Nanoencapsulation of Drug-loaded Lipid by Temperature-induced Phase Transition

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Original scientific paper
Received: June 12, 2012
Accepted: July 27, 2012

Pluronic nanoparticles (NPs) were prepared by means of a temperature-induced phase transition in the mixture composed of Pluronic F-68 and liquid Tween 80/soybean oil containing model drugs such as orlistat, caffeine, and ibuprofen sodium salt. Liquid soybean oil/Tween 80 was used as a solubilizer for model drugs, and Pluronic F-68 was the polymer that stabilizes liquid soybean oil/Tween 80 containing model drugs.

Field-emission scanning electron microscopy and particle size analyzer were used to observe the morphology and size distribution of the prepared NPs. X-ray diffractometer was used to understand relationship between the crystalline state of the model drug and its solubility in the aqueous media. To observe the feasibility of Pluronic NPs as a drug delivery system, the release pattern of model drugs was observed.

Key words:
Nanoencapsulation, pluronic, temperature – induced phase transition, solubility

Introduction

Practically insoluble drugs in the aqueous media still remain as a main issue in the pharmaceutical area and various methods such as solid dispersion, granulation and hot melt extrusion have been developed to improve the solubility[1–3]. Recently, temperature-induced phase transition in the melt state polymer/drug mixtures has been utilized to improve the solubility of hydrophobic anticancer drugs[4–5].

Pluronic-based nanoparticles (NPs) were formed by temperature-induced phase transition in the melt state of Pluronic/poly ethylene oxide/paclitaxel (PTX) mixture.[4] Liquid PEG (molecular weight: 400) was used as a solubilizer of PTX and the polymer that encapsulates the PTX was composed of Pluronics F-68. At the phase transition temperature (120°C for 90 minutes), the polymer mixture was changed to the liquid phase, and stirring the liquid polymer mixture formed emulsions composed of PEG containing PTX and liquidized Pluronic F-68. By cooling to 0°C, PEG containing PTX was encapsulated by Pluronic F-68 on the nanometer scale to form Pluronic NPs with core/shell structure, which was revealed by cry-transmittance electron microscopy. If the same process was applied to docetaxel, which was hydrophobic anticancer drugs belonging to the second generation of the taxoid family, the significant oxidation of DTX was observed during the formation of DTX-loaded Pluronic NPs[5]. Therefore, a strategy to produce NPs at a relatively low transition temperature is necessary. Later, an improvement was made using liquid Tween 80/soybean oil as a solubilizer for DTX and the new type of DTX formulation was prepared in the aqueous media. The melting transition was performed at a relatively low transition temperature (60°C) to avoid the degradation of the drug or the polymer using liquid Tween 80/soybean oil[6].

In this study, Pluronic NPs with Tween80/soybean oil core containing drug were prepared for the sustained drug delivery system by means of temperature-induced phase transition (see Figure 1). Model drugs used were orlistat, caffeine and ibuprofen sodium salt, which were selected with the variation of solubility. Orlistat is known as tetrahydrolipstatin and is a drug designed to treat obesity. The first function of orlistat is blocking the absorption of fats from the human diet, thereby reducing caloric intake[7]. Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) and belongs to the group of 2-arylpropionic acids. Ibuprofen sodium salt is used for the treatment of inflammatory conditions such as rheumatoid arthritis. Furthermore, it is used for treating mild and moderate pain, dysmenorrhea, headache and fever[8]. Caffeine, which belongs to an alkaloid of the methylxanthine family, is a naturally occurring substance found in the leaves, seeds or fruits of over 63 plants species worldwide. Caffeine is an ingredient in various over-the-counter drugs (OTCs) including headache, cold, allergy, pain relief and alerting drugs[9–10].

