# Preparation of Poly(DL-lactide-*co*-glycolide) Nanoparticles Without Surfactant

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**ABSTRACT:** The preparation of poly(DL-lactide-*co*-glycolide) (PLGA) nanoparticles was performed by a dialysis method without surfactant or emulsifiers. The size of the PLGA nanoparticles prepared from dimethylacetamide (DMAc) as an initial solvent was smaller than that from acetone. The sizes of the PLGA nanoparticles from DMAc and acetone were $200.4 \pm 133.0$ and $642.3 \pm 131.1$ nm, respectively. The effects of the initial solvent selected to dissolve the copolymer and the lactide:glycolide ratio were investigated. The PLGA nanoparticles were spherical as revealed by the results of scanning electron microscopy and transmission electron microscopy observations. From these results it was shown that PLGA nanoparticles could be formed by the dialysis method without surfactant. The drug-loading contents and efficiency were also dependent on the lactide:glycolide ratio and initial feeding amount of the drug. A higher lactide ratio resulted in higher drug loading and higher loading efficiency. However, a higher initial feeding amount of the drug resulted in higher drug loading and lower loading efficiency. Clonazepam was released for at least 2 days and the release rate was slower with a higher lactide:glycolide ratio and a larger amount of drug-loading nanoparticles than that with a lower lactide:glycolide ratio and a smaller amount of drug-loading nanoparticles. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 2228–2236, 2001

**Key words:** poly(DL-lactide-*co*-glycolide); nanoparticles; dialysis method; surfactant free; clonazepam

## INTRODUCTION

Nanoparticles have been widely used for intravenous (iv) injection of drugs for drug targeting issues in drug delivery.[1–3] The potential of drug targeting to specific sites of the body would be of great benefit in the therapy of several disease states.[3,4] Therefore, the usage of nanoparticles *in vivo* has attracted considerable interest to achieve these objectives. The fate of nanoparticles after iv injection is greatly influenced by their interaction with the biological environment and their physicochemical properties. The effect of nanoparticle

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size has been shown to be of primary importance.[1,5] Administered particles that are several microns in diameter become filtered by the lung capillaries,[6] and submicron particles are rapidly cleared by the reticuloendothelial system.[7,8] Applications of nanoparticles for drug targeting to specific body sites also have advantages for avoiding surgery, which can be the source of infection. Nanoparticles have also attracted considerable attention in nonparenteral drug delivery systems such as oral, pulmonary, nasal, or ophthalmic delivery of drugs. For attaining these objectives, novel technologies are required to make small nanoparticles.

Although various polymers can be used to make nanoparticles, polymers used to prepare microspheres or nanoparticles for injection into the human body are significantly limited to a few kinds of polymers because of regulatory approval. Among them, poly(DL-lactide-*co*-glycolide) (PLGA) is one of the most widely used biodegradable polymers to make micro- or nanoparticles for controlled drug delivery systems. The nanoparticle preparation method is a critical problem for small-sized particles.[9–11] At present, the emulsion solvent evaporation method is widely employed for preparation of PLGA nanoparticles or microspheres.[11,12] In these cases, surfactants are required to stabilize the dispersed particles. Poly(vinyl alcohol) (PVA) is most frequently used as a stabilizing agent to make micro- or nanoparticles. However, PVA has some problems associated with its use in that it remains at the surface of particles and is difficult to remove. Other surfactants such as Span or Tween, poly(ethylene oxide), and poloxamer are also used to make and stabilize particles.[13,14] Some disadvantages of the conventional methods are the difficulties and necessities of removal of the solvent and surfactant residues because of their toxicities and the solvent properties for the polymer used, low particle yield, excessive steps for preparation, and the necessity to use a high concentration of surfactant for the preparation of small spherical particles.[13–15]

A surfactant-free particulate system was of significant interest to and was investigated by several groups over a period of 10 years.[16–22] Surfactant-free nanocapsules of poly(DL-lactide) (PLA) were developed by Fessi et al. based on the nanoprecipitation technique, and this technique was extensively employed to prepare nanoparticles.[16–20] The nanoprecipitation technique is based on the interfacial deposition of a polymer following displacement of a water-miscible semipolar solvent from a lipophilic solution.[16] There were reports that PLGA or PLA microspheres can be prepared by using PLA oligomers[21] or PLA-poly(ethylene glycol) (PEG) diblock copolymers,[22] which have an amphiphilic surfactant-like structure and behavior, as the surfactant instead of a conventional surfactant. Recently, a dialysis method was developed for the simple preparation of drug carriers such as liposomes and polymeric micelles.[23–25] The dialysis method is a simple and effective preparation method for small and narrow size distributed nanoparticles using block graft copolymers and other amphiphilic materials.[23–25] The application of the dialysis method for the preparation of nanoparticles using PLGA, which is not an amphiphilic material, has not yet been reported in detail.

