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Clonazepam Release from Poly(DL-lactide-co-glycolide) Nanoparticles Prepared by Dialysis Method

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Aim of this work is to prepare poly(DL-lactide-co-glycolide) (PLGA) nanoparticles by dialysis method without surfactant and to investigate drug loading capacity and drug release. The size of PLGA nanoparticles was 269.9±118.7 nm in intensity average and the morphology of PLGA nanoparticles was spherical shape from the observation of SEM and TEM. In the effect of drug loading contents on the particle size distribution, PLGA nanoparticles were monomodal pattern with narrow size distribution in the empty and lower drug loading nanoparticles whereas bi- or trimodal pattern was showed in the higher drug loading ones. Release of clonazepam from PLGA nanoparticles with higher drug loading contents was slower than that with lower loading contents.

Key words : Poly(DL-lactide-co-glycolide), Nanoparticle, Dialysis method, Clonazepam, Controlled release

INTRODUCTION

In the drug delivery systems, nanoparticles or colloidal carriers have been widely accepted for intravenous (i.v.) injection of drugs and for drug targeting issues (Kreuter, 1991; Davis *et al.*, 1993; Alleman, 1993). The possibility of drug targeting to specific organs or tissues would be great benefit in the therapy of several disease states (Leroux *et al.*, 1996; Couvreur *et al.*, 1991; Couvreur *et al.*, 1992). The use of nanoparticles has attracted considerable interest to achieve these objectives. It is widely accepted that the fate of nanoparticles after *i.v.* injection is greatly influenced by their interaction with the biological environment and their physicochemical properties. Among them, the effect of nanoparticle size has been shown to be of primary importance (Davis, 1981; Seijo *et al.*, 1990). For example, administered particles of several micrometers in diameter become filtered by the lung capillaries (Illum *et al.*, 1982; Yoshioka *et al.*, 1981). Also, smaller, submicron particles are normally rapidly cleared by the reticuloendothelial system (RES) such as the Kupffer cells of the liver, a major barrier to effective targeting to other sites (Illum *et al.*, 1986; Muller *et al.*, 1992; Dunn *et al.*, 1994).

On the other hand, preparation method of nanoparticles is a important problem for more small sized particles and for long circulating nanoparticles (Alleman *et al.*, 1993; Seijo *et al.*, 1990; Juliene *et al.*, 1992; Gref *et al.*, 1994; Venier-Juliene *et al.*, 1996). For preparation of poly(DL-lactide-co-glycolide) (PLGA) nanoparticles or microspheres, emulsion solvent evaporation method is a most widely accepted method until present (Venier-Juliene *et al.*, 1996; Jeffery *et al.*, 1991; Scholes *et al.*, 1993; Ciftci *et al.*, 1996). However, several problems may stll exist such as difficulties and necessities of removal of solvent and surfactant due to their toxicity and its solvent properties for polymer used, drug inactivation during the preparation procedure, drug lost during the washing step, low drug loading efficiency, low particle yield, too many steps for the preparation, and necessity of usage of a lot of surfactant for the preparation of small sized spherical shaped particles (Seijo *et al.*, 1990; Sjostrom *et al.*, 1993a; Sjostrom *et al.*, 1993b; Sjostrom *et al.*, 1995; Witschi *et al.*, 1997). Recently, dialysis method was developed for the simple preparation of drug carriers such as liposomes and polymeric micelles, and for application to the drug targeting carriers (Lasic, 1992; Kataoka *et al.*, 1993; Kwon *et al.*, 1995; Nah *et al.*, 1998; Jeong *et al.*, 1998; Cho *et al.*, 1995; Kim *et al.*, 1997). Also, dialysis method is a acceptable simple and ef-

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fective preparation method for small and narrow size distributed nanoparticles using block, graft copolymers and amphiphilic materials (Lasic, 1992; La *et al.*, 1996). However, for the preparation of nanoparticles using PLGA which is not amphiphilic material, the application of dialysis method has not reported until present.