The stability of Pluronic NPs was monitored by observing the change of size distribution of NPs

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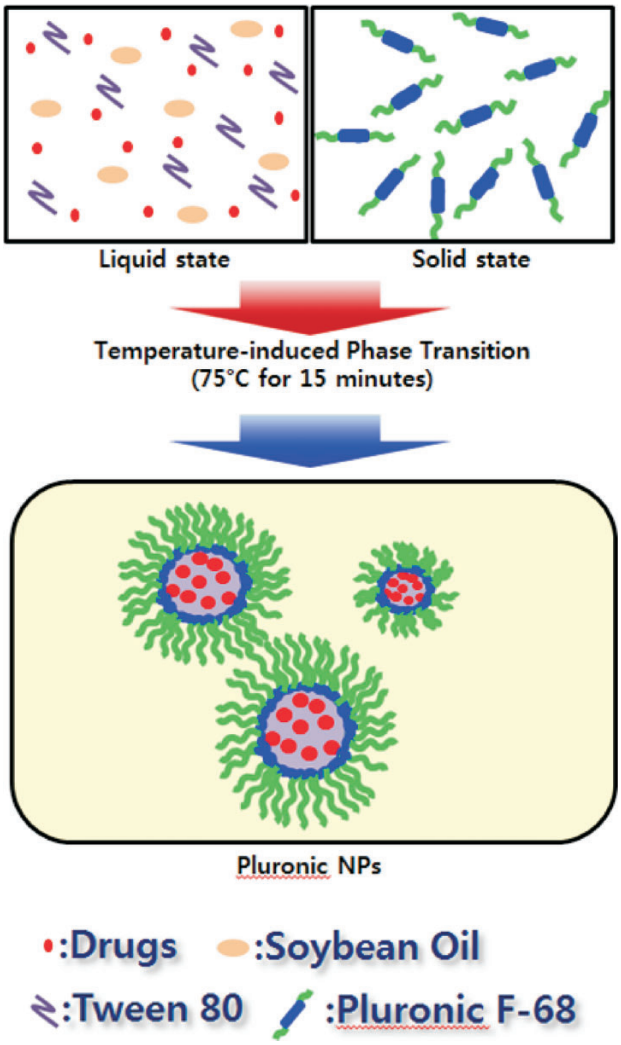


Fig. 1 – *Formation of Pluronic nanoparticles by temperature-induced phase transition*

in the aqueous media. X-ray diffractometer was used to examine the crystalline structure of model drug, which was closely related to the dissolution behavior and release pattern of model drug. The release pattern of model drugs was measured and explained in terms of the size of NPs and the solubility of model drugs.

Materials and methods

Materials

Pluronic F-68, poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer (M_w=8350; $(EO)_{79}(PO)_{28}(EO)_{79}$) was obtained from BASF Corp., Korea, and was used as received. Orlistat, soybean oil, Tween 80, caffeine and ibuprofen (sodium salt) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA).

Preparation of Pluronic NPs

Pluronic NPs were prepared by temperature-induced phase transition. 300 mg of liquid Tween 80/soybean oil and 20 mg of drug (loading amount: 3.2 wt%) were mixed to form a drug-loaded lipid phase, which was subsequently mixed with weighed amount of Pluronic F-68. As the temperature was increased to 75°C, the mixture melted into a liquid phase. After the equilibrium was maintained at 75°C for 15 minutes with stirring, the liquid mixture was then cooled to 0°C for 10 minutes to induce a phase transition.

Particle size and morphology of Pluronic NPs

The average diameter and size distribution of NPs (1 mg/ml of NPs dispersed in phosphate buffered saline (PBS, pH 7.4)) were measured via dynamic light scattering (Zeta Sizer Nano Series) at 632.8 nm and 25°C. Field emission-scanning electron microscopy (FE-SEM) measurements were performed to observe the morphology of Pluronic NPs. To prepare a sample for FE-SEM, 0.1 wt% of aqueous solution of the Pluronic NPs was prepared in distilled-deionized water. Each solution was dropped on a carbon mount and then dried at 25°C in a vacuum oven for 24 h. FE-SEM measurement was performed on a JSM-6700F operating at 5 kV.

Differential scanning calorimetry (DSC) measurements.

Thermal analysis was conducted using a TA Q2000 (TA Instruments, New Castle, DE, USA). Nitrogen was used as purge gas at a flow rate of 50 mL/min for the DSC cell. The temperature scale and cell constants were calibrated by measuring the onset temperature and the enthalpic response of an Indium standard. The aluminum pans were hermetically sealed and the samples were heated from 0°C to 200°C with the heating rate of 10°C/min. Data was acquired and analyzed using Universal Analysis 2000 software (TA Instruments, New Castle, DE, USA).

