Macromolecular Research, Vol. 16, No. 8, pp 717-724 (2008)

All-*trans* Retinoic Acid Release from Surfactant-free Nanoparticles of Poly(DL-lactide-*co*-glycolide)

Young-Il Jeong, Don-Gon Kim, Mi-Kyeong Jang, and Jae-Woon Nah*

Department of Polymer Science and Engineering, Sunchon National University, Jeonnam 540-742, Korea

Yong-Bae Kim

Institute of Photonics & Surface treatment, Q-SYS Co. Ltd., Gwangju 500-460, Korea

Received May 20, 2008; Revised July 9, 2008; Accepted July 11, 2008

Abstract: In this study, we prepared all-trans retinoic acid (ATRA)-encapsulated, surfactant-free, PLGA nanoparticles. The nanoparticles were formed by nanoprecipitation process, after which the solvent was removed by solvent evaporation or dialysis method. When a nanoparticle was prepared by the nanoprecipitation - solvent evaporation method, the nanoparticles were bigger than the nanoparticles of the nanoprecipitation - dialysis method, despite the higher although loading efficiency. Nanoparticles from the nanoprecipitation - dialysis method were smaller than 200 nm in diameter, while the loading efficiency was not significantly changed. Especially, nanoparticles prepared from DMAc, 1,4-dioxane, and DMF had a diameter of less than 100 nm. In the transmission electron microscopy (TEM) observations, all of the nanoparticles showed spherical shapes. The loading efficiency of ATRA was higher than 90 % (w/w) at all formulations with exception of THF. The drug content was increased with increasing drug-feeding amount while the loading efficiency was decreased. In the drug release study, an initial burst was observed for 2~6 days according to the variations of the formulation, after which the drug was continuously released over one month. Nanoparticles from the nanoprecipitation - dialysis method showed faster drug release than those from the nanoprecipitation - solvent evaporation method. The decreased drug release kinetics was observed at lower drug contents. In the tumor cell cytotoxicity test, ATRA-encapsulated, surfactant-free, PLGA nanoparticles exhibited similar cytotoxicity with that of ATRA itself.

Keywords: retinoic acid, surfactant-free nanoparticles, poly(DL-lactide-*co*-glycolide), dialysis, nanoprecipitation.

Introduction

Nanoparticles have been extensively investigated in biomedical field and, especially, in drug delivery systems for drug targeting.[1,2] Due to their reduced particle size, nanoparticles are susceptible for intravenous (i.v.) injection. The advantages of nanoparicles are targeted drug delivery to the desired site of action, prolonged blood circulation of drug, and reduced side effects of anticancer drug, etc.[3,4] 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.[5] 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, etc.[1,3,4,6] Among them, the particle size of nanoparticles is considered primary important factor.[2] Several micrometers of administered particles may become accumulated into the lung capillaries and submicron particles are rapidly cleared by the reticuloendothelial system (RES).[7,8] Furthermore, sub-200 nm particles has advantages to avoid clearance by RES.[7,9]

Despite of these considerable advantages, difficulties and complexities of the preparation method for nanoparticles is one of the major drawbacks for practical application.[1] Practically, too many steps to make nanoparticles are required to reduce their particle size and remove byproducts. Since a lot of amount of surfactant are required to make small size of nanoparticles, surfactant may remained on the surface of nanoparticles and complete removal of surfactant from the

*Corresponding Author. E-mail: jwnah@sunchon.ac.kr

nanoparticle surface is difficult.[10,11] Almost of surfactants used to preparation of nanoparticles are non-biodegradable, non-digestible, and not always biocompatible. Furthermore, these surfactants can be affected to human body such as an allergy-like reaction.

All-trans retinoic acid (ATRA) is known to have various activities in cell fate determination, cell division, cell behavior during development, and cell differentiation.[12] It was reported that ATRA is effective in the treatment of epithelial and hematological malignancies such as breast cancer[13] human malignant gliomas,[14] head and neck cancer,[15] ovarian adenocarcinoma,[16] and acute promyelocytic leukemia (APL).[17] Although ATRA has been proved to be effective against several malignancies in human clinical trials, the cancer relapsed after a brief remission in many patients who were treated with ATRA. Furthermore, ATRA has poor aqueous solubility (0.21 mM in physiological solution, pH 7.3)[18] and short half-lives in blood.[19-21]

For this study, we have prepared ATRA-encapsulated surfactant-free nanoparticles of PLGA by dialysis method and studied possibility of nanoparticles for delivery of ATRA. We have previously reported surfactant-free nanoparticles using PLGA as drug carriers. The advantages of surfactant-free nanoparticles are unnecessary of surfactant removal process, simple preparation method, ease of solvent removal, and minimization of particle size. The drug loading contents and loading efficiency of ATRA, changes of particle size, and physicochemical properties of surfactant-free nanoparticles of PLGA were investigated *in vitro*.

Experimental

Materials. PLGA 50/50 (Resomer RG503H) was purchased from Boehringer Ingelheim, Pharma Co., Germany. All-trans retinoic acid (ATRA) was purchased from Sigma Chem. Co. (St. Louis, USA). The dialysis membrane with a molecular weight cut-off (MWCO) of 12,000 g/mol was purchased from Spectra/Pro™ Membranes. Dimethylformamide (DMF) and acetone as a HPLC grade were purchased from Aldrich Chem. Co. Ltd, USA. All other chemicals and reagents were used as extra reagent grade at all of the experiments.

