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석사학위 논문

Development and characterization of fulvestrant loaded SLNs for enhanced oral bioavailability

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Development and characterization of fulvestrant loaded SLNs for enhanced oral bioavailability

Fulvestrant 의 경구투여 생체이용률 개선을 위한
고형지질 나노입자의 개발 및 특성 평가

2019 년 2 월 25 일

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Development and characterization of fulvestrant loaded SLNs for enhanced oral bioavailability

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국문초록

Fulvestrant 의 경구투여 생체이용률 개선을 위한 고형지질 나노입자의 개발 및 특성 평가

이 용 훈
지 도 교 수 : 지 준 필
조선대학교 대학원 약학과

Fulvestrant 는 유방암 치료에 사용하는 항암제 중 하나로, 호르몬 수용체 양성인 환자에 사용되는 약물이다. 현재 시판중인 유일한 제품은 Faslodex 라는 주사제로, 제형의 특성상 환자가 약물을 투여받기 위해 4 주에 한번씩 주기적으로 병원에 내원하여야 한다는 단점이 있다. 이러한 문제를 해결하고 환자의 편의성을 위해 경구 투여가 가능한 항암제 제형을 개발하고자 하였다. 난용성 약물인 fulvestrant 를 가용화하기 위해 고형 지질과 계면활성제를 이용하여 solid lipid nanoparticle(SLN) 제형을 제조하였다.

SLN 은 소장 내에서 지용성 물질의 흡수와 이동에 관여하는 Peyer’s patch 경로를 통해 체내에 흡수되며, 림프계를 통해 체내에 순환되므로 간 초회 통과를 회피할 수 있다는 장점이 있다. 또한 계면활성제로 사용한 TPGS 는 체내 흡수시 p-gp 단백질에 의한 efflux 를 억제하여 난용성 약물의 흡수를 도와 생체 이용률을 증가시키는 효과가 있다.

고형 지질의 chain length 에 따라 SLN 의 흡수 정도를 비교하기 위해 3 가지의 고형 지질(triluarin, trimyristin, precirol ATO 5)를 사용하여 SLN 을 제조하고, 고형 지질에 따른 SLN 의 특성을 비교하였다. 구성 성분의 최적의 조성비를 구하기 위해 고형 지질과 계면활성제의 비율을

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달리하여 SLN 을 제조하였고, 각 제형의 particle diameter 와 zeta potential, PDI 값을 토대로 최종 제형을 결정하였다.

최적의 조성으로 제조된 fulvestrant 함유 고형 지질 나노 입자는 particle diameter, zeta potential, PDI 그리고 약물의 봉입률 등을 측정함으로써 물리 화학적 성질을 확인하였다. 또한 고형 지질 나노입자로부터 약물이 방출되는 특성을 알아보기 위해 in vitro release 평가를 인공위액 및 인공장액에서 진행하였고, PAMPA assay 와 Caco-2 cell 투과 실험을 통하여 경구투여시 약물의 체내 흡수를 예측 평가하고자 하였다.

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2. Introduction

Breast cancer is a common cancer diagnosed in women. The main treatment strategies for breast cancer are surgical intervention and radiotherapy, chemotherapy or hormone therapy. Approximately 70% of breast cancers are hormone dependent, and intrinsically estrogen receptor-α (ERα) is involved. Therefore, hormone therapy is used as primary chemotherapy in patients with hormone receptor positive breast cancer [1]. Tamoxifen, a selective estrogen receptor modulator (SERM) and aromatase inhibitors (AIs), which block estrogen synthesis are effective primary endocrine therapies that significantly improve recurrence and overall survival of all stages of ERα-positive cancer. However, acquired resistance to tamoxifen and AI typically occurs after prolonged therapy in the majority of early reactive breast cancers and almost 50% of patients with advanced ER-positive breast cancers do not respond to tamoxifen or AI in the first line treatment [2].

Fulvestrant (FLV), one of selective estrogen receptor down regulators (SERD) plays a critical role in these acquired resistance cancers. FLV demonstrates their activity through completely inhibiting both ERα-modiated genomic and non-genomic signaling, so that it can be used to patients resistant to previous hormone therapy [3]. However, FLV has a low bioavailability when administrated to oral route because of its poor solubility, so only one commercial product (Faslodex) that is administrated by intramuscular injection is used [1].

Solid lipid nanoparticle (SLN) is one of a lipid based drug delivery system that is absorbed into the body by the lymphatic system of the small intestine [4]. There are several advantages over the oral absorption via the lymphatic system. For intense, drugs enter the systemic circulation without first passing through the liver. It is effective for drugs with very low oral bioavailability because most of them are metabolized due to hepatic first pass effect. Also, intestinal lymphatic transport has been reported to be more effective for immunomodulating and chemotherapy drugs because the lymphatic system is the primary pathway for solid tumors and the transport pathway for T and B lymphocytes. Thus, SLN is being used to develop not only anti-cancer drugs but also

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immunomodulatory such as human immunodeficiency virus(HIV), hepatic B and C virus [5, 6, 7].

