Effect of solvent on the preparation of surfactant-free poly(DL-lactide-co-glycolide) nanoparticles and norfloxacin release characteristics

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Abstract

The surfactant-free nanoparticles of poly(DL-lactide-co-glycolide) (PLGA) were prepared by dialysis method without surfactant and physicochemical properties such as particle size and drug contents were investigated against used initial solvent. The size of PLGA nanoparticles and drug contents were significantly changed by used initial solvent. The size of PLGA nanoparticles prepared from dimethylacetamide (DMAc), dimethylformamide (DMF), and dimethylsulfoxide (DMSO) as a initial used solvent was smaller than that of acetone. Selected initial solvent used to dissolve the copolymer significantly affects the size of nanoparticles and drug contents. It was shown that PLGA nanoparticles have spherical shapes from the results of scanning electron microscopy (SEM) and transmission electron microscopy (TEM) observations. It was thought that surfactant-free nanoparticles of PLGA entrapping norfloxacin (NFX) has nice drug loading capacity without free-drug on the surface of nanoparticles through the analysis of X-ray powder diffraction. From these results, it was showed the potential that the PLGA nanoparticles could be formed successively by dialysis method without surfactant. Release kinetics of NFX used as a model drug was governed by not only drug contents but also particle size parameter. The higher the drug contents and the larger the particle size resulted in slower the drug release. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Poly(DL-lactide-co-glycolide); Surfactant-free nanoparticles; Used initial solvent; Dialysis method; Norfloxacin; Controlled drug release

1. Introduction

Nanoparticles or colloidal carriers have been extensively investigated in biomedical and biotechnological areas and, especially, in drug delivery systems for drug targeting (Alleman et al.,

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1993; Davis, 1981; Davis et al., 1993) because their particle sizes (ranged from 10 to 1000 nm) are acceptable for intravenous (i.v.) injection (Kreuter, 1993). The advantages of targeted drug delivery to the specific site of body are in the therapy of several disease states such as anticancer treatment, gene therapy, viral disease, and bacterial infection in the specific body sites (Couvreur et al., 1991, 1992; Leroux et al., 1996). Therefore, the application of nanoparticles for drug targeting in vivo has attracted considerable interest 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 of nanoparticles, morphology, and hydrophilicity, etc. Among them, the effect of nanoparticle size has been shown to be of primary importance (Davis, 1981; Seijo et al., 1990). Administered particles with several micrometers in diameter become mainly accumulated into 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 advantages to avoid any surgery which can always be the source of infection. Also, nanoparticles have been much attention in non-parenteral 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. Also, novel preparation methods are needed for development of effective nanoparticulate drug delivery vehicles.

Although various polymers are possible to make nanoparticles for drug delivery system in vitro, polymeric materials used to prepare microspheres or nanoparticles for administration into the human body are significantly limited to a few kinds of acceptable polymers. 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 one of the most widely used biodegradable polymers to make micro- or nanoparticles for controlled drug delivery systems. The preparation method of nanoparticles is a critical problem for small sized particles (Gref et al., 1994; Juliene et al., 1992; Venier-Julienne and Benoit, 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 and Benoit, 1996) at present. In these methods, serious amount of surfactants are required to stabilize the dispersed particles. Especially, poly(vinyl alcohol) as a stabilizing agents is most frequently used to make micro- or nanoparticles (Lee et al., 1999; Shakesheff et al., 1997). Poly(vinyl alcohol) have some problems that PVA remains at the surface of particles and then difficult to remove. It was known that PVA existed on the surface of PLGA micro- or nanoparticles change the biodegradability, biodistribution, and drug release behavior of drug carrier (Landry et al., 1996, 1997; Lavelle et al., 1995; Tabata and Ikada, 1991). Other surfactants such as Span series or Tween series, poly(ethylene oxide) (PEO), and poloxamer (PEO-poly(propylene oxide) block copolymer), etc. are also used to make and stabilize particles (Sjostrom et al., 1993a,b). Also, some disadvantages of these methods are difficulties and necessities of removal of solvent and surfactant due to their toxicity and its solvent properties for polymer used, low particle yield, too many step for the preparation, and necessity of usage of a lot of surfactant for the preparation of small sized spherical particles (Sjostrom et al., 1993a,b; Witschi and Doelker 1997). Almost of these surfactants are non-biodegradable, non-digestible, and not always biocompatible. Also, these surfactants can be affected to human body such as an allergy-like reaction.