In this study we prepared PLGA nanoparticles by a dialysis method without surfactant and evaluated the potential of the nanoparticles as drug carriers using clonazepam (CNZ) as a model drug. CNZ is an anticonvulsant benzodiazepine that has a considerable hydrophobic character [solubility $< 14.66$ $\mu$g/mL in PBS (pH 7.5, 37°C)][26] and high interaction with proteins *in vivo*.[27] The changes of the physicochemical properties of PLGA nanoparticles after drug entrapment are being investigated as a function of the solvent and the composition used to prepare the nanoparticles.

## EXPERIMENTAL

### Materials

The PLGA and CNZ were purchased from Sigma Chemical Co. (St. Louis, MO). The molecular weight of PLGA was analyzed by GPC as described below. The respective weight-average molecular weight ($M_w$), number-average molecular weight ($M_n$), and polydispersity ($M_w/M_n$) of each polymer were as follows: 85:15 (lactide:glycolide) PLGA was 48,400, 37,200, and 1.30; 75:25 PLGA was 47,500, 37,100, and 1.28; 50:50 PLGA was 40,100, 30,200, and 1.33. The solvents tetrahydrofuran (THF), dimethylformamide (DMF), dimethylsulfoxide (DMSO), dimethylacetamide (DMAc), and acetone (reagent grade) were used without further purification.

### Methods

The preparation of PLGA nanoparticles was carried out by the dialysis method without surfactant. Twenty milligrams of PLGA were dissolved in 10 mL of various solvents and subsequently 10–20 mg of CNZ were added. The solution was stirred at room temperature and solubilized completely. The

solution was introduced into the dialysis tube (molecular cutoff 12,000 g/mol) and dialyzed 3 times against 1.0 L of distilled water for 3 h and then distilled water was exchanged at intervals of 3–4 h during 24 h to remove organic solvent and unloaded drug. The resulting suspension was used for immediate analysis or freeze-dried.

For measurement of the drug loading the freeze-dried samples of the CNZ-loaded PLGA nanoparticles were suspended in methanol, vigorously stirred for 3 h, and sonicated for 15 min. The resulting solution was centrifuged (12,000 $\times$ *g* for 20 min) and the supernatant was taken for measurement of the drug concentration using a UV spectrophotometer (Shimadzu UV-1201) at 310 nm. The drug loading and loading efficiency were calculated with the following equation: drug loading (wt %) = [(weight of remaining drug in nanoparticles)/(weight of remaining drug in nanoparticles + polymer weight)] $\times$ 100; loading efficiency (wt %) = [(amount of remaining drug in nanoparticles)/(initial feeding amount of drug)] $\times$ 100.

The release experiment *in vitro* was carried out as previously reported.[25,28] Five miligrams of CNZ-loaded PLGA nanoparticles were suspended in 2 mL of PBS (0.1*M*, pH 7.4) by sonication for 30 s at 15 W using a bar-type sonicator (Ultrasonic homogenizer, UH-50, SMT Co. Ltd.) and then put into dialysis tubes (molecular weight cutoff: 12,000). The dialysis tube was placed into a 100-mL bottle with 50 mL of PBS, and the media was stirred at 100 rpm at 37°C. The whole-media change method was used for prevention of saturation of the drug. At each sampling time the whole medium (50 mL) was taken out and replaced with the same volume of fresh PBS (50 mL). The concentration of the released CNZ in the PBS was determined by UV spectrophotometer at 310 nm.

### Measurements

The MW of PLGA was measured with a Waters LC system coupled with a Waters 410 differential refractometer using Waters Styragel™ HR1, HR2, and HR4 columns at a flow rate of 1 mL/min. THF was used as an eluent. The average MW was evaluated with polystyrene as a standard.[29]

The morphology of the nanoparticles was observed using scanning electron microscopy (SEM, Jeol JSM-5400). One drop of the nanoparticle suspension was placed on a graphite surface. After freeze-drying the sample was coated with gold/palladium using an ion sputterer (Jeol JFC-1100). Coating was performed at 20 mA for 4 min. The observation was performed at 25 kV.

For the transmission electron microscopy (TEM) observations we placed one drop of nanoparticle suspension containing 0.01% of phosphotungstic acid on a carbon film coated on a TEM copper grid. The observation was done at 80 kV in a Jeol JEM-2000 FX II.

The size of the PLGA nanoparticles (0.1 wt % concentration) prepared by the dialysis method was found using photon correlation spectroscopy (PCS, Zetasizer 3000, Malvern Instruments) with a He-Ne laser beam at a wavelength of 633 nm at 25°C (scattering angle of 90°).

X-ray powder diffractograms were obtained with a Rigaku D/Max-1200 (Rigaku) using Ni-filtered CuK$\alpha$ radiation at 35 kV and 15 mA.