In this study, we developed FLV-loaded solid lipid nanoparticle(FLV-SLN) to deliver FLV through oral administration and compare the properties of SLN prepared with different solid lipids that composed with different carbon chain lengths [10]. Three different solid lipids (trilaurin(TL), trimyristin(TM), precirol ATO 5(ATO)) were selected to prepare SLN and evaluate the characteristics to decide a final formulation. Kollisphor TPGS and solutol 15 HS were used as surfactant. TPGS has advantages in various nanocarriers models including increase of the drug half-life in plasma and enhancing the cellular uptake by avoiding P-gp efflux [8, 9]. There are several reports that the intestinal absorption and oral bioavailability of poor soluble drugs can be improved when the drug is delivered by encapsulation into nanocarriers composed with TPGS. So, it is valuable to note that SLNs prepared with TPGS can enhance the oral bioavailability of FLV.

The optimal composition of SLNs was determined by preparation of SLNs with different ratio of lipids, surfactants and water. The developed FLV-SLNs were characterized the properties such as particle diameter, polydispersity index and zeta potential. In vitro release studies and Caco-2 cell permeability test were performed to predict absorption of FLV in GI tract.

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3. Materials and Methods

3-1. Materials

Fulvestrant (FLV), trilaurin (Glyceryl tridodecanoate) and trimyristin (Glyceryl tritetradecanoate) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Precirol ATO 5 (Glyceryl distearate) was gifted from Gatteosse SAS (Saint Priest Cedex, France). Kolliphor TPGS (Vitamin E Polyethylene Glycol Succinate) and Solutol HS 15 (Polyoxyl 15 hydroxystearate) were obtained from BASF (Ludwigshafen, Germany). Acetonitrile (ACN) and methanol were HPLC grade and purchased from Avantor Performance Materials, Inc (Center valley, PA, USA). All other reagents used in this study were laboratory grade.

3-2. Determination of the composition ratio of SLNs

As shown in Table 1, SLNs were prepared with different solid lipid/surfactant ratios. Fifty formulations for each lipid were prepared and stored at RT and 4° C. The SLNs were prepared by the section of '3-3 preparation of SLNs' and evaluated the particle diameter at designated periods (0, 7th and 30th day). Based on the results, the optimum ratio was determined and used for subsequent experiments.

3-3. Preparation of SLNs

FLV loaded SLNs were prepared by hot-melting sonication method. In brief, 10 mg of FLV and weighed solid lipid, TPGS and solutol were mixed and heated at 65℃ for 40 min in a water bath. Purified water heated at same degree was added into the mixture and mixed at 65℃. Homogenization was performed using a probe sonicator (VCX-500, Sonics & Materials, Inc., USA) for 10 min in 65℃ water bath. The obtained SLNs dispersion was filtered through 0.8 μm pore size syringe filter and cooled down at room temperature or 4℃ during 12 hrs. Blank SLNs were prepared in a same process without FLV.

3-4. Particle diameter, polydispersity index, zeta potential

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The mean particle diameter, polydispersity index(PDI) and zeta potential of FLV-loaded SLNs were measured by Zeta potential & Particle size Analyzer ELSZ-2000 series (Otsuka Electronics Co., Ltd, Japan). The SLNs dispersion was diluted with purified water to reach a concentration to suit the measurement. All analysis was performed at room temperature.

3-5. Encapsulation efficiency (EE), loading capacity (LC)

To calculate EE and LC, concentrations of FLV encapsulated in SLNs were analyzed. In brief, 100 μl of SLNs was mixed with 900 μl ACN and sonicated using bath sonicator for 30 min. The obtained solution was centrifuged at 12,000 g for 15 min at 4℃. FLV in the supernatant was analyzed using high performance liquid chromatographic system (Azura, Germany). For HPLC analysis, C18 column (Luna C18, 4.6 mm × 150 mm, 5 μm; Phenomenex, Torrance, CA, USA) was used and heated at 40℃. The mobile phase consisted of a 20:80 v/v mixture of water and ACN with 0.1% phosphoric acid. The pH was adjusted to 3.0 with 0.1 N sodium hydroxide. The flow rate was adjusted to 1.0 ml/min and injection volume was 20 μl. The detection wavelength was fixed at 215 nm. The chromatograms were analyzed using ClarityChrom® software (Version 6.1.0.130, Knauer, Germany) provided with the system.