Recently, dialysis method was developed for the simple preparation of drug carriers such as liposomes and polymeric micelles (Jeong et al., 1998; Kwon et al., 1995; Lasic 1992; Nah et al., 1998). 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 (Jeong

et al., 1998; Kim et al., 1999; Kwon et al., 1995; Lasic 1992; Nah et al., 1998).

For this study, we have prepared surfactant-free PLGA nanoparticles by dialysis method without surfactant using various solvent and studied possibility of nanoparticles as a drug carriers using norfloxacin (NFX) as a hydrophobic drug. The drug loading contents, loading efficiency, changes of particle size, and physicochemical properties of PLGA nanoparticles after drug entrapped into the nanoparticles are investigated against various solvents.

2. Materials and methods

2.1. Materials

PLGA and NFX were purchased from Sigma Chemicals, USA. Molecular weight of PLGA 85:15, 75:25 and 50:50 was 48 400, 47 500 and 40 100 Da from our GPC measurements as described below. Various solvents, i.e. dimethylformamide (DMF), dimethylsulfoxide (DMSO), dimethylacetamide (DMAc) and acetone, as a reagent grade were used without further purification.

2.2. Gel permeation chromatography (GPC) measurement

Molecular weight 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 molecular weight was evaluated by polystyrene as a standard.

2.3. 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. The solution was introduced into dialysis tube (molecular cutoff 12 000 g/mol) and dialyzed against 1.0 l × 3 of distilled water

for 3 h and then distilled water exchange at intervals of 3 ∼ 4 h during 24 h. The resultant solution was used for analysis or freeze-dried.

Drug loading procedure was carried out as followed: 20 mg of PLGA was dissolved in 10 ml of various solvents and subsequently 20 mg of NFX was added. The solution was stirred at room temperature. The solution was dialyzed using dialysis tube (molecular cutoff 12 000 g/mol) against 1.0 l × 3 of distilled water for 3 h and then distilled water exchange at intervals of 3 ∼ 4 h during 24 h. The solution was then used for analysis or freeze-dried.

For measurement of drug-loading content, freeze-dried samples of NFX-loaded PLGA nanoparticles were suspended into methanol and vigorously stirred for 3 h and sonicating 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) at 322 nm.

2.4. Scanning electron microscope (SEM) observation

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.

2.5. Transmission electron microscope (TEM) observation

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.

2.6. 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.

2.7. X-ray diffractometer measurement

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

2.8. In vitro release studies

The release experiment in vitro was carried out as previous report (Nah et al., 1998; Peracchia et al., 1997). Seven micrograms of NFX-loaded PLGA nanoparticles were suspended in 2 ml phosphate buffered saline (PBS, 0.1M, pH 7.4) by sonication for 30 s at 15 W using bar type sonicator (Ultrasonic homogenizer, UH-50, SMT Co. Ltd., Japan) and then put into a dialysis tubes (MWCO: 12 000). The dialysis tube was placed into a 100 ml bottle with 50 mL PBS and the media was stirred at 100 rpm at 37°C. Whole-media change method on the drug release study was used for prevention of saturation of drug. At specific time intervals, whole medium (50 ml) was taken and replaced with same volume of fresh PBS (50 ml). The concentration of the released NFX into PBS was determined by UV spectrophotometer (Shimadzu UV-1201) at 322 nm. Drug contents and loading efficiency was calculated as following equation:

$$\text{Drug contents} = \frac{\text{(weight of remained drug in the nanoparticles)}}{\text{(weight of remained drug in the nanoparticles} + \text{polymer weight)}} \times 100$$

$$\text{Loading efficiency} = \frac{\text{(amount of remained drug in the nanoparticles)}}{\text{(initial feeding amount of drug)}} \times 100$$

3. Results and discussion

The PLGA nanoparticles was 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 on the NFX loading contents, their physicochemical properties, and in vitro release kinetics. To evaluate the effect of initial solvent, various water-miscible solvents were used to preparation of PLGA nanoparticles.