X-Ray diffraction analysis

The crystalline characteristic of Pluronic NPs was examined using a Bruker X–ray diffractometer D8 Advance with Davinci (Bruker AXS GmbH, Germany) equipped with Ni-filtered Cu K_α radiation (λ = 1.54056 Å) and a LynxEye detector. The samples were placed in a quartz sample holder and scanned from 4 to 40° (2θ value) at a scanning rate of 0.2 sec/step.

In vitro drug release characteristics from the Pluronic NPs

To measure the release pattern of model drugs from the NPs, 10 mg of NPs was dispersed in 10 mL of PBS and put into a dialysis bag (MWCO: 500,000, Spectrum®, Rancho Dominquez, CA), which was immersed in 20 mL of PBS. The experimental setup was placed in a shaking water bath maintained at 37°C and shaken horizontally at 100 rpm. At predetermined time intervals, 2 mL of aliquots of release medium (PBS) were withdrawn, and the total release medium was replaced with 20 ml of fresh PBS to maintain the sink conditions. The quantification of released drug was determined by reverse-phase high performance liquid chromatography (RP-HPLC) using a Capcell-pack C_{18} column. The mobile phase for orlistat was acetonitrile/water (95/5, v/v) with 0.1 % phosphoric acid mobile phase over 15 minutes at a flow rate of 1.5 ml/ minute. The eluent was monitored by UV absorption at 227 nm [11]. In the case of caffeine, the mobile phase was methanol/water (40/60, v/v) mobile phase over 15 minutes at a flow rate of 0.5 ml/minute and the eluent was monitored by UV absorption at 272 nm. In the case of ibuprofen, the mobile phase was methanol/acetonitrile/water (85/15/5, v/v) mobile phase over 15 minutes at a flow rate of 1.0 ml/minute and the eluent was monitored by UV absorption at 220 nm [12].

Statistical analysis

Data are expressed as the mean±S.E.M. of at least three experiments. All data processing was performed using the ORIGIN®8.0 statistical software program (OriginLab Corp., Northampton, MA, USA).

Results and discussion

Temperature-induced phase transition was demonstrated in the melt state of Pluronic F-68/Tween 80/soybean oil with model drugs to perform the nanoencapsultion of drug-loaded lipid. Liquid Tween 80/soybean oil was selected for solubilizing drugs to form the drug-loaded lipid and Pluronic F-68 was selected to encapsulate the drug-loaded lipid on the nanometer scale. To decide the proper temperature for transition, the transition behavior of Pluronic F-68 and Pluronic F-68/Tween 80/soybean oil containing drug were performed, which showed the melting transition around 53°C and 50°C, respectively (see Figure 2).

According to Figure 2, the mixture composed of Pluronic F-68/Tween 80/soybean oil containing drug showed the melting transition at 50°C and the formation of liquidized mixture was observed. However, it took 2 hours to induce the formation of the homogeneous solution mixture and increase of melting temperature was required to set up for fast and convenient process. Therefore, the melting transition was performed at 75°C. The formation of drug-loaded Pluronic NPs was induced when the temperature was decreased to 0°C. The morphology and size distribution of Pluronic NPs were examined by FE-SEM and particle size analyzer as shown in Figure 3. Pluronic NPs had the spherical form with approximately 220 nm in diameter. The size distribution of drug-loaded Pluronic NPs does not change significantly after 24-hour release experiment. This indicates that the stability of Pluronic NPs was maintained in the aqueous media during the release experiment.