EE and LC were presented according to the equations, respectively.

$$\mathrm{EE}(\%) = \frac{\text{Encapsulated drug } (\mu g/ml)}{\text{Total drug } (\mu g/ml)} \times 100$$

$$\mathrm{LC}(\%) = \frac{\text{Encapsulated drug } (\mu g/ml)}{\text{Total lipid } (\mu g/ml)} \times 100$$

3-6. Freeze-drying

To prepare the most representative oral dosage form tablets or capsules, the liquid state of SLNs should be transformed into solid powder state. Freeze-drying method was

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selected as the transformation method of SLNs state. As a cryoprotectant, sucrose and HP-β-CD were selected. The SLNs dispersions were diluted (1:1) with cryoprotectant solutions and frozen at -21℃ overnight. The freeze-drying process was carried out at –80℃ at a pressure of 50 mTorr during 24 h. The particle diameter of SLNs obtained by re-dispersing in pure water was measured to determine the type and concentration of the cryoprotectant.

3-7. Differential scanning calorimetry

Thermal analysis of pure FLV, solid lipid, FLV loaded and blank form of solid lipid nanoparticles were carried out with Universal V4.5A Instruments.

3-8. Transmission electron microscopy

A mixtures of FLV-loaded SLNs and cryoprotectant solution were characterized their shapes by Tecnai G2 Spirit TWIN Transmission Electron Microscope(FEI company, USA) As a negative staining, a copper grid coated with a carbon and uranyl acetate (2% v/v) were used.

3-9. *In vitro* release of FLV from SLNs

In vitro release of FLV from FLV-loaded SLNs was determined. According to the Korean Pharmacopoeia Ⅹ Ⅰ, solution 1 (2.0 g of sodium chloride in 7.0 ml hydrochloric acid and 1000 ml water, pH 1.2) and solution 2 (a mixture of phosphoric buffer solution and water, pH 6.8) were used to simulate the pH condition of stomach and intestine, respectively. A 0.25% (w/w) tween 80 was added to maintain a sink condition. A 200 ml of release medium in a dissolution vessel was heated and maintained at 37℃. The SLNs dispersion containing 10 mg of FLV was added to release medium without dialysis bag and stirred at 100 rpm. One milliliter of the solution was taken at determined time (5, 10, 15, 30, 60, 90, 120, 180 min) and centrifuged (12,000 rpm, 30 min, 4℃). The precipitate was re-dispersed in 1 ml of release medium and placed in a dissolution vessel and the concentration of FLV in supernatant was analyzed by HPLC. The experiments were performed in triplicate.

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3-10. Permeability study of SLNs

3-10-1. PAMPA assay

The parallel artificial membrane permeability assay was carried to using 96-well microtiter plate and a 96-well filter plate (Millipore, Bedford, MA, USA). Two plates were assempled like a sandwich. The donor wells were filled with 200 μl of SLNs dispersion (200 μg/ml) and the acceptor wells were filled with 300 μl PBS containing 2.5%(v/v) DMSO to maintain the sink condition. Then, the plate was incubated at room temperature for 5 hours. FLV concentrations of acceptor wells were analyzed by HPLC.

3-10-2. Caco-2 cell study

A monolayer of well-differentiated human intestinal epithelial cell line Caco-2 was used as a model to study drug absorption through the intestinal epithelium. Caco-2 cells were cultured in DMEM/HIGH GLUCOSE media with 10% fetal bovine serum, 1% penicillin/streptomycin in a humidified atmosphere of 5% CO2 in air at 37℃ for 7 days. A 400 μl volume of 1.5×10^5 cells was seeded at apical side of transwell insert and a 100 μl of media was added. And a 1.5 ml of cell culture medium was added to the basolateral side. The cells were cultured in transwell for 4 weeks and permeability test was performed.

The effect of SLNs and free form of FLV on cell permeability were evaluated using Caco-2 cell. Culture medium was removed from both apical and basolateral side. Then, add 0.5 ml of HBSS on the apical side and 1.5 ml of HBSS containing 2.5%(v/v) DMSO at basolateral side and incubate for 30 min in an incubator for washing remained culture medium. Following incubation, solution was removed from both sides. After that, a 200 μl of SLNs dispersion (200 μg/ml) was added on the apical side. On the basolateral side, 1.5 ml of HBSS containing 2.5%(v/v) DMSO was added as release medium and incubated at 37℃ for 5 h.

At predetermined time intervals (0.5, 1, 2, 3, 4, 5 h), 200 μl of aliquots were taken at basolateral side and immediately replaced with an equal volume of fresh medium. FLV concentration was analysis using HPLC and the experiments were preformed in triplicate.

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4. Results and Discussion

4-1. Preparation of SLNs

In order to determine the optimum ratio of composition and the cooling temperature, 50 formulations for each lipid were prepared by hot-melting sonication method (Table 1) and evaluated the stability of SLNs stored at 4℃ or room temperature for 1 month (data not shown). The formulations were excluded when SLNs turned into gel immediately, during storage of had unusual particle diameters. The particle diameter was affected by the ratio of surfactants. When the ratios of TPGS and solutol were 50/50, uniform and consistent diameters of SLNs were obtained.