DMF, DMSO, DMAc, and acetone as a initial solvent for preparation was used to make nanoparticles of PLGA by dialysis without addition of any other surfactant. Dialysis procedure was performed for 24 h to remove the solvent completely and make the surfactant-free nanoparticles. Milky like suspension was observed after the end of dialysis procedure of PLGA against distilled water. To know whether or not to be formed nanoparticle, particle size was analyzed using photon correlation spectroscopy and their morphology was observed by TEM and SEM. TEM photographs of PLGA 85:15 (a), 75:25 (b), and 50:50 (c) nanoparticles prepared from DMF (Fig. 1), and PLGA 50:50 nanoparticles prepared DMSO (Fig. 1(d)), and DMAc (Fig. 1(e)) as a initial solvent was shown in Fig. 1. SEM photograph of PLGA 50:50 nanoparticles prepared from DMF (a) and acetone (b) as a initial solvent was shown in Fig. 2. As shown in Fig. 1 and 2, PLGA nanoparticles can be ease to make by dialysis procedure without surfactant or emulsifying system. The particle size of PLGA nanoparticles from DMF, DMSO, and DMAc were about 200–400 nm. In these results, the differences of particle size against polymer composition and used initial solvent system in the case of DMF, DMSO, and DMAc were not significantly changed. In the SEM observations, the PLGA nanoparticles prepared from DMF (Fig. 2(a)) was 200–400 nm and has nice spherical shapes which is almost same results to TEM observation. But, when acetone (Fig. 2(b)) as an initial solvent was used to make particles, PLGA particle size was

increased and their size were 500–1000 nm (Fig. 2) and also have nice spherical shapes. The difference of particle size of PLGA nanoparticles prepared from acetone may be caused by various physicochemical properties between polymer and solvents such as solubility difference of polymer to the solvent, viscosity of solvents and polymer, miscibility difference of solvent and water. Also, we are going to investigate the physicochemical properties between polymer, solvent, and water and will report in the near future. These results have shown that nanoparticles (in the case of DMF, DMSO, and DMAc) and sub-micron particles (Acetone) of PLGA were successfully prepared by dialysis procedure without surfactant and their morphology have nice spherical shapes. All of the solvents used to dissolve the polymers

were resulted in milky-like suspension without any other precipitate.