For this study, we have prepared PLGA nanoparticles by dialysis method without surfactant and studied potential of nanoparticles as a drug carriers using clonazepam (CNZ) as a model drug. CNZ is an anticonvulsant benzodiazepine which is efficacious for the treatment of panic disorder and has considerable hydrophobic character (water solubility: <14.7 μg/ml) and, especially, has high interaction with proteins *in vivo* (Mura *et al.*, 1990; White, 1995). Therefore, the CNZ has need to extend the half-life to avoid protein adsorption and rapid clearance by the unwanted organs or tissues when injected into the body and to solve the drug solubility. Also, shapes and sizes of the nanoparticles were investigated and CNZ release from the nanoparticles was performed *in vitro*.

MATERIALS AND METHODS

Materials

Poly(DL-lactide-co-glycolide) (PLGA) 50:50 (Molecular weights: 40,000~75,000) was purchased from Sigma Chemical Co., USA. From the result of GPC measurement as described below, *Mw* and *Mn* of PLGA 50:50 was 40,100 and 30,200, respectively, which was showed a slight difference with manufacturer's data and *Mw/Mn* was 1.33. Clonazepam (CNZ) was obtained from ROCHE, Switzerland. Dimethylformamide (DMF) as a reagent grade was used without further purification.

Gel permeation chromatography measurement

M.W. of PLGA was measured by GPC method (Shimadzu, GPC-800 series). 20 μl of PLGA solution (in THF, 2 mg/mL) was injected to GPC. Flow rate of tetrahydrofuran (THF 99.5%, Sigma Chemical Co., USA) as a mobile phase was 1 ml/min. Average M.W. was evaluated by polystyrene as a standard.

Preparation of PLGA nanoparticles and drug loading procedure

Preparation of PLGA nanoparticles was carried out by a dialysis method without any other surfactant. Briefly, 20 mg of PLGA was dissolved in 10 ml of DMF. The solution was introduced into dialysis tube (molecular cut-off 12,000 g/mol, Sigma Chemical Co., USA) and dialyzed against 1.0 L×3 of distilled water for 3 hrs and then distilled water exchanged at intervals of 3~4 hrs during 24 hrs. Then, the resultant solution was used for analysis.

Drug loading procedure was carried out as followed: 20 mg of PLGA was dissolved in 10 mL of DMF and subsequently 10~40 mg of clonazepam was added. The solution was solubilized at room temperature. The solution was dialyzed using molecular cutoff 12, 000 g/mol dialysis tube against 1.0 L×3 of distilled water for 3 hrs and then distilled water exchanged at intervals of 3~4 hrs during 24 hrs. The solution was freeze-dried using freeze-vacuum dryer (Freezone 6, LABCONCO Co., USA).

For measurement of drug-loading content, freeze-dried samples of CNZ-loaded PLGA nanoparticles were suspended into methanol and vigorously stirred for 2 hrs and sonicated for 15 min. Resulting solution was centrifuged with 12,000 g for 20 min and supernatent was taken for measurement of drug concentration using Ultraviolet (UV) spectrophotometer (Shimadzu UV-1201, Japan) at 310 nm.

Scanning electron microscope (SEM) measurements

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

Transmission electron microscope (TEM) measurements

A drop of nanoparticles 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 FX II, Japan.

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 diafiltration method was used for particle size measurement (concentration: 0.1 wt.-%) and measured without filtering.

In vitro release studies

The release experiment in vitro was carried out as follows: 5 mg of CNZ loaded PLGA nanoparticles and 1 mL phosphate buffered saline (PBS, 0.1 M and pH 7.4) were put into a dialysis tubes (MWCO:12, 000) and the tube was introduced into a vial with 10 mL PBS and the media were stirred at 100 rpm at 37 °C. At specific time intervals, medium was taken and replaced with fresh PBS. The concentration of the released CNZ into PBS was determined by UV spectro-

photometer (Shimadzu UV-1201) at 310 nm.

RESULTS AND DISCUSSION

To investigate the possibility of nanoparticle formation of PLGA by dialysis method without any other surfactant, particle characterization was performed by measurement of PCS and by observation of SEM and TEM.