When the lipid was 5%, the diameter was very small or not uniform. In addition, the EE and LC were low when the lipid content was low. Whereas, the lipid content was 15%, SLNs were gelled immediately after preparation or within one week.

The cooling temperature did not affect on the initial particle diameter of SLNs, whereas, the diameter of SLNs cooled at RT increased after 1 week. Re-crystallization of solid lipids in SLNs was affected by the temperature of cooling process, and the crystalline form of solid lipid was more stable at 4℃ than at room temperature [16]. Based on the stability data for 1 month, the composition ratio of SLNs was decided as of lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5 and cooling temperature was selected as 4℃.

4-2. Evaluation of FLV-loaded SLNs properties

4-2-1. Particle diameter, zeta potential, PDI

The mean particle diameter, zeta potential and PDI of SLNs which were made using three different lipids were evaluated (Table 2). SLN(TL) and SLN(TM) were in range of 98.13 ± 1.97 and 94.59 ± 2.30 nm, respectively. Whereas SLN(ATO) showed smaller diameter of 63.62 ± 4.63 nm and the appearance was slightly transparent compared to SLN(TL) and SLN(TM). From the obtained data, it was suggested that the physical state of the lipid played an important role in the particle diameter of the SLNs. SLNs composed of diglyceride (ATO) showed smaller in diameter than SLNs composed

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of triglyceride (TL,TM). In all formulations, PDI remained within the acceptable range (<0.3).

In all prepared SLNs, zeta potential was in range of -12 to -20 mV. In general, a preferred zeta potential value for achieving particle stability only by electrostatic stabilization is > 30 mV. The produced SLNs have a lower zeta potential than requirements but are higher than the zeta potential required to stabilize with electrostatic and steric stabilization (approximately 8 to 9 mV) [18].

4-2-2. Encapsulation of FLV into SLNs

The amount of FLV encapsulated into SLNs was analyzed by HPLC analysis. The calculated EE and LC of FLV-loaded SLNs were presented at Figure 1. SLN(TL), SLN(TM) and SLN(ATO) had EE values of 94.11 ± 2.28, 97.85 ± 5.92 and 99.28 ± 8.94, respectively. SLNs with longer chain lipid had higher EE values. However, it was not significantly different. LC value was in range about 4.5 ~ 5%, which showed similar tendency to EE value. The results indicated that FLV was successfully incorporated into the solid lipid nanoparticles.

4-2-3. Freeze-drying

The commonly used cryoprotectants sucrose and HP-β-CD were chosen because of their buffer stabilizer and collapse temperature modifier effects, respectively [11]. FLV-loaded SLNs were mixed with various concentration of cryoptotectants followed by lyophilization. The effects of cryoprotectants on the particle diameter of SLNs were shown in Table 3. The stabilizing effect of cryoprotectant in particle diameter was stronger in HP-β-CD than in sucrose. When 20% HP-β-CD was used, the lyophilized SLNs had the smallest diameter (Figure 3).

EE and LC values were decreased after lyophilization in all cryoprotectants (Figure 3). Especially in 5% sucrose, there is no difference compared with SLNs without cryoprotectant. In the case of HP-β-CD, there was no significant difference in EE value and LC value according to the concentration of cryoprotectant. Therefore, 20% HP-β-CD was selected considering the mean particle diameter of SLNs.

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4-2-4. Thermal analysis

DSC analysis results of pure FLV, pure lipid and FLV-loaded SLNs were represented in Figure 4. The pure form of FLV had a broad melting endotherms at 108℃, proving the melting points (T_m) of the FLV. The endothermic crystalline peak of pure lipid also showed similar to the melting point of lipid. However, the peak of pure TM and TL had a one sharp peak, whereas ATO showed a broad separated peak. It was also seen in the peaks of SLNs made with each lipid. It was suggested that this property of ATO influenced on the properties of SLN(ATO) such as small diameter and transparent appearance which was different with other SLNs.

The endothermic peak of FLV at 108℃ was disappeared in all FLV-loaded SLNs formulations. The results suggested that FLV was incorporated into the SLNs part an amorphous form or solid structure in the matrix.

4-2-5. TEM images of FLV-loaded SLNs

Figure 5 showed the TEM photographs of FLV-loaded SLNs before lyophilization and after redispersion. The mean particle diameter of SLNs by the TEM images was consistent with the results measured using DLS. The morphologies of SLN(TL) and SLN(TM) were discrete particles with sharp boundaries and near-spherical morphology which was typical of SLNs systems. However, the morphology of SLN(ATO) was an irregular shape. It was suggested that the chain length of the lipid became longer, the physical shape of the SLNs was also distorted[16].

From the TEM images, we also conclude that lyophilization did not affect particle shape and size.