The crystalline structure of drug causes limited solubility in the aqueous media [13]. With the nanoencapsulation of drug-loaded lipid, drug-loaded

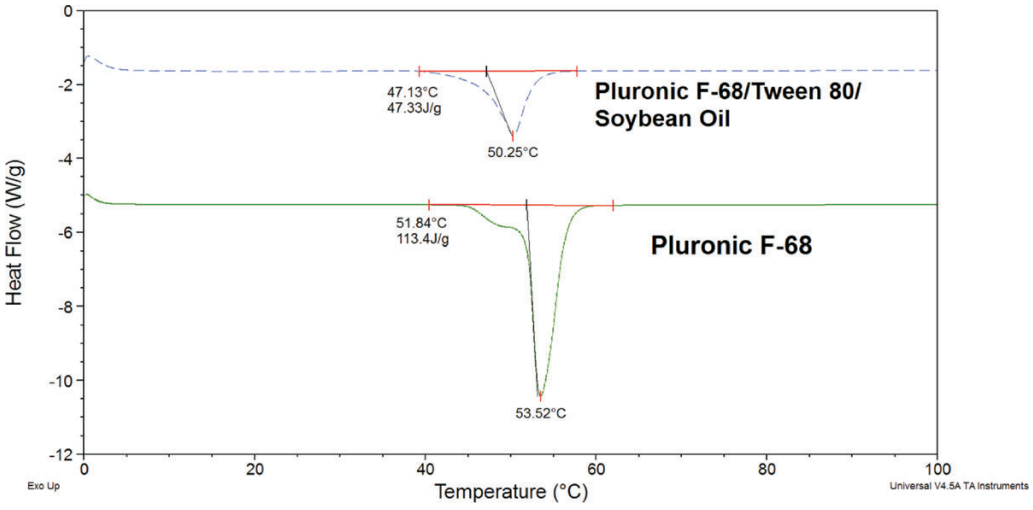
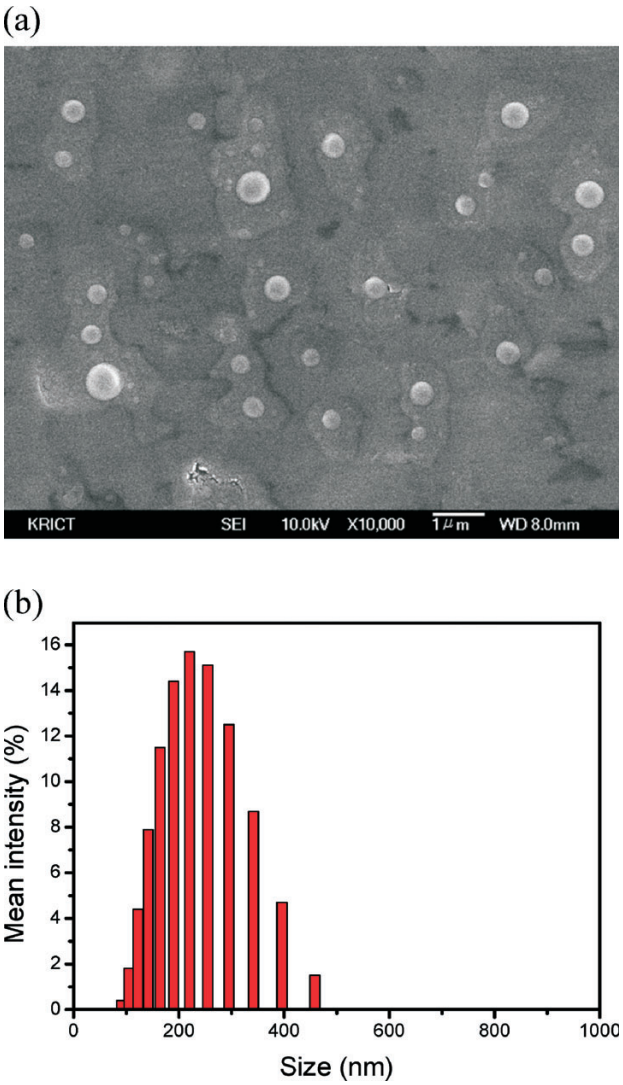