Particle size distribution of PLGA 50:50 nanoparticles was shown in Fig. 1. The size of PLGA nanoparticles was 269.9±118.7 nm in intensity average. These results indicated that the particle size of PLGA nanoparticles prepared by dialysis method without surfactant was revealed small and narrow size distribution. Also, the particle yield of PLGA after freeze-drying was about 97.0 wt.-%. It is indicated that the initial amount of polymer was almost changed to nanoparticles. The morphology of PLGA nanoparticles was shown in Fig. 2 and 3. Fig. 2 shows SEM photograph of PLGA nanoparticles. These results indicated that PLGA nanoparticles prepared by dialysis method has good spherical shapes and ranged about 100~500 nm which is almost similar with the results of particle size analysis. TEM observation of PLGA nanoparticles was also shown in Fig. 3. Their shapes were showed nice sphericity and ranged about 200~500 nm which was almost same with results of PCS and SEM. Interestingly, it was found that double walled-structure was seen in the PLGA nanoparticles which has about 40~50 nm thickness of outerwall and 100~300 nm of innercore. From the results of Fig. 1, Fig. 2, and Fig. 3,

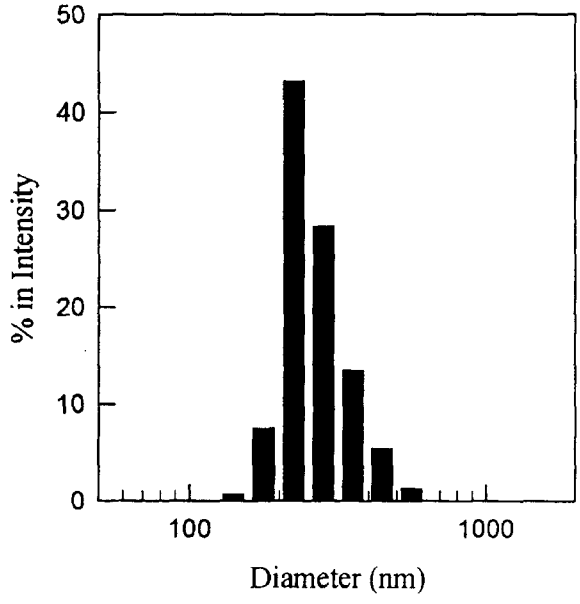

Fig. 1. Particle size distribution of PLGA nanoparticles. Size distribution was measured by photon correlation spectroscopy at 25°C (Sample concentration: 0.1 wt.-%.).

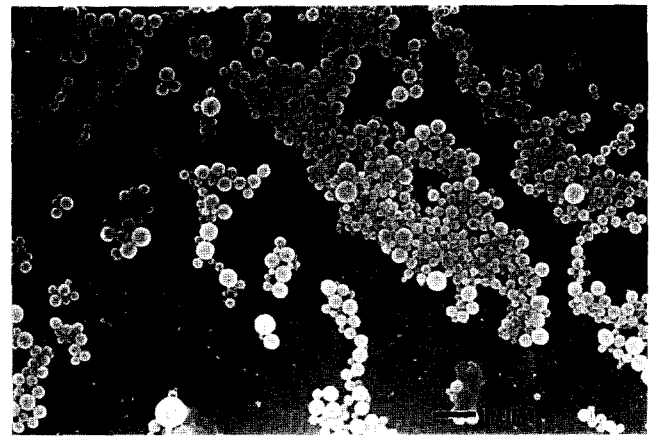

Fig. 2. Scanning electron microphotograph of PLGA nanoparticles. A drop of the nanoparticle suspension was placed on a graphite surface. After freeze-drying, the sample was coated with gold/palladium.

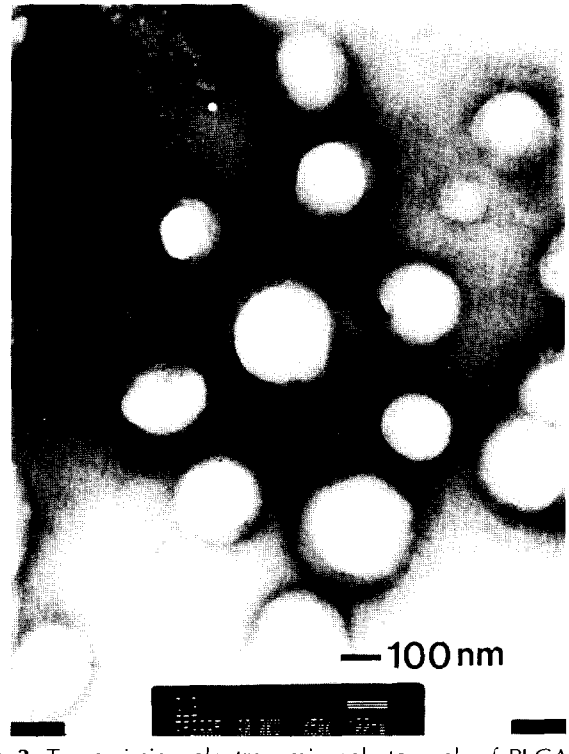

Fig. 3. Transmission electron microphotograph of PLGA nanoparticles. A drop of nanoparticles suspension was placed on a carbon film coated on a copper grid and negatively stained with phosphotungstic acid (0.01 wt.-%).