## RESULTS AND DISCUSSION

A surfactant-free nanoparticulate system has some advantages such as ease of preparation, preventing the possible side effects of the nanoparticle surface-located surfactant to the human body, and avoiding complexation of physicochemical properties of the polymer nanoparticulate system by the surfactant on the drug release mechanism and polymer degradation. Nanocapsules using PLA as a surfactant-free system were first developed by Fessi et al.[16] by interfacial polymer deposition following solvent displacement. Since their report, several research groups used the Fessi et al. method to make nanoparticles and nanocapsules using PLA, PLGA, or polycaprolactone.[16–22] Gref et al.[10] developed a one-step procedure to make nanospheres without the use of any other surfactant or emulsifiers; their nanosphere systems were based on block copolymers of PLA-PEG or PLGA-PEG, which have amphiphilic surfactant-like behavior and character and are different than the PLA homopolymer or PLGA random copolymer. Since Lasic[23] reported the dialysis procedure to make liposomes using amphiphilic materials there have been two groups[24,25] who used his method to make core–shell type nanoparticles or polymeric micelles. The successful investigation by Fessi et al.[16] and Lasic[23] led us to try the preparation of surfactant-free PLGA nanoparticles by the dialysis method. These nanoparticles were prepared without any additives such as surfactants, emulsifiers, or stabilizers and were characterized by PCS and electron microscopy. We also investigated the effect of the solvents used, copolymer composition, and drug-loading content on the size of the nanoparticles and their physicochemical properties. In the

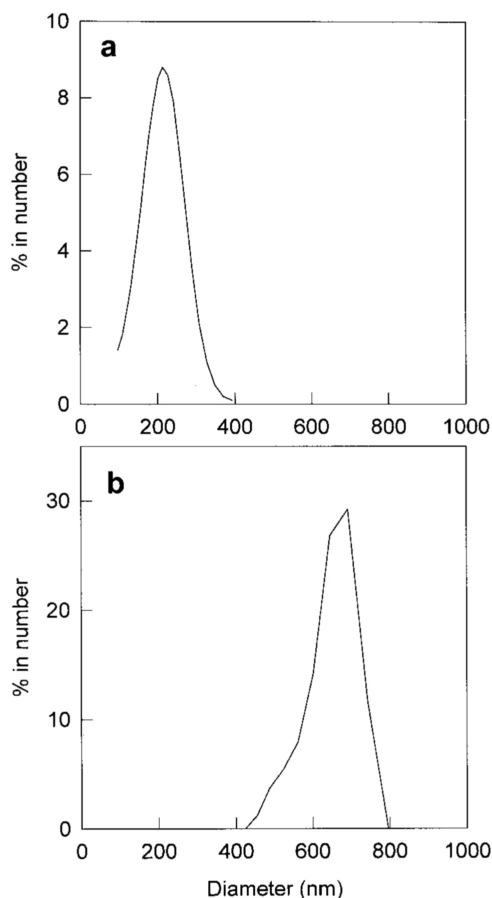


**Figure 1** The particle size distribution of 50:50 PLGA nanoparticles prepared from (a) DMAc or (b) acetone as an initial solvent.

dialysis system, solvent systems to make nanoparticles are limited to water-miscible solvents, which can dissolve both the polymer and drug, because water-immiscible solvents such as dichloromethane or chloroform cannot diffuse out or evaporate from the dialysis membrane to the outer aqueous environment.

The initial solvents DMAc and acetone were used to make 50:50 PLGA nanoparticles by the dialysis method. After the dialysis procedure, a milky suspension of 50:50 PLGA was observed when DMAc or acetone were used as the initial solvent. When DMAc was used, the resulting suspension was more transparent than that of acetone. To confirm nanoparticle formation, the particle size was analyzed using PCS. The particle size distribution of 50:50 PLGA prepared from DMAc and acetone is shown in Figure 1. The PLGA nanoparticles prepared from DMAc were smaller than nanoparticles prepared using acetone. The sizes of the PLGA nanoparticles prepared using DMAc and acetone were 200.4 ± 133.0 and 642.3 ± 131.1 nm, respectively. These results indicated that the selected initial solvent used to dissolve the copolymer had a slight affect on the size of the nanoparticles. Figure 2 shows SEM micrographs of PLGA nanoparticles prepared from DMAc and acetone. The PLGA nanoparticles prepared by the dialysis method had good spherical shapes in both cases. The 50:50 PLGA nanoparticles prepared from DMAc and acetone ranged between 100 and 400 and 400 and 1000 nm, respectively, which was similar to the particle size analysis. The TEM observation of 50:50 PLGA nanoparticles prepared from DMAc is shown in Figure 3. They showed uniform spherical shapes and the size range was about 200–500 nm, which were not significantly changed in comparison with the PCS and SEM results. In addition to the DMAc and acetone, other water-miscible organic solvents such as THF, DMSO, and DMF were used to make the PLGA nanoparticles. Table I shows the particle size of the PLGA against the various solvents used and the copolymer composition such as