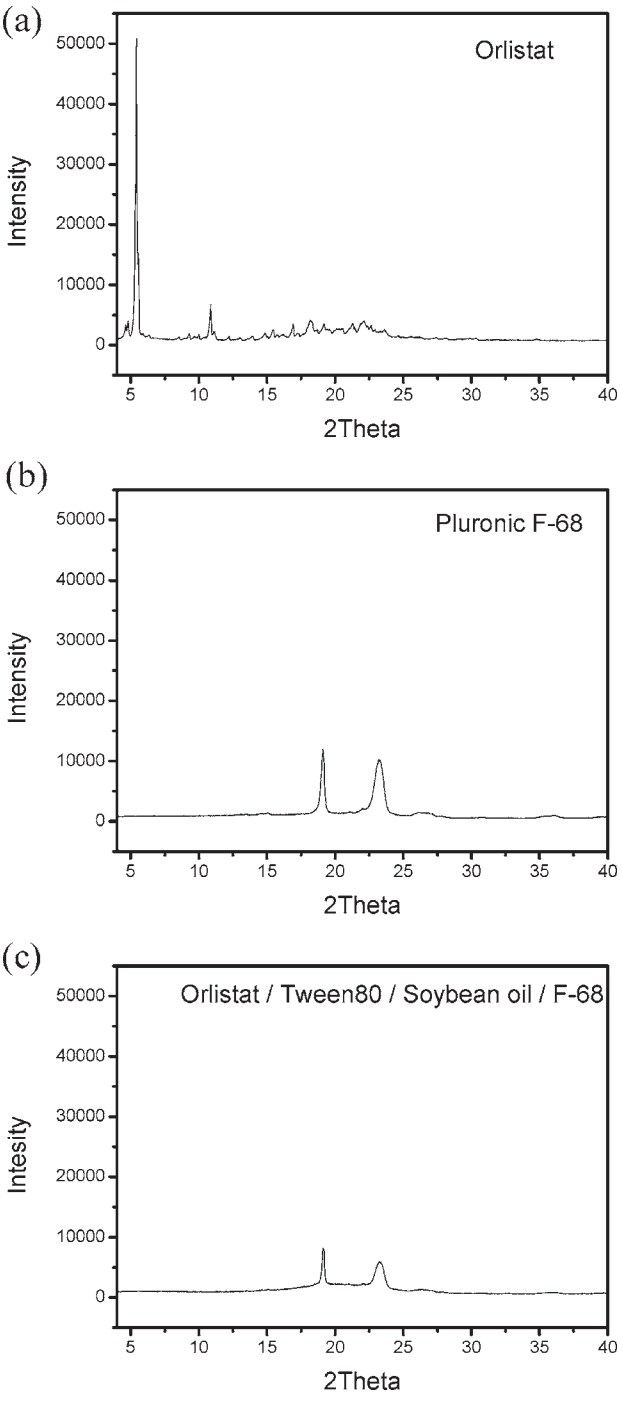
Fig. 2 – *DSC thermograms*



F i g . 3 – *(a) FE-SEM images of Pluronic NPs and (b) the size distribution of Pluronic NPs after 24-hour release experiment*

lipid was coated with hydrophilic Pluronic F-68 and this led to the improved stability of drug in the aqueous media. As shown in Figure 3, the nanoencapsulated drug-loaded lipids (Pluronic NPs) maintained their nano-sized diameter indicating the improved stability of drug (or drug-loaded lipid) in the aqueous media. To understand the relationship between the crystallinity and the dissolution behavior more in detail, X-ray diffraction analysis was used by observing the change of crystallinity during the nanoencapsulation. In this study, orlistat, caffeine and ibuprofen sodium salt were used as model drugs. Because caffeine and ibuprofen sodium salt are soluble in water (The solubilities of caffeine and ibuprofen sodium salt are 22 mg/ml and 100 mg/ml, respectively [14–15]), X-ray diffraction analysis was not required. Therefore, X-ray diffraction analysis was performed with orlistat.

Orlistat and Pluronic F-68 showed the characteristic crystalline structures as shown in Figures 4 (a) and 4(b). With liquid Tween 80/soybean oil as a solubilizer for orlistat, the crystalline structure from orlistat was completely disappeared, indicating that orlistat mixed homogeneously in Tween 80/soybean as shown in Figure 4 (c). This led to suppressing the crystallization of orlistat in the Pluronic NPs with the increased stability in the aqueous media.



F i g . 4 – *X-ray analysis for (a) free orlistat, (b) Pluronic F-68, (c) melted mixture of orlistat/Tween 80/Soybean oil/Pluronic F-68*

Also, the increased solubility of orlistat in the aqueous media was expected, as the amorphous state of drug had higher penetration of water molecules due to the disordered state of intermolecular chains than the crystalline state [13, 16–17].