Preparation of ATRA-Encapsulated PLGA Nanoparticles. For nanoprecipitation-solvent evaporation method, 40 mg of PLGA was dissolved in 7 mL of acetone and then 5 mg of ATRA was added to this solution. To make nanoparticle, this solution was poured into 20 mL of deionized water and stirred for 10 min. The solvent was evaporated using by rotary-evaporator (Rotary Vacuum Evaporator, Type N-N, EYELA, Tokyo Rikakikai, Co. Ltd., Japan) under reduced pressure for 1 h. After that, nanoparticle solution was harvested and the volume of nanoparticle solution was adjusted to 40 mL. This solution was used to analysis or lyophilized.

For nanoprecipitation-dialysis procedure, 40 mg of PLGA was dissolved in 5 mL of solvent and then 2~10 mg of ATRA was added to this solution. To make nanoparticle, this solution was poured into 20 mL of deionized water and stirred for 10 min. The solvent was removed by dialysis (molecular weight cut-off (MWCO) of dialysis tube: 12,000 g/mol) against deionized water for 24 h. During dialysis procedure, deionized water was exchanged for every 2 h. After that, dialyzed nanoparticle solution was harvested and the volume of nanoparticle solution was adjusted to 40 mL. This solution was used to analysis or lyophilized.

Empty PLGA nanoparticles were prepared without addition of ATRA and then same procedure was employed to make nanoparticles.

To determine drug contents and loading efficiency, the volume of the dialyzed nanoparticle solution was adjusted to 40 mL with deionized water (i.e. 40 mg of PLGA/40 mL of water). The 100 μL of adjusted solution was diluted with acetone. ATRA concentration was measured with UV spectrophotometer at 365 nm (UV-1201, Shimadzu Co., Ltd., Japan). For blank test, empty PLGA nanoparticle solution was adjusted to 40 mL (i.e. 40 mg of PLGA/40 mL of water) and 0.1 mL of this solution was diluted with acetone. All experiments were triplicated. The equation of drug contents and loading efficiency were as follows:

$$\text{Drug contents} = \frac{\text{Amount of ATRA in the nanoparticles}}{\text{Weight of nanoparticles}} \times 100$$

$$\text{Loading efficiency} = \frac{\text{Residual amount of ATRA in the nanoparticles}}{\text{Feeding amount of ATRA}} \times 100$$

Analysis of PLGA Nanoparticles. The particle size and the zeta potential of the polymeric micelles were measured with an ELS-8000 electrophoretic LS spectrophotometer (NICOMP 380 ZLS zeta potential/particle sizer, Otsuka electronics Inc., Japan) equipped with a He-Ne laser beam at a wavelength of 632.8 nm at 25 °C (scattering angle of 90°). A sample solution prepared was used for the particle size measurement (concentration: 1 mg/mL).

Morphological observation was performed using electron microscope. For observation with FESEM (S-4800, Hitachi Co. Ltd. Japan), nanoparticle solution was put on a cover slide and freeze-dried for 1 day. This cover glass was placed on a double-sided tape attached onto graphite surface. The sample was coated with gold/palladium using an Ion Sputter. Coating was provided at 20 mA for 4 min. Observation was performed at 25 kV.

For observation with TEM, a drop of nanoparticle suspension containing 0.05% (w/v) of phosphotungstic acid was placed on a TEM copper grid coated with carbon film and dried at room temperature. Observation was performed at

80 kV with JEM-2000 FX II (Jeol, Japan). For observation with SEM, nanoparticle solution were dropped onto a cover glass and dried at room temperature. 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.

X-ray powder diffractograms were obtained with a X' pert PRO/MPD(Netherlands) using Ni filtered CuKα radiation (40 kV, 20 mA) to determine the crystallinity of drug. All experiments were performed at room temperature. The conditions of powder XRD measurement was as follows:

Data Type = Binary; Goniometer = 1; Attachment = 1; Scan mode = Continuous.

Mode 2 (R/T) = Reflection; Scan axis = 2-Theta/Theta.

Start angle = 10.000; Stop angle = 80.000; Scan speed = 5.000; Sampling interval = 0.050; Theta angle = 5.000; 2Theta angle = 10.000; Fixed time = 0.01; Full scale = 1000; Counting unit = CPS; Target = Cu.

Wave length Kal = 1.540510; Wave length Ka2 = 1.544330; Wave length Ka = 1.541780; Wave length Kb = 1.392170; 40.0 kV; 20.0 mA.

To measure powder XRD of ATRA-incorporated nanoparticles, ATRA as a solid powder, empty PLGA nanoparticles, and ATRA-encapsulated nanoparticles were used. For physical mixture of ATRA and PLGA nanoparticles, 90 mg of empty nanoparticles was mixed with 10 mg of ATRA.