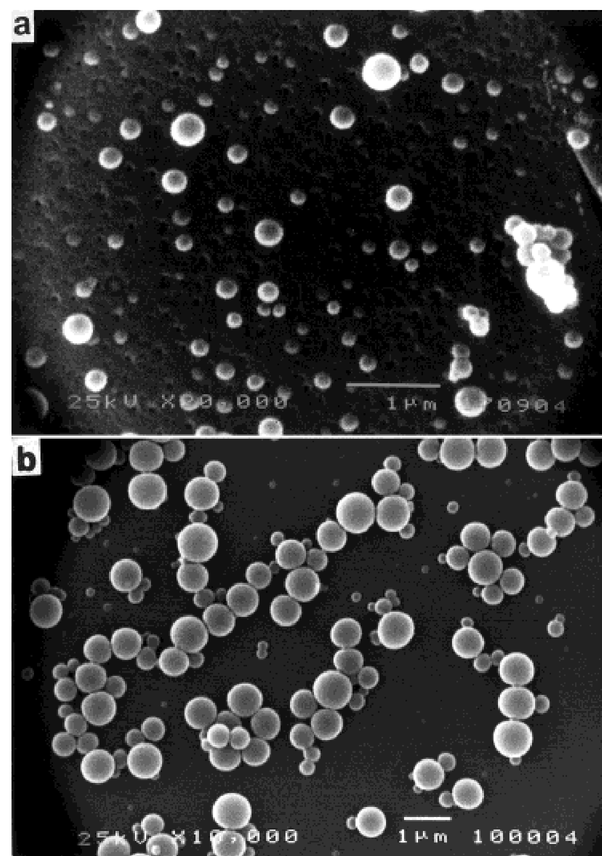


**Figure 2** Scanning electron microphotographs of 50:50 PLGA nanoparticles prepared from (a) DMAc or (b) acetone as a function of the initial solvent.

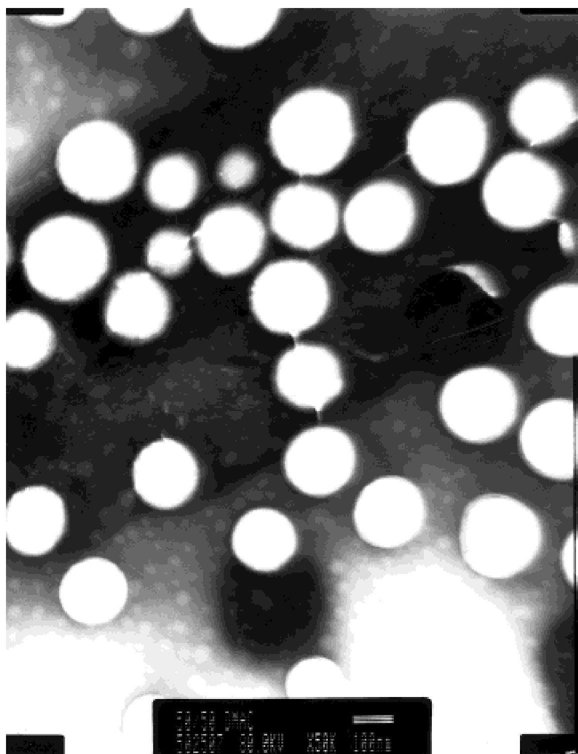

**Figure 3** A transmission electron micrograph of 50:50 PLGA nanoparticles prepared from DMAc as an initial solvent.

the 85:15 and 75:25 PLGAs. Among them, the 50:50 PLGA nanoparticles prepared from DMAc showed a relatively small particle size. However, the particle size results from DMAc, DMSO, and DMF were actually not significantly changed because of their large size distribution. The particle size of the PLGA nanoparticles prepared from THF and acetone were relatively larger than that of other solvents. These phenomena could be explained by differences of solubility and miscibility between the polymer and solvent or the water and solvent or viscosity of the solvent itself. Also, the particle size of the PLGA nanoparticles against the lactide:glycolide ratio was not significantly changed. The results of Figures 1 and 2 and Table I obviously confirm the formation of PLGA nanoparticles by the dialysis method without surfactant, and the particle size can be controlled by solvents used to dissolve the polymer. DMAc was used to dissolve the PLGA in the following experiments.