Table 1 shows the particle size and NFX loading contents of the PLGA nanoparticles against used initial solvent and copolymer composition. Size of PLGA nanoparticles from acetone was relatively larger than that of other solvents. Also, PLGA nanoparticles from DMAc, DMSO, and DMF as an used initial solvent were showed relatively more transparent in sight than that of acetone. These results indicated that selected initial solvent used to dissolve the copolymer significantly affects on the size of nanoparticles as same as morphological results in Fig. 1 and Fig. 2. Drug contents of surfactant-free nanoparticles was relatively lower than that of our expectations. It might be because of norfloxacin as a hydropho-
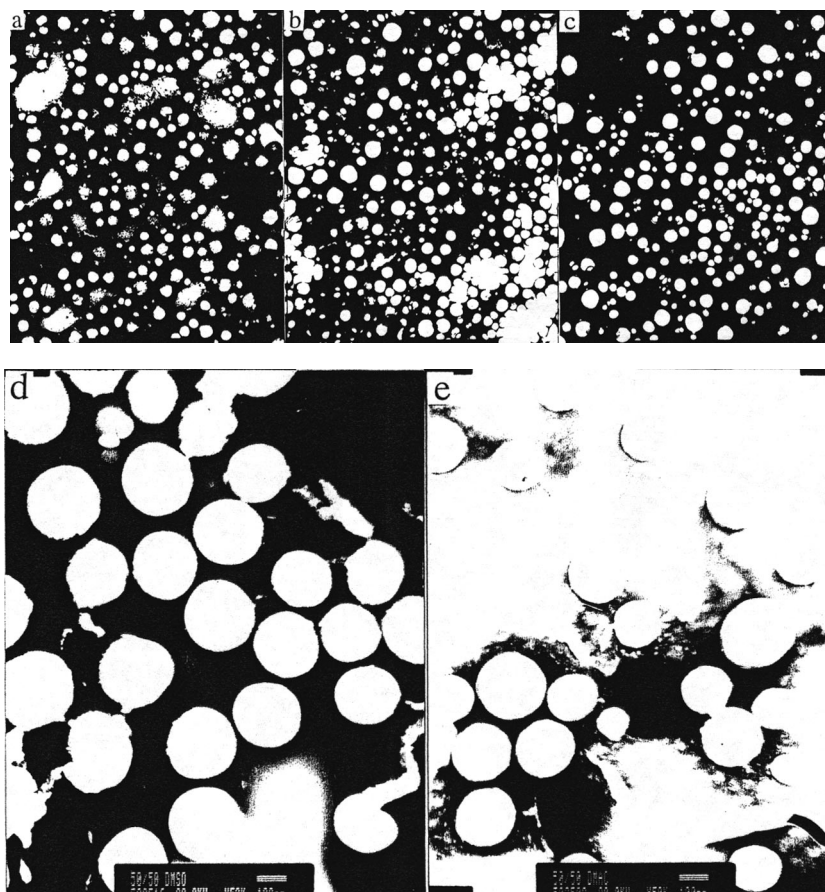

Fig. 1. Transmission electron microphotograph of PLGA 85:15 (a), 75:25; (b), and 50:50 (c) nanoparticles prepared from DMF (bar = 200 nm) and PLGA 50:50 nanoparticles prepared from DMSO (d), and DMAc (e) (bar = 100 nm) as an used initial solvent.
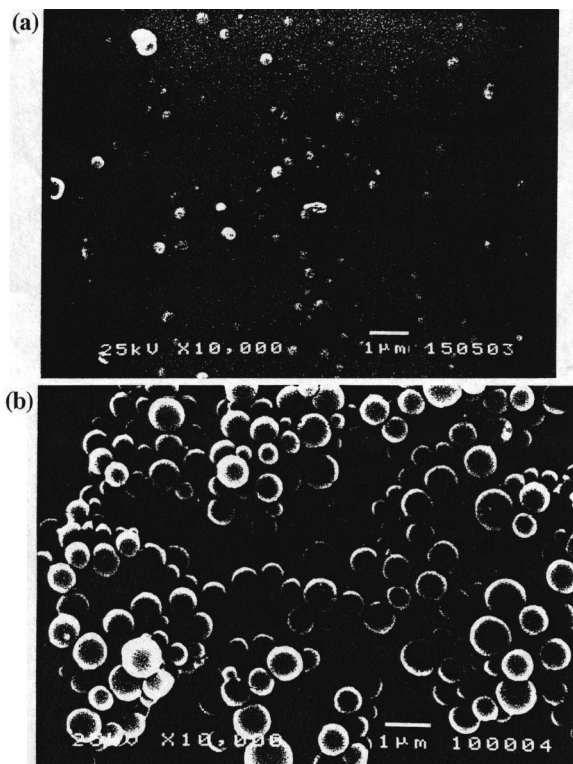
Fig. 2. Scanning electron microphotograph (SEM) of PLGA 50:50 nanoparticles prepared from DMF (a) and acetone (b) as an initial solvent.