Drug Release Study. Drug release tests are performed as follows: ATRA-encapsulated nanoparticles were prepared as described above and final aqueous solution adjusted to 40 mL (i.e. 40 mg of polymer/40 mL of water). 5 mL of adjusted solution was introduced into dialysis tube (M.W. cut-off: 12,000 g/mol) and dialysis tube introduced into a bottle with 195 mL of phosphate buffered saline (PBS, 0.1 M, pH 7.4). Release test was performed at 37 °C with stirring rate of 100 rpm. The whole release medium was exchanged with fresh medium at predetermined time intervals. Released amount of drug was measured at 365 nm with UV spectrophotometer (UV-1200, Shimadzu Co., Ltd., Japan).

Cell Cytotoxicity Test *In Vitro*. To test the anti-proliferation effect of ATRA and ATRA-encapsulated nanoparticles, HT 1080 cells was used. HT 1080 cells were maintained at 5 % CO_2 incubator at 37 °C. The effect of free ATRA and ATRA-encapsulated nanoparticles on the tumor cell proliferation was determined using an MTT cell proliferation assay. ATRA was dissolved in 100% DMSO and diluted 100 times using DMEM (supplemented with 10% serum). ATRA-encapsulated nanoparticles was distributed in the DMEM (supplemented with 10% serum) and diluted to adjust the equivalent concentration of the free ATRA. The tumor cell lines were seeded at a density of 5×10^3 per well in 96-well plates using 100 μL of DMEM supplemented with 10 % serum in a CO_2 incubator (5% CO_2 at 37 °C) for 12 h. After that, 100 mL of DMEM (supplemented with 10% serum) containing free ATRA or ATRA-encapsulated nanoparticles was added. After 1 or 2 days of incubation, MTT was added to the 96 wells and incubated for 4 h in a CO_2 incubator (5% CO_2 at 37 °C). After that, the supernatant was discarded and 100 μL of DMSO was added to the 96 wells. The absorbance was measured at 560 nm using a microtiter plate reader (Thermomax microplate reader, Molecular Devices).

Results and Discussion

Characterization of ATRA-Encapsulated Surfactant Free Nanoparticles of PLGA. Nanoprecipitation method is a common method for nanoparticle preparation and widely used to make nanoparticles.[1,22] Nanoprecipitation method was used to prepare surfactant-free nanoparticles of PLGA and remove of solvent was varied such as solvent evaporation under reduced pressure and dialysis against deionized water. For solvent evaporation method, solvent is limited to volatile solvent such as acetone, THF, methylene chloride, chloroform, and etc.. For this study, acetone was used to prepare nanoparticles by solvent evaporation method. ATRA-encapsulated PLGA nanoparticles were prepared nanoprecipitation-solvent evaporation method as shown in Figure

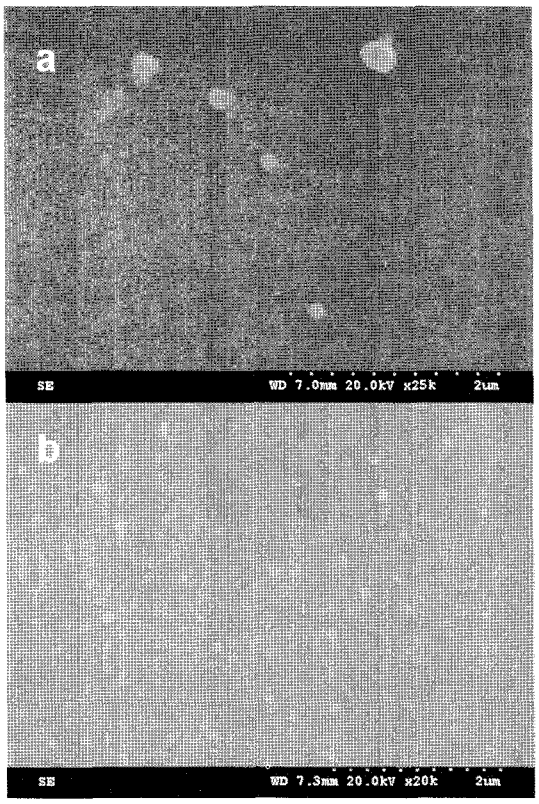

Figure 1. SEM image of ATRA-encapsulated surfactant-free nanoparticles of PLGA prepared by nanoprecipitation-solvent evaporation method (a) and nanoprecipitation-dialysis method (b).

1(a). As shown in Figure 1(a), nanoparticles showed spherical shape and their size was ranged about 100~500 nm in diameter. Furthermore, drug contents and loading efficiency was 11.0 and 98.9% (w/w) as shown in Table I. Especially, loading efficiency was very high together with reduced particle size. Choi *et al.*[23] reported that ATRA was encapsulated into microspheres of poly(L-lactide) (PLA)/PLA-poly(ethylene glycol) (PEG) block copolymer and obtained pseudo-zero order release of ATRA for 5 weeks. Even if they have also obtained high loading efficiency higher than 90% w/w, size of their microspheres was higher than 5 micrometer.

Since the body distribution of nanoparticles after intravenous (i.v.) injection is significantly influenced by their particle size,[2,24] reduced particle size for i.v. administration must be primarily considered. Especially, sub-200 nm particles should be[9,24] primarily considered for drug targeting issues and site-specific drug delivery using nanoparticles.