4-2-6. In vitro release

The FLV release profiles from FLV-loaded SLNs are shown in Figure 6. The in vitro release experiment was performed in sink conditions, by putting 3 ml SLNs dispersion (containing 10 mg of FLV) in 200 ml release media (AGF and AIF, respectively) without dialysis membrane bag. As a control, FLV suspended in 0.5% MC solution was used. The graphs (a) and (b) showed the release profile of SLNs before

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freeze drying in solution 1 and solution 2. In all experiments, approximately 90% of FLV was released from FLV-loaded SLNs within 5 min, whereas only 12.7% of FLV was dissolved in the release medium during 180 min from control. Based on the curve obtained from in vitro release study, FLV was effectively solubilized by encapsulation of SLNs.

4-3. Permeability study

PAMPA assay is one of the permeability study that it evaluates the transmission by passive drug absorption. Figure 7 showed the results of the PAMPA assay. SLN-n was pure SLNs with no addition, SLN-b was a mixture of HP-β-CD and SLNs, and SLN-a was lyophilized and re-dispersed of SLN-b.

The results of drug permeation by passive transport showed a similar tendency to the results of permeation experiment using Caco-2 cell. The control group, as same with Caco-2 cell results, was not detected in acceptor wells. The drug was permeated due to the effect of the formulation on the drug delivery carrier.

Caco-2 cell model was used to evaluate oral permeability of FLV and predict oral absorption of FLV. Figure 8 showed the permeability profiles of FLV-loaded SLNs. FLV dissolved in DMSO was used as control. The experiment sample was the same as that used in PAMPA assay. In this experiment, FLV as a control group was not detected in basolateral side of transwell. Whereas FLV-loaded SLNs was permeated through Caco-2 cells and the drug was detected on the basolateral side. The graphs of SLN(TL) and SLN(TM) showed that permeation of SLN-a and b was better than SLN-n (Figure 7. a, b). It was due to the absorption-promoting effect of HP-β-CD. Compared to SLN-a and SLN-b, the permeability of FLV was increased after freeze-drying. However, in the case of SLN(ATO), FLV of SLN-n did not penetrate Caco-2 cells at all, while permeability of SLNs mixed with HP-b-CD was improved because of HP-β-CD. SLN(ATO) showed different properties compared with other SLNs. SLN-b before freeze drying had higher permeability than SLN-a. These results suggest that the structure of the nanoparticle was destroyed after lyophilization, as shown in the TEM image, and it was not adequate carrier for FLV formulation.

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This result indicated that SLNs played as a drug delivery carriers. Studies have shown that SLNs prepared with long chain lipid absorbed better than short chain lipid when administrated orally [5]. However, SLN(ATO) showed the lowest permeability among the formulations in Caco-2 cell study and PAMPA assay. This result was thought to be due to the physical properties of the ATO described in DSC data and TEM images. So, in vivo study is necessary to determine the lipid of final formulation by comparing the absorption rate of the drug with the different chain length of the lipid.

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5. Conclusion

FLV-loaded SLNs were successfully developed by hot melting sonication method and the properties of optimized formulations were characterized. It had a suitable particle diameter as a nanocarrier.

The results suggested that the length of the carbon chain of fatty acids in solid lipids played an important role on physical properties of formulations. DSC and TEM results suggested that ATO with a long lipid chain length had a negative effect on the stability of the formulations. EE and release profiles showed that the drug was well encapsulated into the formulation and increased the solubility of the drug through the formulations. However, the Caco-2 cell permeability study and PAMPA assay suggested that the additional modification of FLV-loaded SLNs should be performed to improve the oral BA of FLV.

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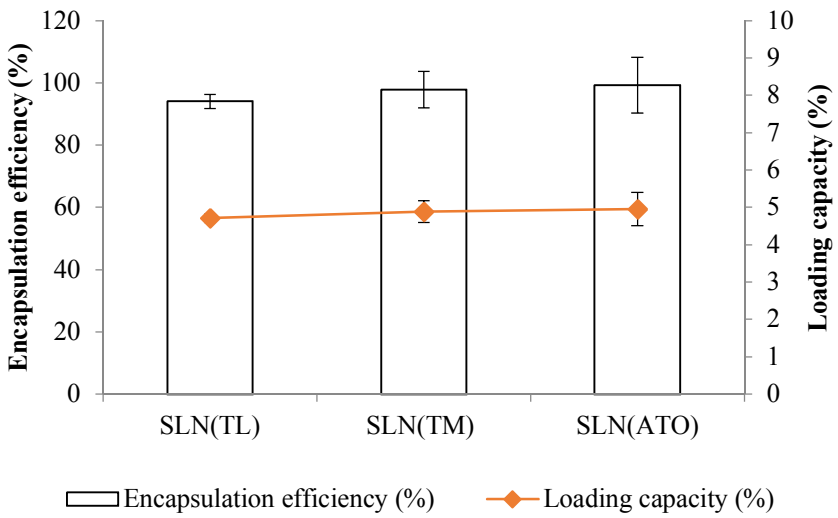

Figure 1. Encapsulation efficiency (EE) and loading capacity (LC) of SLN(TL), SLN(TM) and SLN(ATO). The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5. There was no significant lipid-specific difference in drug encapsulation (n=3).