Generally, in self-assembling nanoparticulate systems such as the core–shell type nanoparticles[10] or polymeric micelles[24,25] using block or graft copolymers, the mechanism of nanoparticle formation is believed to be the hydrophobic interaction between the hydrophobic domains of the block copolymers. Fessi et al.[16] reported that the origin of the mechanism of nanocapsule formation could be explained in terms of interfacial turbulence or spontaneous agitation of the interface between two equilibrated liquid phases involving flow, diffusion, and surface processes. Although the mechanism is not fully understood at present, it was thought that the principle of PLGA nanoparticle formation by the dialysis procedure without surfactant may be based on a mechanism similar to the Fessi et al. method.[16] Additionally, the PLGA nanoparticles might be formed by hydrophobic interaction between polymeric chains and they would be stabilized solely by the presence of charged groups at the surface of the PLGA nanoparticles.[20]

The loading process of hydrophobic drugs into the particles is thought to involve the hydrophobic interaction between the drug and the hydrophobic segment of the polymeric chains. Also, the drug-loading process into the nanoparticles might controlled by the hydrophobic interaction. Table II shows the effect of the lactide:glycolide ratio and the initial drug feeding amount on the PLGA nanoparticles. The sizes of the PLGA nanoparticles against the lactide:glycolide ratio were not significantly changed after drug loading, although the size of the 50:50 PLGA nanoparticles against the drug loading increased slightly with higher drug loading than that of lower drug loading. The drug loading and loading efficiency were slightly dependent on the lactide:glycolide ratio: the higher the lactide ratio, the higher the drug loading and the loading efficiency. Also, the drug loading increased according to the increased amount of initial drug feeding, although the loading efficiency was decreased. Interestingly, the loading efficiency of PLGA nanoparticles prepared by the dialysis method (10–20 wt % of the initial concentration) was very low compared to the Fessi et al. method[16] (100 wt % of the initial concentration). It was thought that the major reason for the low loading efficiency of PLGA nanoparticles prepared by the dialysis procedure may have been due to the continuous removal of unwanted organic solvent and unloaded free drug from the solution during the dialysis procedure. Also, the entrapped drug might be released from the PLGA nanoparticles because the distilled water was exchanged at intervals of 3–4 h during 24 h to remove the solvents and unloaded drug. Basically, high loading efficiency of drugs into PLGA nanoparticles cannot be achieved by the dialysis method, although our drug loading was

**Table I Particle Size Distribution of PLGA Nanoparticles as Function of Lactide : Glycolide Ratio and Initial Solvent Used**

| PLGA | Solvent | Particle Size Distribution (nm) Intensity Average | Volume Average | Number Average |
|---|---|---|---|---|
| 50 : 50 | DMSO | 236.4 ± 94.4 | 266.7 ± 186.8 | 210.1 ± 116.7 |
| 50 : 50 | DMF | 269.6 ± 118.7 | 364.2 ± 269.6 | 261.8 ± 164.2 |
| 50 : 50 | DMAc | 241.7 ± 102.2 | 240.2 ± 121.6 | 200.4 ± 133.0 |
| 50 : 50 | THF | 431.8 ± 156.7 | 422.8 ± 212.2 | 411.1 ± 201.5 |
| 50 : 50 | Acetone | 634.1 ± 134.5 | 658.3 ± 120.5 | 643.2 ± 131.1 |
| 75 : 25 | DMAc | 249.3 ± 20.3 | 307.2 ± 186.6 | 251.3 ± 177.2 |
| 85 : 15 | DMAc | 423.8 ± 273.7 | 437.7 ± 218.9 | 409.0 ± 213.6 |

slightly higher than that of the Fessi et al.[16] method. Therefore, we thought that the major drawback of the dialysis procedure for preparation of surfactant-free PLGA nanoparticles was low loading efficiency. X-ray powder diffraction was employed to confirm the characteristics of the CNZ-loaded PLGA nanoparticles. Figure 4 shows the X-ray diffraction scans of the CNZ-loaded PLGA nanoparticles and the corresponding physical blend. It can be observed that the X-ray diffraction peaks characteristic of CNZ, which were visible in the pattern obtained for the physical blend, disappeared in the scans corresponding to the CNZ-entrapped nanoparticles with low drug loading (10.5 wt % CNZ loading). These results indicated that the CNZ existed as a molecular dispersion in the polymeric nanoparticles at low drug loading. It was thought that free drug did not exist on the surface of the surfactant-free nanoparticles. However, contrary to our expectations, drug crystal peaks were observed in the peaks of the X-ray diffraction scan of PLGA nanoparticles with high drug loading (15.7 wt % CNZ loading), indicating that the drug cannot be completely entrapped in the nanoparticles in the high initial feeding concentration of the drug and free drug crystals may have existed on the nanoparticle surface.