bic model drug is not so much hydrophobic (NFX solubility is 0.28 mg/ml in water at 25°C) to maintain high drug contents and dialysis for 24 h to remove the solvents is also too long period. In our case, to make small sized nanoparticles, we have used 10 ml amount of solvents to dissolve the polymer and drug, and then dialysis procedure was performed for longer period to remove the solvents and unloaded drug, completely, than that period of other report (La et al., 1996; Kim et al., 1999). That may be the reason for lower drug contents of our case than that of other report (La et al., 1996; Kim et al., 1999). Drug contents were also quite different according to used solvents. The orders of drug contents were DMAc > DMF > DMSO = acetone. It was expected that acetone would be induced high drug contents due to their large particle size but the actual results were significantly different to our expectations,

i.e. lowest drug contents in spite of their largest particle size. Also, drug contents and loading efficiency were dependent on the lactide/glycolide ratio, i.e. the higher the lactide ratio, the higher the drug contents and loading efficiency. These phenomena could be expected that differences of solubility and miscibility between polymer, drug, and solvent, or water and solvent would be affect the size and drug loading contents of nanoparticles. From all of these results of Figs. 1 and 2, and Table 1, formation of nanoparticles of PLGA by dialysis method without surfactant was confirmed. Particle size and drug content of PLGA nanoparticles can be controlled by used initial solvents. DMF as a selective solvent was used to dissolve the PLGA in the following experiments.

As a hydrophobic model drug, NFX 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, entrapment process of hydrophobic drug into the particles are thought to be 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 to be formed by hydrophobic self-aggregation through hydrophobic interaction between each polymeric chains. Additionally, they would be stabilized solely by the presence of charged groups at the surface of the PLGA nanoparticles (Govender et al., 1999). Also, drug loading process into the nanoparticles might to be hydrophobic interaction.

X-ray powder diffractometer was employed to confirm the characteristics of NFX-loaded PLGA nanoparticles. Fig. 3 shows the X-ray diffraction scans of NFX-loaded PLGA nanoparticles and the corresponding physical blend. It can be observed that the X-ray diffraction peaks characteristics of NFX (drug crystal peaks), which were visible in the pattern obtained for the physical blend, disappeared in those corresponding to NFX-entrapped nanoparticles. It is indicated that NFX is existed to molecular dispersion in the

polymeric nanoparticles and also showed that surfactant-free nanoparticles of PLGA can entrapped drug without free drug in the surface of nanoparticles and in the nanoparticle formulations. This result showed the potential of surfactant-free PLGA nanoparticles as a drug carrier as same as other PLGA nanoparticle system prepared by O/W or W/O/W emulsion evaporation methods by use of PVA or other surfactant system such as Span series, Tween series, and Poloxamer series.

Fig. 4 shows the NFX release from PLGA nanoparticles against initial solvent used (a) and copolymer composition (b). Generally, the drug release rate from the nanoparticles is relatively faster than that of other microsphere systems because of the high surface area and their small size. As shown in Fig. 4, almost of them were showed burst initial release of NFX for 3–4 h and then pseudo zero-order release kinetics until 12 or 24 h. In the case of DMAc which has highest drug loading contents, the drug release kinetics showed that burst release during initial 4 h was showed and then zero-order release over 24 h. In the case of DMSO which is lowest drug loading contents, however, the initial burst release was observed during about initial 1–2 h and then drug was constantly release until 12 h. The drug release rate kinetics was order of DMSO > DMF > DMAc except for case of acetone. These results indicated that the higher the drug contents, 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). (Gref et al., 1994; Peracchia et al., 1997) reported that hydrophobic drug was occurred crystallization inside the nanoparticles and a phase separation occurs at higher drug contents of drug in the nanoparticles, leading to the crystallization of part of the drug (Gref et al., 1994; Peracchia et al., 1997). As shown in Fig. 4(b), it was found that the higher the lactide ratio, the slower the drug release. These results may be due to the large particle size, higher drug contents, and larger hydrophobic interaction between hydrophobic lactide segments and drug than that of glycolide segments. Also, the larger hydrophobic lactide of polymer could be lead to the stronger hydrophobic interaction. Hydrophobic drug loaded into nanoparticles is slower release at higher drug contents which is differenced with hydrophilic water-soluble drugs. Our results were observed that NFX release was slower rate kinetics from the nanoparticles with higher drug contents. At low drug content, NFX is relatively present as a molecular dispersion inside the nanoparticles (Gref et al., 1994). These results could be supported from the results of XRD patterns in Fig. 3. At lower drug content, XRD patterns were almost similar with the patterns of empty nanoparticles and not showed in peaks of drug crystal. But, at higher drug contents, peaks of crystallized drug in the nanoparticles were slightly increased at higher