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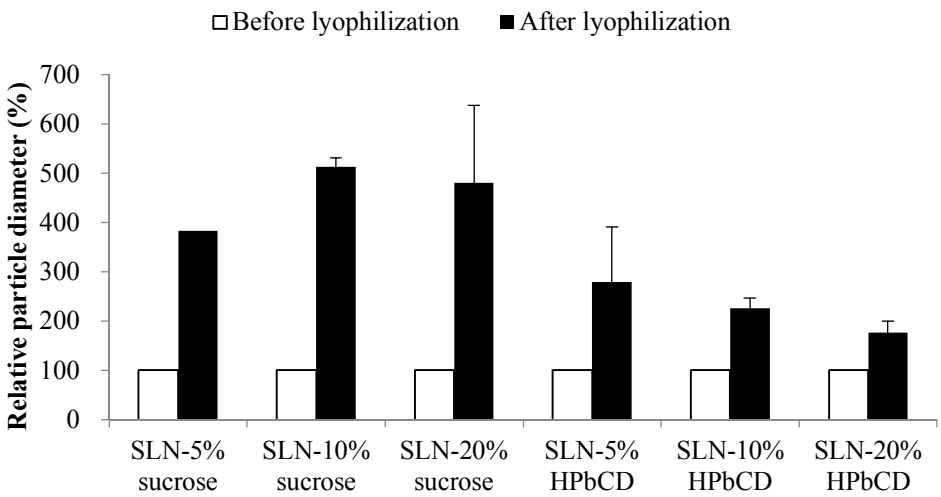

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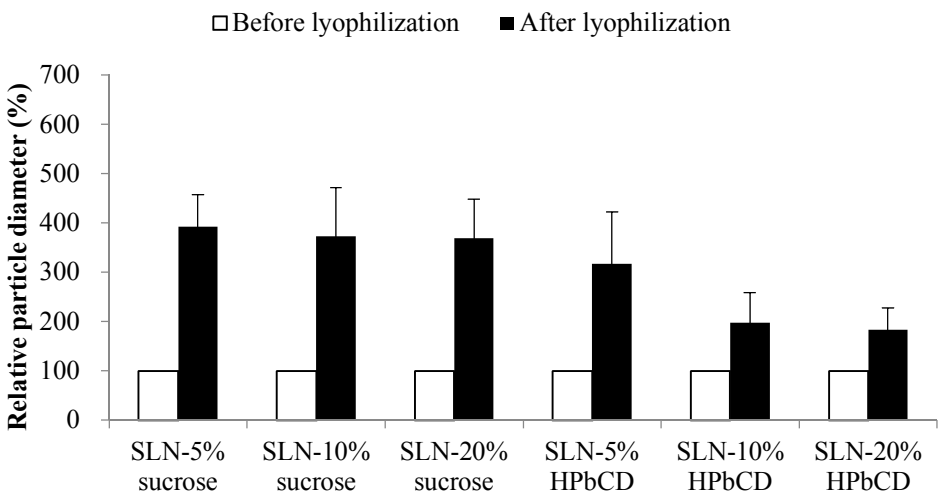

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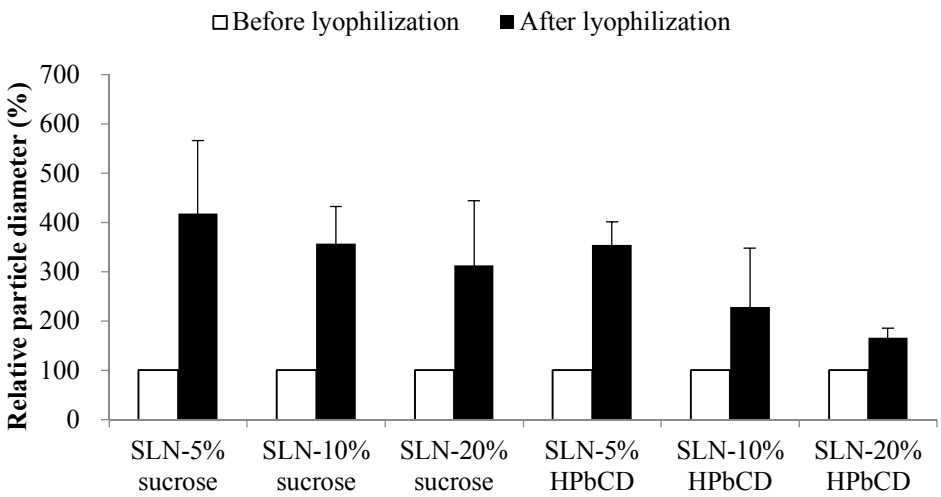

Figure 2. Effect of cryoprotectant on particle diameter of SLNs before and after freeze drying (n=3). The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5.