To optimize the composition of the Pluronic NPs, the size change of the Pluronic NPs with orlistat-loaded lipid cores were observed in PBS with a variation of Tween 80/soybean oil ratio (see Figure 5). The weight ratio of solubilizer (Tween 80/soybean oil mixture) and Pluronic was maintained 1:1 in this experiment. With the increase of Tween 80, the size of Pluronic NPs was decreased and the minimum size was obtained at the ratio of 1.0. The size change of Pluronic NPs was also observed as a function of Pluronic content in the NPs with maintaining the ratio of Tween 80/soybean oil at 1. The size change of NPs was minimal with the variation of Pluronic content in the NPs irrespective of model drugs as shown in Figure 6.

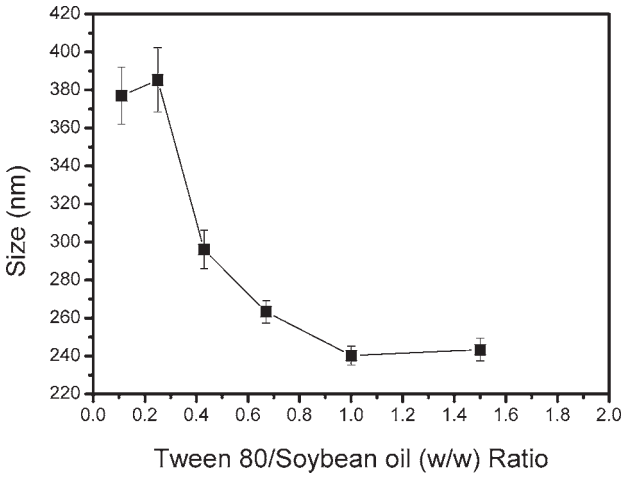


Fig. 5 – *The size changes of Pluronic NPs as a function of Tween 80/Soybean oil (w/w) ratio. (The total number of experiments is three.)*

Figure 7 shows the drug release patterns of model drugs. Drug solubility played an important role in determining the drug release pattern. In the case of model drugs with high solubility such as caffeine (the solubility: 22 mg/ml) and ibuprofen salt (the solubility: 100 mg/ml), the released amount was significantly high comparing with that of orilistat (the solubility: practically insoluble[18]). However, the sustained release pattern was demonstrated indicating that Pluronic controls the release of drugs from the Pluronic NPs. In the case of model drug with low solubility such as orlistat, the released amount was quite low comparing with caffeine and ibuprofen salt. However, the Pluronic NPs improved the stability of orlistat in the aqueous media significantly and we could expect that