From this point of view, we tried to make PLGA nanoparticles by nanoprecipitation-dialysis method. As shown in Figure 1(b), size of nanoparticles were certainly decreased when nanoprecipitation-dialysis method were used using acetone as a solvent. Furthermore, nanoparticle morphology showed spherical shapes as well as nanoparticles by nanoprecipitation-solvent evaporation method. Their particle size were around 100~150 nm.

To study the effect of used solvent variation on the particle size, various solvent such as acetone, THF, DMSO, DMAc, 1,4-dioxane, and DMF were used to make nanoparticles by nanoprecipitation - dialysis method. The particle size against the solvent used was shown in Figure 2 and their loading efficiency was abbreviated in Table I. As shown in Table I, loading efficiency was higher than 90% at all of the solvent used with the exception of THF. When THF were used, nanoparticles were significantly aggregated and, after filtration of aggregated particles, loading efficiency was lower than other solvent used as shown in Table I. Loading efficiency was higher at acetone, DMSO, and DMF than THF, DMAc, and 1,4-dioxane. As shown in Figure 2(a), particle size was decreased when nonvolatile solvent such as DMSO, DMAc, 1,4-dioxane, and DMF was used. Volatile solvent such as acetone and THF resulted in larger particle size even if nanoprecipitation – dialysis method was used. Generally, drawback of dialysis method is loss of encapsulated drug in the nanoparticles outside the dialysis tube during dialyzed procedure. Therefore, dialysis method for nanoparticle preparation is difficult to apply to water-soluble drug. Furthermore, even if water-insoluble drug was used, encapsulated drug in the nanoparticles also

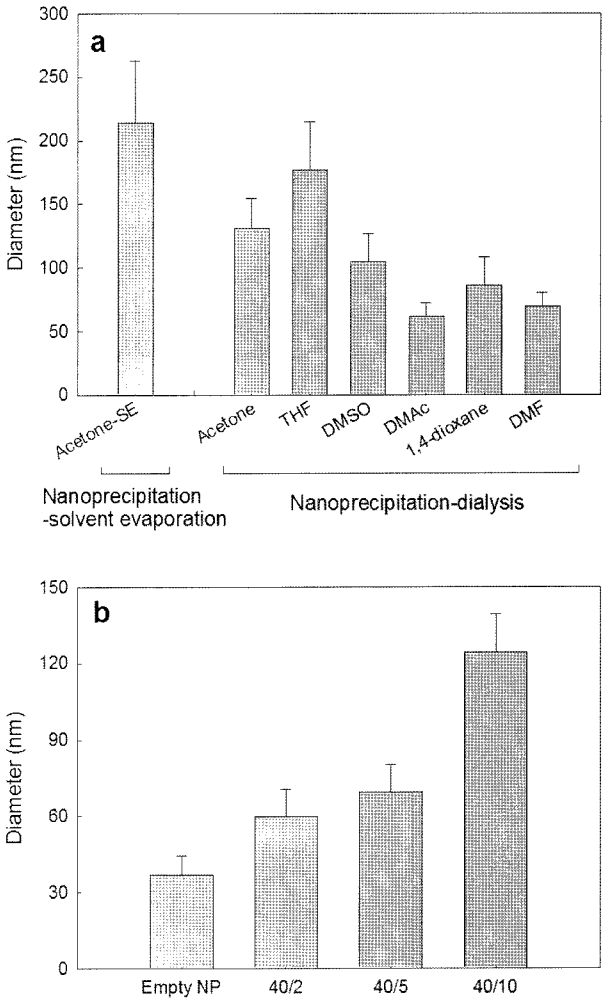

Figure 2. Particle size of ATRA-encapsulated surfactant-free nanoparticles of PLGA. Particle size changes according to the used solvent (a); the effect of drug contents (used solvent : DMF) (b).

Table I. Characterization of ATRA-Encapsulated PLGA Nanoparticles Against Used Organic Solvent

Preparation Method	Used Solvent	Weight Ratio of PLGA/ ATRA (mg/mg)	Drug Contents (%, w/w)		Loading Efficiency (%, w/w)
			Theoretical	Experimental	
Nanoprecipitation-solvent evaporation	Acetone	40/5	11.11	11.0	98.9
Nanoprecipitation-dialysis	Acetone	40/5	11.11	10.0	97.9
	THF	40/5	11.11	4.5	37.9
	DMSO	40/5	11.11	10.7	95.7
	DMAc	40/5	11.11	10.3	91.9
	1,4-dioxane	40/5	11.11	10.2	90.9
	DMF	40/2	4.76	4.7	98.6
		40/5	11.11	10.8	96.9
		40/10	20.0	18.9	93.2