In the nanoparticle system one of the major advantages is the ease of long-term storage of drug-loaded nanoparticles in a freeze-dried form until used. Therefore, it is necessary to confirm the reconstitution of freeze-dried surfactant-free nanoparticles; this is an important factor for administering it through intravenous injection. Figure 5 shows the particle size and the SEM images of the PLGA nanoparticles after redistribution (0.1*M* PBS, pH 7.4) using a sonicator. After the reconstitution of freeze-dried PLGA nanoparticles, the particle size was significantly increased with a broad and bimodal size distribution pattern [Fig. 5(a)]. The size of the reconstituted PLGA nanoparticles was 338.6 ± 155.7 nm for a 76.4% area and 580.2 ± 123.2 nm for a 23.6% area. In addition to the particle size results, the observation of SEM images showed aggregated and increased particles. Fessi et al.[16] reported that a highly aqueous soluble surfactant such as poloxamer was needed for physical stability of the nanocapsule suspension, although it was possible to make nanocapsules in the absence of any surfactant (i.e., surfactant-free nanocapsules). They

**Table II Effects of Lactide : Glycolide Ratio and Drug Loading on Particle Size Distribution of PLGA Nanoparticles**

| Sample | Initial Polymer Amount (mg) | Initial Drug Feeding Amount (mg) | Drug Loading (wt %) | Loading Efficiency (wt %) | Particle Size Distribution (nm) Intensity Average | Volume Average | Number Average |
|---|---|---|---|---|---|---|---|
| 85 : 15 | 20 | 20 | 11.7 | 13.3 | 453.7 ± 175.8 | 470.5 ± 220.1 | 421.2 ± 186.9 |
| 75 : 25 | 20 | 20 | 10.6 | 11.9 | 281.3 ± 145.8 | 354.7 ± 217.7 | 276.9 ± 125.6 |
| 50 : 50 | 20 | 20 | 10.5 | 11.7 | 297.1 ± 119.6 | 336.5 ± 173.3 | 289.1 ± 190.2 |
| 50 : 50 | 20 | 40 | 15.7 | 18.6 | 275.8 ± 118.9 | 343.6 ± 202.9 | 296.8 ± 202.6 |