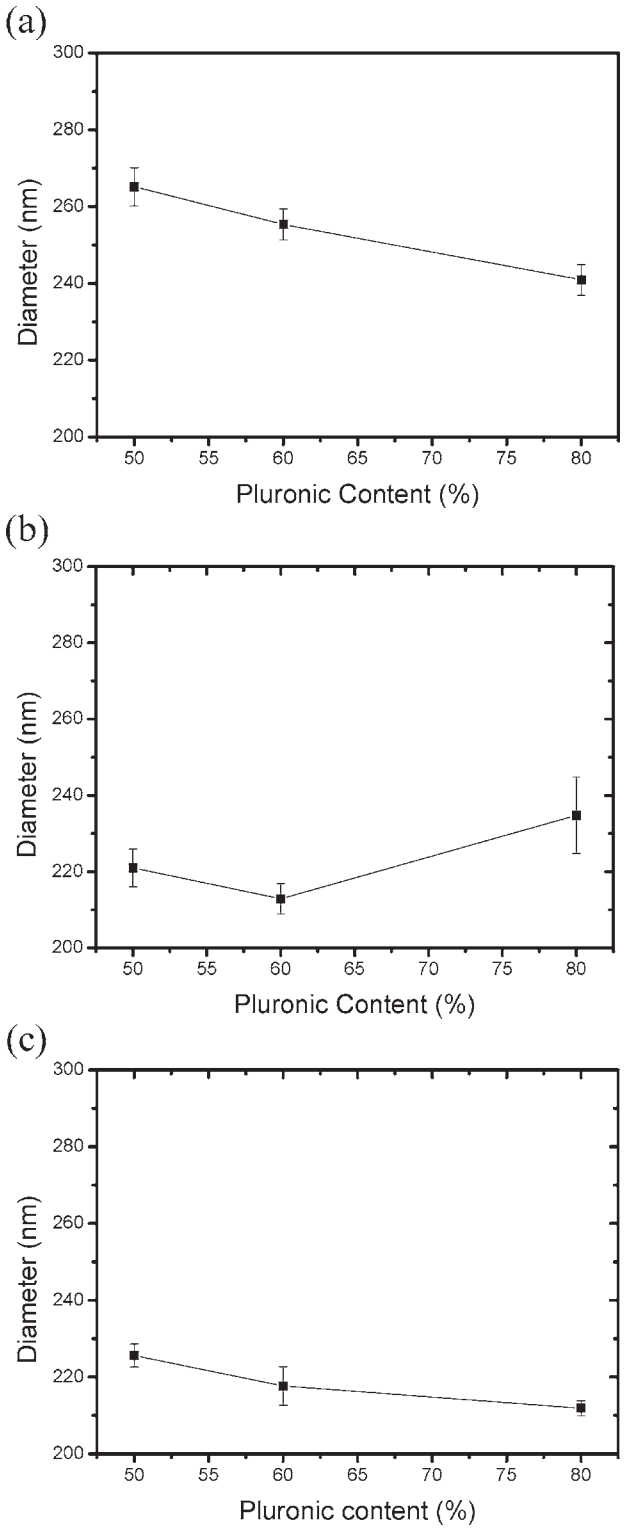
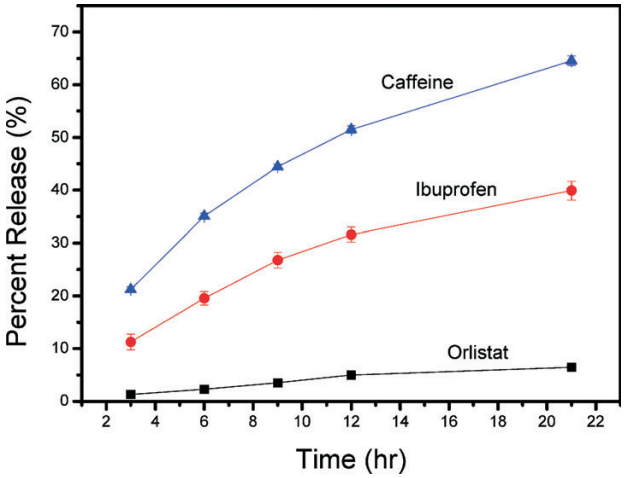


Fig. 6 – *The size changes of Pluronic NPs as a function of Pluronic content in the NPs*

Pluronic NPs could be utilized to accomplish the sustained release of drugs with low solubility for long period of time.

In the conventional preparation of NPs or microparticles based on the emulsification/solvent evaporation technique[19–20], methylene chloride,



F i g . 7 – *The release pattern of model drugs from Pluronic NPs. (The number of experiments is three.)*

ethyl acetate, or the mixture of methylene chloride and methanol (or acetone) were used to prepare for the homogeneous organic mixtures composed of polymer, drug and surfactant. Although the solvent is evaporated during the preparation, special care should be taken to remove the residual solvent completely for the clinical application. By successful demonstration of NPs formed by temperature-induced phase transition, a new preparation method for drug-loaded NPs was proposed without using organic solvents.

Conclusions

Temperature-induced phase transition was performed in the melt state of polymer/lipid/drug mixture and the nanoencapsulation was successfully demonstrated to form nanocarriers for the drug delivery system. With the presence of polymer (Pluronic F-68) around the drug-loaded lipid core, the stability of model drug with low solubility was improved in the aqueous media and the release pattern of water-soluble drug was controlled. These preliminary results indicate that temperature-induced phase transition may be very useful process for the preparation of a particle-type drug carrier without using organic solvent.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (MEST) (20110027932 and 2012028831).

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