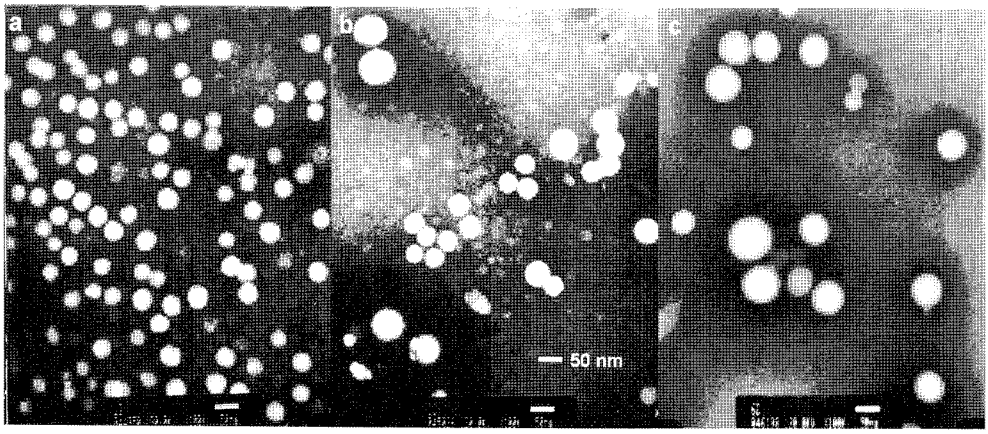

Figure 3. TEM images of surfactant-free nanoparticles of PLGA. ATRA-encapsulated nanoparticles prepared by nanoprecipitation-dialysis method using DMF. Empty nanoparticles (a); PLGA/ATRA weight ratio = 40/2 (b); PLGA/ATRA weight ratio = 40/5 (c) in Table I.

can be liberated outside of dialysis tube during dialysis procedure. This drawback of dialysis method induced lower loading efficiency compared to solvent evaporation method. As shown in Table I, ATRA was relatively high loading efficiency even though nanoprecipitation - dialysis method was used to make nanoparticles. These results might be due to the very low aqueous solubility of ATRA (0.1 μM at pH 7.3)[18] The feeding amount of ATRA was changed to know the effect of feeding ratio of polymer/drug on the particle size and loading efficiency as shown in Table I and Figure 2(b). In this case, DMF was used to make nanoparticles because of DMF was induced higher loading efficiency with small particle size. When drug feeding amount was increased, drug contents and particle size was increased but loading efficiency was decreased. Especially, particle size was higher than 100 nm at 40/10 of polymer/drug ratio. The morphology of nanoparticles prepared by DMF was observed with TEM as shown in Figure 3. As shown in Figure 3, empty nanoparticles were smaller than 50 nm while ATRA-encapsulated nanoparticles have both of > 50 nm particles and < 50 nm particles. At all of the formulations, nanoparticles showed spherical shapes in their morphology.

Figure 4 showed XRD patterns of ATRA-encapsulated surfactant-free nanoparticles of PLGA. As shown in Figure 4, ATRA has its intrinsic crystalline peaks (Figure 4(a)) and empty nanoparticles (Figure 4(b)) showed broad peak characteristics. ATRA-encapsulated nanoparticles prepared by nanoprecipitation - dialysis procedure (polymer/drug ratio = 40/5, DMF in Table I) showed almost similar peak characteristics with empty nanoparticles (Figure 4(c)), indicating that drug was successively encapsulated into nanoparticles. However, when feeding amount of drug was increased, drug crystalline peaks were slightly observed (Figure 4(d)). These results indicated that some of free drug can be remained in the nanoparticle surfaces when drug feeding ratio was increased. Furthermore, drug crystalline peaks was certainly observed when nanoparticles was prepared by nanoprecipitation - solvent

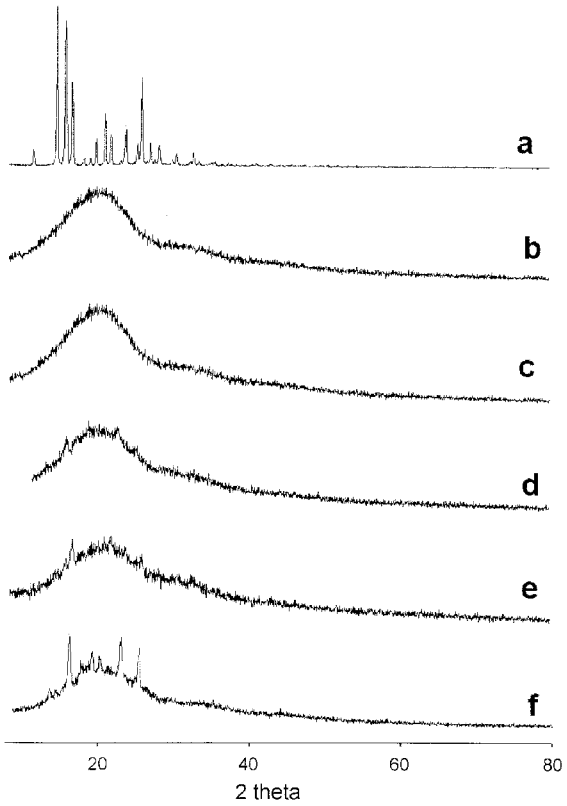

Figure 4. X-ray powder diffraction pattern of ATRA-encapsulated PLGA nanoparticles. ATRA (a); empty nanoparticles (b); ATRA-encapsularated PLGA nanoparticles prepared by nanoprecipitation - dialysis : 40/5 - DMF as a solvent (c); 40/10 - DMF as a solvent (d); nanoparticles prepared by nanoprecipitation - solvent evaporation (detailed condition in Table I) (e); empty nanoparticles:ATRA physical mixture (weight ratio = 90:10) (f).