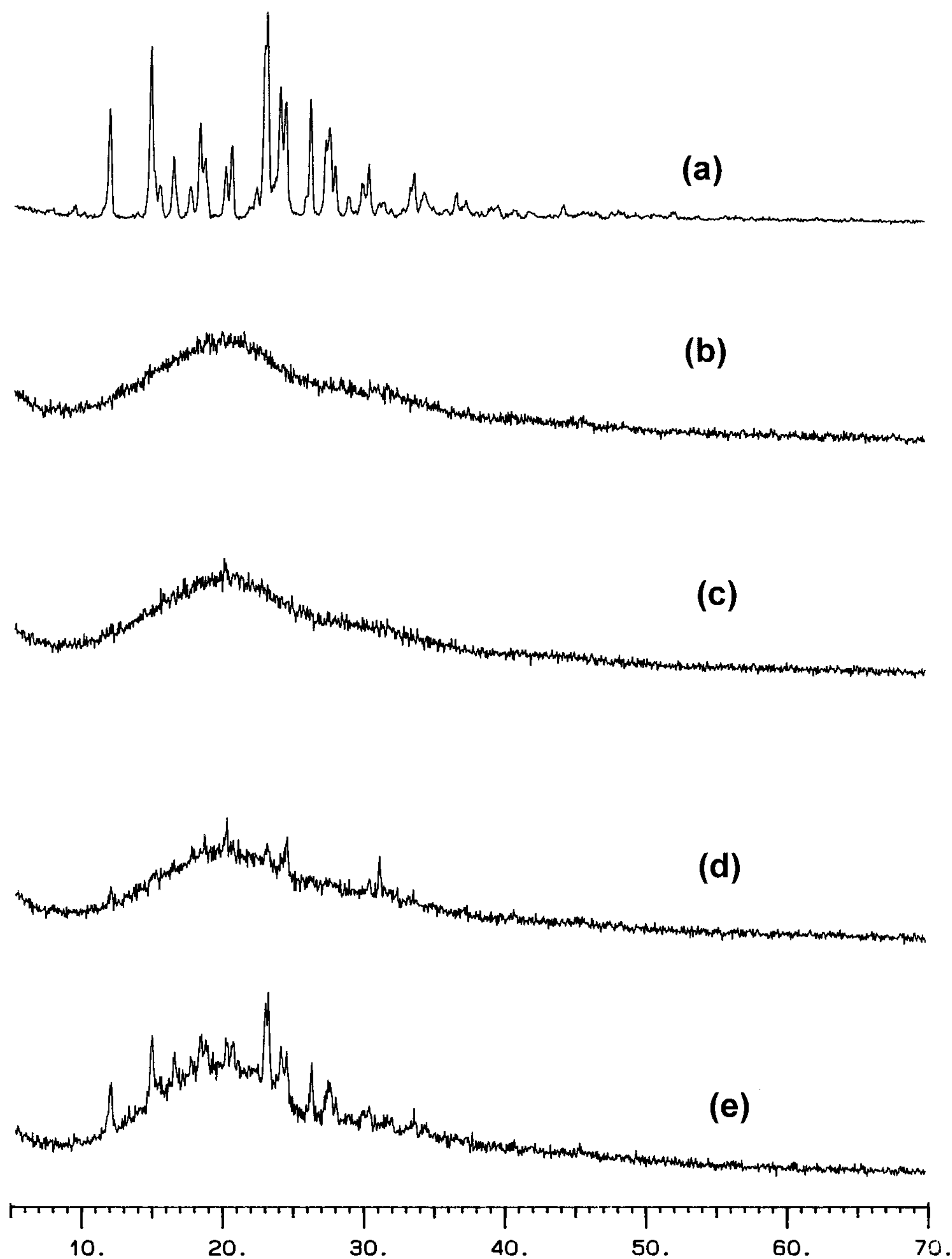


**Figure 4** X-Ray diffractometer patterns of 50:50 PLGA nanoparticles: (a) clonazepam, (b) empty PLGA nanoparticles, (c) CNZ-loaded PLGA nanoparticles (10.5 wt % drug loading), (d) CNZ-loaded PLGA nanoparticles (15.7 wt % drug loading), and (e) a physical mixture of CNZ and empty PLGA nanoparticles (weight ratio of CNZ/polymer = 1/10).

also reported that the presence of at least one of the emulsifiers was needed for wall coating formation and for suspension stabilization; a maximum suspension stability was achieved with a combination of both emulsifiers because, when prepared with only one emulsifier, nanocapsules would sediment and form a cake that was difficult to redisperse.[16] In this report, we tried to reconstitute the cake after freeze-drying, but the physical stability was not maintained after reconstitution (i.e., surfactant-free PLGA nanoparticles were largely aggregated by reconstitution after freeze-drying). Of course, we can store the surfactant-free nanoparticles or conventional nanoparticles in a freezing state without drying, but drugs or biological agents in the frozen nanoparticles

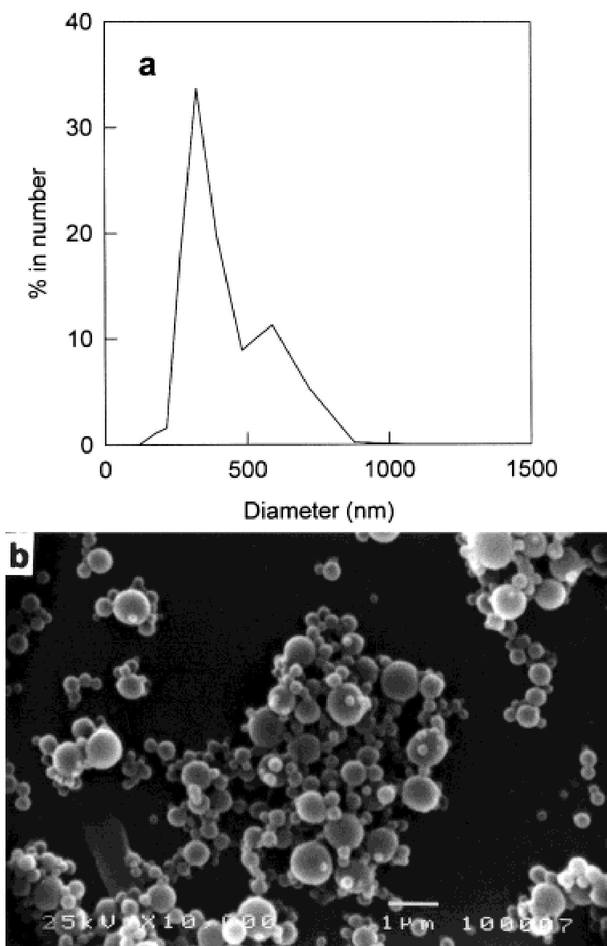


**Figure 5** (a) The particle size distribution and (b) a scanning electron microscope image of reconstituted 50:50 PLGA nanoparticles prepared from DMAc as an initial solvent.

may easily lose their biological activities in long-term storage. We are going to investigate the reconstitution factor of surfactant-free PLGA nanoparticles using dispersing agents such as poloxamer, carboxy methyl cellulose, or albumin and without the use of dispersing agents.