PLGA nanoparticles with small size, spherical sphape and narrow size distribution were simply and succefully made by dialysis method without any other surfactant.

Table I shows the effect of drug loading contents on the particle size of PLGA nanoparticles. The particle size of PLGA nanoparticles in the lower drug loading (10.0 wt.-%) was not sigfinicantly changed when compared with empty nanoparticles. However,

Table I. Particle size distribution of clonazepam loaded-PLGA nanoparticles against drug loading contents

Drug-loading contents (wt.-%)	Particle size distribution (nm) (area-%)		
	Intensity average	Volume average	Number average
0*	269.6±118.7	364.2±269.6	261.8±164.2
10.0	290.9±123.3	345.3±300.7	273.1±193.9
24.6	196.9±54.1 (43.2)	334.5±157.6 (86.3)	191.1±42.8 (37.0)
	333.2±134.5 (52.1)	721.4±310.0 (13.7)	329.8±183.7 (63.0)
	763.1±162.2 (4.7)		

*Empty nanoparticles

in the higher drug loading (24.6 wt.-%) PLGA nanoparticles, the particle size was slightly increased compared to empty and lower drug loading nanoparticles. Also, the pattern of particle size was showed bi- or trimodal distribution as shown in Table I.

CNZ release from PLGA nanoparticles against drug loading content are shown in Fig. 4. These results indicated that the more the drug content, the slower the drug release. At lower loading, CNZ may be present as a dispersed state in the nanoparticles whereas a crystallization of drug in the nanoparticles occurs at higher loading (Gref *et al.*, 1994; Jeong *et al.*, 1998). As reported elsewhere (Gref *et al.*, 1994; Nah *et al.*, 1998), the crystallized drug should be dissolved more slowly and diffused into the outer aqueous phase. CNZ release was followed the zero-order kinetics about 2 days for 10.0 wt.-% drug loading and about 3 days for 24.6 wt.-% drug loading, indicating that control of drug release kinetics can be achieved by changing drug loading contents.

In conclusion, PLGA nanoparticles were prepared by dialysis method without using surfactant. The size of PLGA nanoparticles was 269.9±118.7 nm in intensity average and the morphology of PLGA nanoparticles was spherical shape from the observation of SEM and TEM. In the effect of drug loading contents on the particle size distribution, PLGA nanoparticles were monomodal pattern with narrow size distribution in the empty and lower drug loading PLGA nanoparticles whereas bi- or trimodal pattern was showed in the higher drug loading. Release of CNZ from PLGA nanoparticles with higher drug loading contents was slower than that with lower drug loading contents.

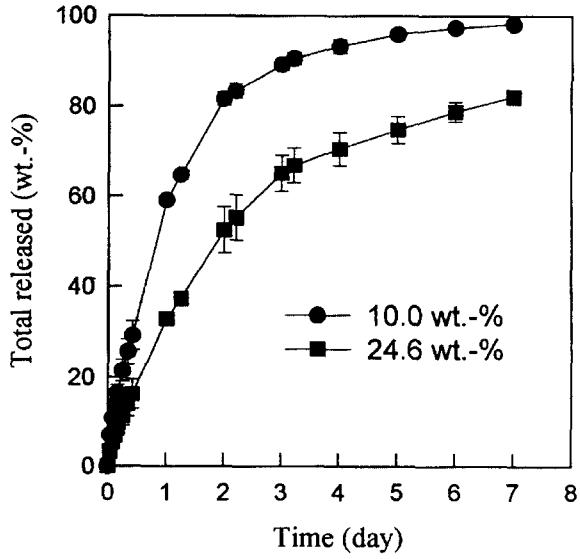

Fig. 4. Clonazepam release from PLGA nanoparticles against drug loading contents. Release experiment was performed with PBS (0.1 M, pH 7.4) at 37°C.

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