evaporation method (Figure 4(e)), indicating that certain amount of free drug can be remained at nanoprecipitation - solvent evaporation procedure even though loading efficiency was highest at all formulation. Physical mixture of

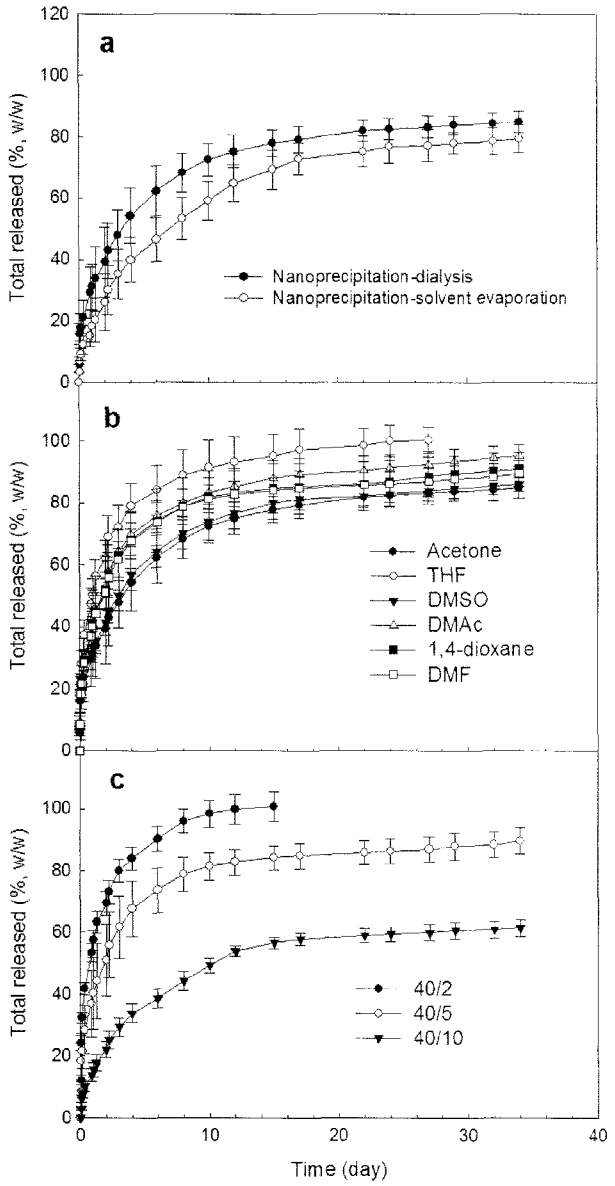

Figure 5. The drug release from surfactant-free nanoparticles of PLGA. (a) The effect of preparation method. Drug release was compared prepared by nanoprecipitation-solvent evaporation method and nanoprecipitation-dialysis method from acetone as a used solvent; (b) the effect of used solvent. Nanoparticles prepared by nanoprecipitation-dialysis method; (c) the effect of drug contents. Nanoparticles were prepared from DMF as a used solvent.