Figure 6 shows the CNZ release from PLGA nanoparticles against the lactide:glycolide ratio and drug loading of 50:50 PLGA nanoparticles. The drug release rate from the nanoparticles would be faster than that of other microsphere systems because of the significantly larger surface area and smaller size of the nanoparticles than those of a microsphere system. Drugs entrapped in the nanoparticles can be more rapidly diffused into the outer aqueous environment than microsphere systems. As shown in Figure 6, the drug release was faster than we expected (i.e., the drug release was almost finished in 2–3 days). These results may be attributable to the small particle size and possible existence of free drug on the nanoparticle surface as explained in Figure 4. As shown in Figure 6(a), we found that the higher the lactide ratio, the slower the drug release. These results may be due to the large particle size and the stronger interaction between the hydrophobic lactide segments and drug than that of the glycolide segments.[10,25,28] The CNZ releases from 50:50 PLGA nanoparticles against the drug loading are shown in Figure 6(b). These results indicated that the higher the drug loading, the slower the drug release. These phenomena were reported by several authors.[10,25] Gref et al.[10] and Peracchia et al.[28] reported that a hydrophobic drug such as lidocaine crystallized inside the nanoparticles and a phase separation occurred at higher drug-loading contents. Therefore, the hydrophobic drug loaded into the nanoparticles could be slowly released at higher drug-loading contents,

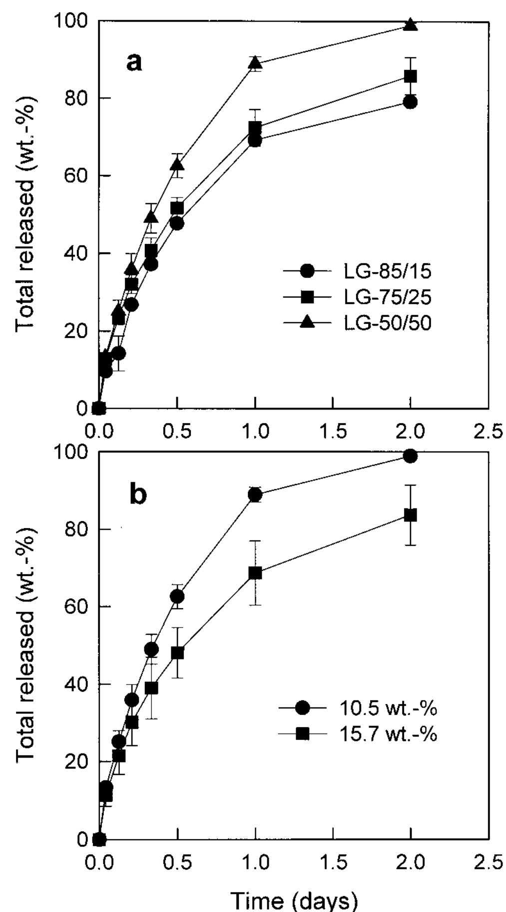


**Figure 6** The clonazepam release from PLGA nanoparticles against (a) the copolymer composition (drug-loading contents as described in Table II) and (b) the drug loading of 50:50 PLGA nanoparticles.

which is different than the hydrophilic water-soluble drugs. A drug is often present as a molecular dispersion inside the nanoparticles at low drug loading.[10] The crystallized drug should dissolve and diffuse into the outer aqueous phase more slowly than a molecular dispersion. As shown in Figure 6(a,b), the CNZ release rate was observed almost as a pseudo zero-order kinetics for 2 days. The drug release kinetics were affected by drug loading, particle size, and polymer degradation rate because of the differences in drug diffusivity to the outer aqueous phase. The drug release from large nanoparticles would exhibit slower rate kinetics than those of the small nanoparticles. In conclusion, the drug release kinetics can be controlled by optimizing the chemical nature of the polymers used, the drug loading, and the nanoparticle size.

## CONCLUSIONS

PLGA nanoparticles were successively prepared by a dialysis method without surfactant, even though there were some drawbacks such as the instability of the reconstituted freeze-dried PLGA nanoparticles and the low loading efficiency of the drugs. The formation and size of PLGA nanoparticles by a dialysis method was confirmed by PCS measurement and electromicroscopic observation using TEM and SEM. PLGA nanoparticles prepared from DMAc were smaller than those prepared from acetone. The sizes of the PLGA nanoparticles using DMAc and acetone were 200.4 $\pm$ 133.0 and 642.3 $\pm$ 131.1 nm, respectively. The solvent used to dissolve the copolymer had a slight affect on the size of the nanoparticles. Also, the sizes of the PLGA nanoparticles were not significantly changed according to the lactide:glycolide ratio. The PLGA nanoparticles were spherically shaped as ascertained from the SEM and TEM observations. The drug-loading contents and loading efficiency were also dependent on the lactide:glycolide ratio and the initial feeding amount of the drug. The higher the lactide ratio, the higher the drug loading and loading efficiency. The CNZ release rate was slower at higher lactide:glycolide ratio. Also, the release rate of CNZ from the PLGA nanoparticles with high drug-loading nanoparticles was slower than that from the lower drug-loading nanoparticles.

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