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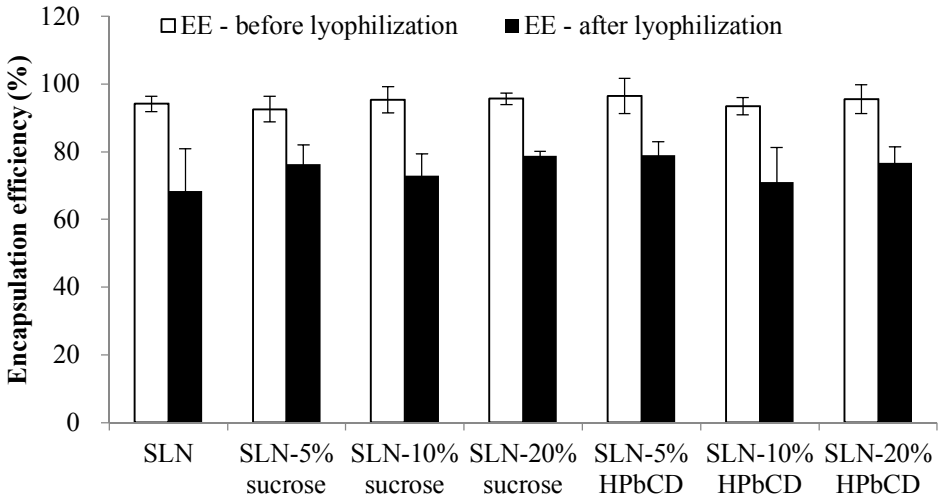

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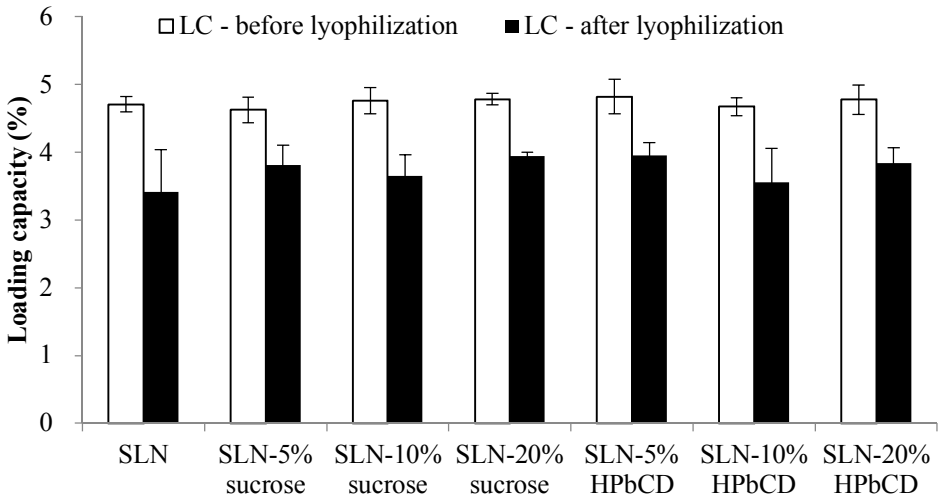

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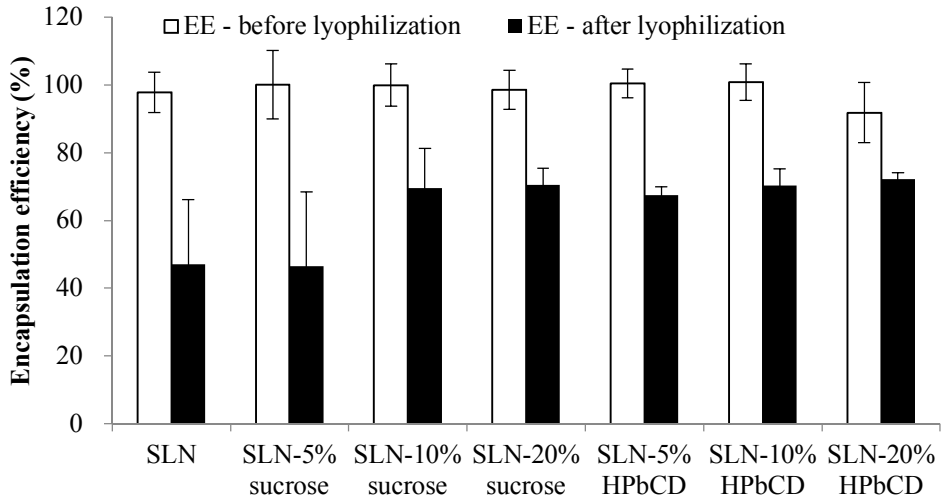

