, B., Bergenstahl, B. and Kronberg, B., "A Method for the Preparation of Submicron Particles of Sparingly Water-Soluble Drugs by Precipitationin Oil-in Water Emulsions. II: Influence of the Emulsifier, the Solvent, and the Drug Substance," *J. Pharm. Sci.*, **82**, 585 (1993b).

Sjostrom, B., Kaplun, A., Talmon, Y. and Cabane, B., "Structure of Nanoparticles Prepared from Oil-in Water Emulsions," *Pharm. Res.*, **12**, 39 (1995).

Sjostrom, B., Kronberg, Br. and Carlfors, J., "A Method for the Preparation of Submicron Particles of Sparingly Water-Soluble Drugs by Precifitation in Oil-in Water Emulsions, I. Influence of Emulsification and Surfactant Concentration," *J. Pharm. Sci.*, **82**, 579 (1993a).

White, H. S., "Antiepileptic Drugs," In The Science and Practice of Pharmacy, Remington, Vol. 2, 19th ed.; A. R. Gennaro, Eds.; Mack Publishing Co.: Easton, Pennsylvania, USA, 1173 (1995).

Venier-Julienne, M. C. and Benoit, J. P., "Preparation, Purification and Morphology of Polymeric Nanoparticles as Drug Carriers," *Pharm. Acta Helv.*, **71**, 121 (1996).

Witschi, C. and Doelker, E., "Residual Solvents in Pharmaceutical Products: Acceptable Limits, Influences on Physicochemical Properties, Analytical Methods and Documented Values," *Eur. J. Pharm. Biopharm.*, **43**, 215 (1997).

Yoshioka, T., Hashida, M., Muranishi, S. and Sezaki, H., "Specific Delivery of Mitomycin C to the Liver, Spleen and Lung: Nano- and Microspherical Carriers of Gelatin," *Int. J. Pharm.*, **81**, 131 (1981).

Zhang, X., Jackson, J. K. and Burt, H. M., "Development of Amphiphilic Diblock Copolymers as Micellar Carriers of Taxol," *Int. J. Pharm.*, **132**, 195 (1996).