Table 1
Effect of various initial solvent on the particle size distribution of PLGA nanoparticles

| PLGA	Used initial solvent	Drug contents (wt. −%)	Loading efficiency (wt. −%)	Particle size (nm)		
				Intensity average	Volume average	Number average
50:50	DMSO	7.73	8.4	236.5 ± 148.1	248 ± 232.9	195 ± 112.9
50:50	DMF	9.74	10.8	185.8 ± 35.6 (80.8%)	185.8 ± 68.8 (52.4%)	183.0 ± 70.6 (95.0%)
				516.5 ± 97.7 (19.2%)	480.5 ± 195.6 (47.6%)	471.2 ± 183.0 (5.0%)
50:50	DMAc	12.97	14.9	304.2 ± 129.1	322.8 ± 181.6	306.4 ± 178.2
50:50	Acetone	7.73	8.4	634.0 ± 263.5	679.7 ± 333.8	663.7 ± 342.7
75:25	DMF	10.75	12.0	241.0 ± 116.5	246.5 ± 121.7	242.7 ± 97.7
85:15	DMF	12.17	13.9	287.7 ± 109.5	307.4 ± 157.8	287.5 ± 147.6
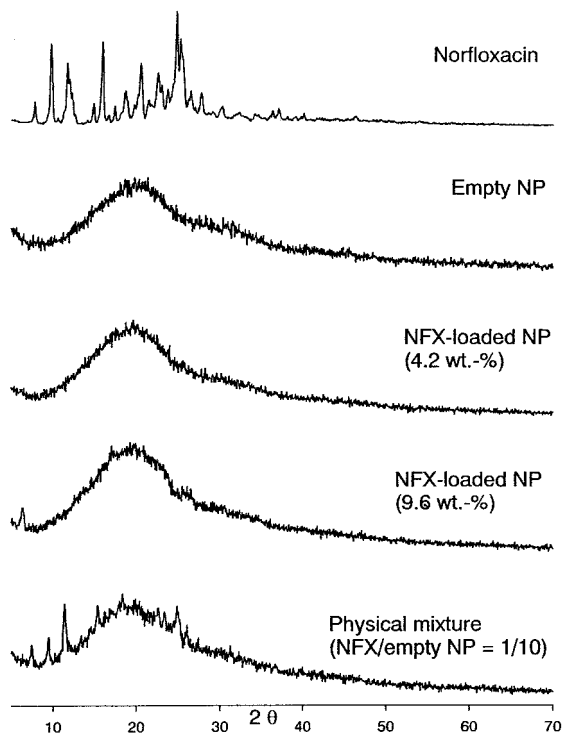
Fig. 3. X-Ray diffractometer patterns of PLGA 50:50 nanoparticles. Norfloxacin (a), PLGA nanoparticles (empty) (b), NFX loaded PLGA nanoparticles (drug loading contents: 4.2 wt.%) (c), NFX loaded PLGA nanoparticles (drug loading contents: 9.6 wt.%) (d), and physical mixture of NFX/empty PLGA nanoparticles (weight ratio of NFX/polymer = 1/10) (e), repetively.