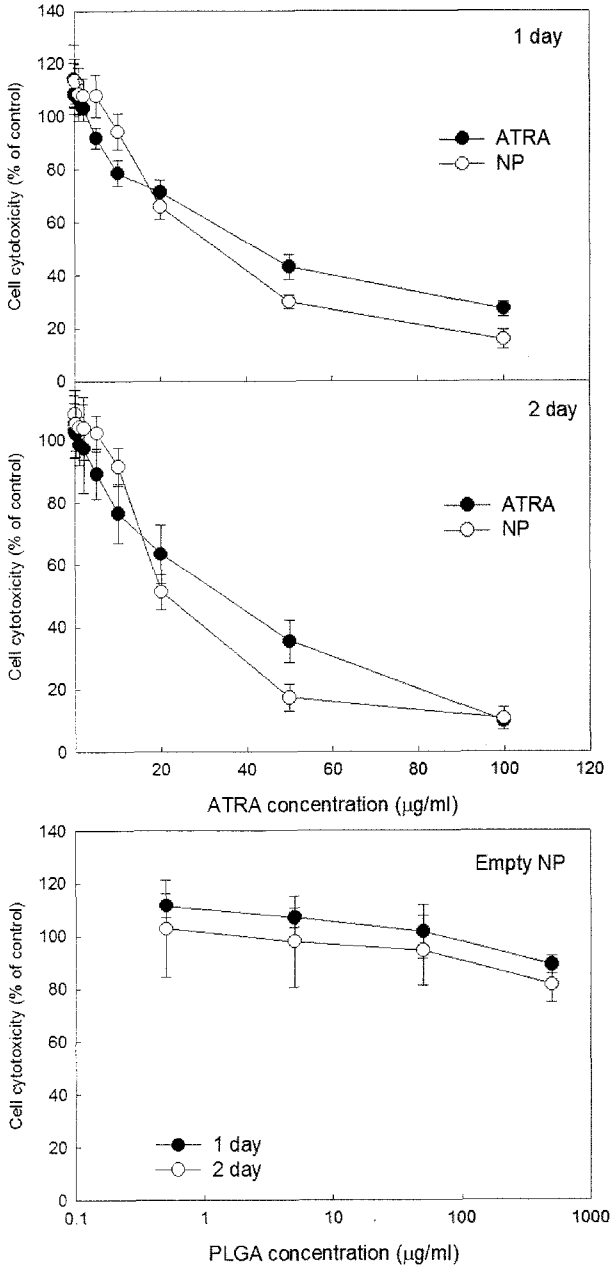

Figure 6. Cell cytotoxicity of ATRA-encapsulated PLGA nanoparticles against HT 1080 tumor cells. HT 1080 (5×10^3 cells/well) were treated with ATRA and ATRA-encapsulated PLGA nanoparticles for 1 day (top) and 2 day (middle). Empty nanoparticles were also employed for comparison of cell cytotoxicity (bottom). Empty nanoparticles were treated to HT 1080 tumor cells (5×10^3 cells/well).

empty nanoparticles and drug showed both of intrinsic peaks of empty nanoparticles and drug.

Drug Release Study. Figure 5 shows the ATRA release kinetics from nanoparticles. The effect of preparation method was shown in Figure 5(a). As shown in Figure 5(a), initial burst was observed for 4~6 days and then drug was controlled release for over one month. When nanoprecipitation - dialysis method was used to make nanoparticles, drug was released faster than nanoprecipitation - solvent evaporation method. These results might be due to the smaller particle size and lower drug contents of nanoparticles from nanoprecipitation - dialysis method. There were reported that the drug release rate from large nanoparticles was slower than that of small sized nanoparticles.[1,4] Furthermore, hydrophobic drug can be crystallized in the nanoparticles at higher

drug contents.[3] Crystallized drug in the nanoparticles can be released slowly rather than molecular dispersion of drug in the nanoparticles. In this case, nanoparticles prepared by nanoprecipitation - solvent evaporation has both of larger particle size and higher drug contents. These factors might affect to the slow release of drug from nanoparticles. Figure 5(b) showed the effect of solvent used on the drug release rate from nanoparticles. As shown in Figure 5(b), drug release from nanoparticles prepared by THF was faster than other solvents, indicating that drug contents of THF was lower than other solvents and this factor might affected to the increased release of drug from nanoparticles, even though size of nanoparticles was larger than other solvents. The initial burst effect was observed for 4 days at nanoparticles prepared by THF, DMAc, 1,4-dioxane and DMF, and for 6 days against DMSO and acetone. Drug release rate was not significantly changed at nanoparticles prepared by THF, DMAc, 1,4-dioxane and DMF. These nanoparticles showed slightly faster drug release pattern than that of acetone and DMSO one. The effects of drug contents on the drug release kinetics are showed in Figure 5(c). As shown in Figure 5(c), the higher the drug contents the slower the release rate of drug. At lower drug contents (40/2), initial burst was observed about for 2 day and drug was continuously released for 10 days. At higher drug contents (40/10), initial burst was observed for 4 days and drug was released over one month. At low drug content, ATRA is relatively present as a molecular dispersion inside the nanoparticles.[3] These results could be supported from the XRD results in Figure 4. At lower drug content, XRD patterns were almost similar with the empty nanoparticles. As described above, hydrophobic drug can be crystallized in the nanoparticles at higher drug contents. The crystallized drug should be dissolved more slowly and diffused into the outer aqueous phase than that of molecular dispersion.

Cytotoxicity of ATRA-Encapsulated Nanoparticles Against Tumor Cell. Figure 6 showed cytotoxicity of ATRA-encapsulated surfactant-free nanoparticles of PLGA against HT1080 cells. As shown in Figure 6(c), empty nanoparticles did not significant cytotoxicity against HT1080 although cytotoxicity was increased according to the increase of concentration of nanoparticles. ATRA showed higher cytotoxicity at lower ATRA concentration than 20 mg/mL while nanoparticles were more cytotoxic at higher than 20 mg/mL. At 2 day treatment, survivability of tumor cells was lower than 20% at 100 mg/mL ATRA concentration both of ATRA and nanoparticles. These results indicated that intrinsic cytotoxicity of ATRA against tumor cells was not affected by encapsulation process into nanoparticles.

Conclusions

The surfactant-free PLGA nanoparticles were prepared by nanoprecipitation - solvent evaporation method and nanoprecipitation - dialysis method. When nanoparticles was prepared by nanoprecipitation - solvent evaporation method, size of nanoparticles was higher than nanoparticles of nanoprecipitation - dialysis method although loading efficiency was higher. Nanoparticles from nanoprecipitation - dialysis method was relatively smaller particle size while loading efficiency was not significantly changed. At all formulation, loading efficiency of ATRA was higher than 90% (w/w) with exception of THF. At variations of the solvent used, DMAc, 1,4-dioxane, and DMF was relatively smaller particle size than other solvent used. The drug contents were increased according to the increased feeding amount of drug while loading efficiency was decreased. At XRD results, drug was completely encapsulated into nanoparticles at lower drug contents while some free drug was observed at higher drug contents. At drug release study, initial burst was observed for 2~6 days according to the variations of formulation and then drug was continuously released over one month. Nanoparticles from nanoprecipitation - dialysis method showed faster drug release than nanoprecipitation - solvent evaporation method. The decreased drug release kinetics was observed at lower drug contents. At tumor cell cytotoxicity test, ATRA-encapsulated surfactant-free nanoparticles of PLGA have similar cytotoxicity with ATRA itself, indicating intrinsic potential of ATRA against tumor cell was not affected by encapsulation process.