drug loading. The crystallized drug should be dissolved more slowly and diffused into the outer aqueous phase than that of molecular dispersion. Despite of the lowest drug contents, PLGA nanoparticles prepared from acetone which has highest particle size as shown in Fig. 2(b) and in Table 1 was resulted in slower release rate kinetics than that of DMSO and DMF. For same drug contents, there were reported that the drug release rate from large nanoparticles was slower than that of small sized nanoparticles (Alleman et al., 1993; Leroux et al., 1996). Interestingly, however, in spite of that PLGA nanoparticles prepared from acetone has lower drug contents than that of DMF, the release kinetics of acetone was slower than that of DMF, indicating that particle size differences are significant factor for drug release

rate kinetics in the nanoparticulate drug delivery system. But the release kinetics of PLGA nanoparticles prepared from acetone was not slower than that of DMAc. It was thought that because of the size differences of PLGA nanoparticles between acetone and DMAc was relatively smaller than differences between acetone and DMSO or DMF. Another reason of these results is large differences in the drug contents between acetone and DMAc, i.e. the drug contents of PLGA nanoparticles prepared from DMAc was significantly higher than that of acetone. From these results, it can be suggested that drug release kinetics from PLGA nanoparticles prepared as a surfactant-free system was controlled by size difference when differences of drug contents is not significantly different. When drug content was significantly different, however, drug release kinetics of PLGA nanoparticles were relatively
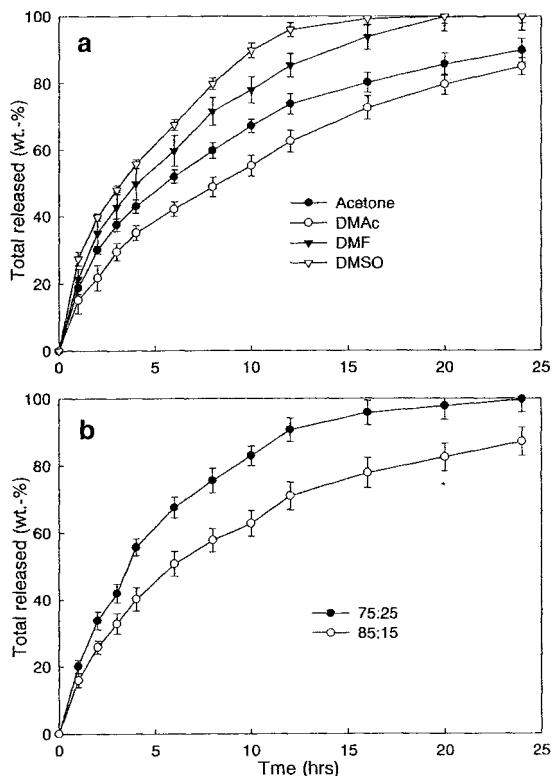

Fig. 4. Norfloxacin release from PLGA nanoparticles against initial solvent used (a) and copolymer composition (b). The drug contents and particle size were described in Table 1.

dominated by drug contents rather than particle size factor. Resultantly, because of differences in the diffusivity of drug molecules to the outer aqueous phase, drug-release kinetics is affected not only by drug contents but also the size of nanoparticles. Of course, further investigations on the drug release characteristics are needed for small sized nanoparticles as a function of drug contents, the size of nanoparticles and polymer used. Resultantly, control of drug release kinetics can be achieved by optimizing the chemical nature of the used polymers, drug contents, the used initial solvents, and the size of nanoparticles.

4. Conclusion

The surfactant-free PLGA nanoparticles were prepared by dialysis method and their physico-chemical properties were investigated against used an initial solvent. The size of PLGA nanoparticles prepared from DMAc, DMF, and DMSO as a initial used solvent was smaller than that of acetone. Selected initial solvent used to dissolve the copolymer significantly affects the size of nanoparticles and drug contents. PLGA nanoparticles have spherical shapes from the observations of SEM and TEM. The analysis of X-ray powder diffraction patterns showed potential of surfactant-free nanoparticles of PLGA as a drug carrier system. Release kinetics of NFX used as a model drug was governed by not only drug loading contents but also particle size parameter. The higher the drug contents and the larger the particle size resulted in slower the drug release.

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