Acknowledgment. This study was supported by Academic Industrial Joint Center for Technological Development(2008) in Sunchon National University. We thank Korea Basic Science Institute (KBSI, Sunchon Branch) for the use of XRD and FESEM.

References

(1) E. Alleman, R. Gurny, and E. Doelker, *Eur. J. Pharm. Biopharm.*, **39**, 173 (1993).
(2) S. S. Davis, L. Illum, S. M. Moghimi, M. C. Davies, C. J. H. Porter, I. S. Muir, A. Brindley, N. M. Christy, M. E. Norman, P. Williams, and S. E. Dunn, *J. Control. Release*, **24**, 157 (1993).
(3) R. Gref, Y. Minamitake, M. T. Peracchia, V. Trubetskoy, V. Torchilin, and R. Langer, *Science*, **263**, 1600 (1994).
(4) J. C. Leroux, E. Allemann, F. D. Jaeghere, E. Doelker, and R. Gurny, *J. Control. Release*, **39**, 339 (1996).
(5) J. W. Bae, D. H. Go, K. D. Park, and S. J. Lee, *Macromol. Res.*, **14**, 461 (2006).
(6) D. G. Kim, C. Y. Choi, Y. I. Jeong, M. K. Jang, S. K. Kang, M. S. Bang, and J. W. Nah, *Macromol. Res.*, **14**, 66 (2006).
(7) L. Illum, S. S. Davis, C. G. Wilson, M. Frier, J. G. Hardy, and N. W. Thomas, *Int. J. Pharm.*, **12**, 135 (1982).
(8) T. Yoshioka, M. Hashida, S. Muranishi, and H. Sezaki, *Int. J. Pharm.*, **81**, 131 (1981).
(9) P. D. Scholes, A. G. A. Coombes, L. Illum, S. S. Davis, M. Vert, and M. C. Davies, *J. Control. Release*, **25**, 145 (1993).

(10) S. C. Lee, J. T. Oh, M. H. Jang, and S. I. Chung, *J. Control. Release,* **59**, 123 (1999).
(11) B. Sjostrom, Br. Kronberg, and J. Carlfors, *J. Pharm. Sci.*, **82**, 579 (1993).
(12) G. Morris-Kay, Ed., *Retinoids in Normal Development and Teratogenesis*, Oxford, Oxford Science Publications, 1992.
(13) G. P. Kalmekerian, R. K. Jasti, P. Celano, B. D. Nelkin, and M. Marby, *Cell Growth Differ.,* **5**, 55 (1994).
(14) G. L. Defer, H. Adle-Biassette, F. Ricolfi, L. Martin, F. J. Authier, C. Chomienne, L. Degos, and J. D. Degos, *J. Neurooncol.*, **34**, 169 (1997).
(15) F. Giannini, R. Maestro, T. Vukosa, T. Vljevic, F. Pomponi, and M. Boiocchi, *Int. J. Cancer,* **70**, 194 (1997).
(16) G. Krupitza, W. Hulla, H. Harant, E. Dittrich, E. Kallay, and H. Huber, *Int. J. Cancer,* **61**, 649 (1995).
(17) E. J. Huang, Y. C. Ye, S. R. Chen, J. R. Chai, J. X. Lu, L. Zhoa, L. J. Gu, and Z. Y. Wang, *Blood,* **72**, 567 (1988).
(18) E. Z. Szuts and F. I. Harosi, *Arch. Biochem. Biophys.*, **287**, 297 (1991).
(19) C. C. Achkar, J. M. Bentel, J. F. Boylan, H. I. Scher, L. J. Gudas, and W. H. Miller, *Drug. Metab. Disp.,* **22**, 451 (1994).
(20) H. S. Lin, C. S. Chean, Y. Y. Ng, S. Y. Chan, and P. C. Ho, *J. Clin. Pharm. Ther.*, **25**, 265 (2000).
(21) E. Estey, P. F. Thall, K. Mehta, M. Rosenblum, Jr. T. Brewer, V. Simmons, F. Cabanillas, R. Kurzrock, and G. Lopez-Berestein, *Blood*, **87**, 3650 (1996).
(22) T. Govender, S. Stolnik, M. C. Garnett, L. Illum, and S. S. Davis, *J. Control. Release,* **57**, 171 (1999).
(23) Y. Choi, S. Y. Kim, S. H. Kim, K. S. Lee, C. Kim, and Y. Byun, *Int. J. Pharm.,* **215**, 67 (2001).
(24) B. Seijo, E. Fattal, L. Roblot-Treupel, and P. Couvreur, *Int. J. Pharm.*, **62**, 1 (1990).