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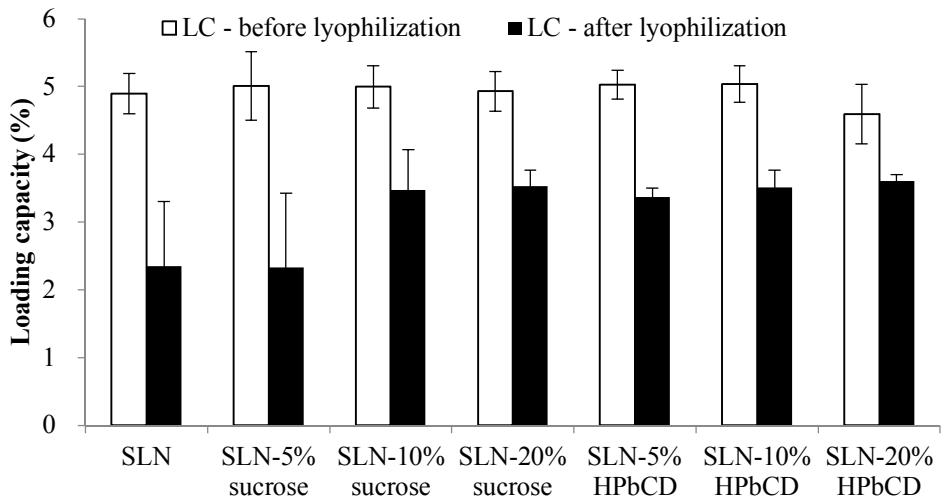

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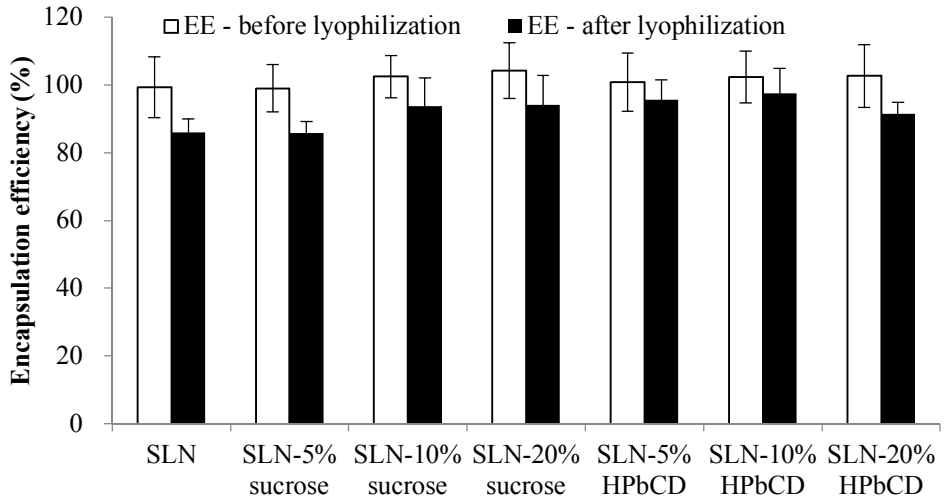

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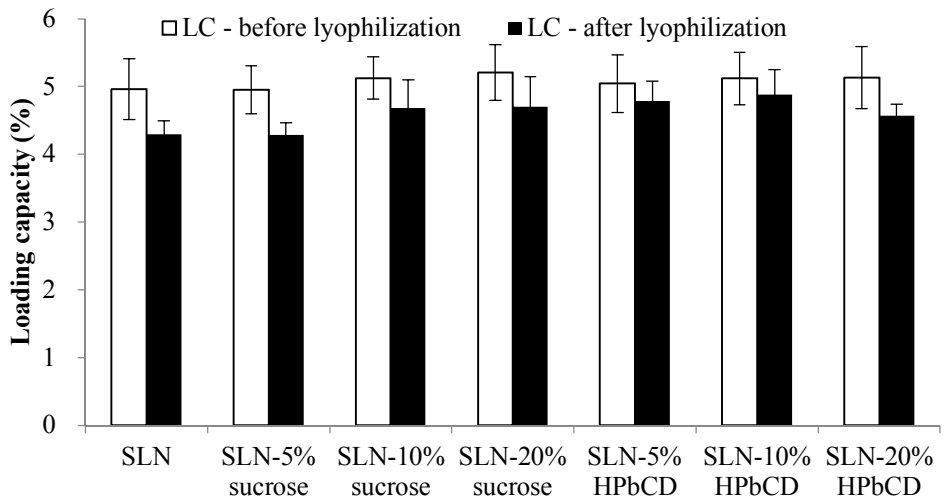

Figure 3. Effect of cryoprotectant on EE and LC value of SLNs before and after freeze drying. The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5. (a) and (b) : SLN(TL), (c) and (d) : SLN(TM) and (e) and (f) : SLN(ATO) (n=3).

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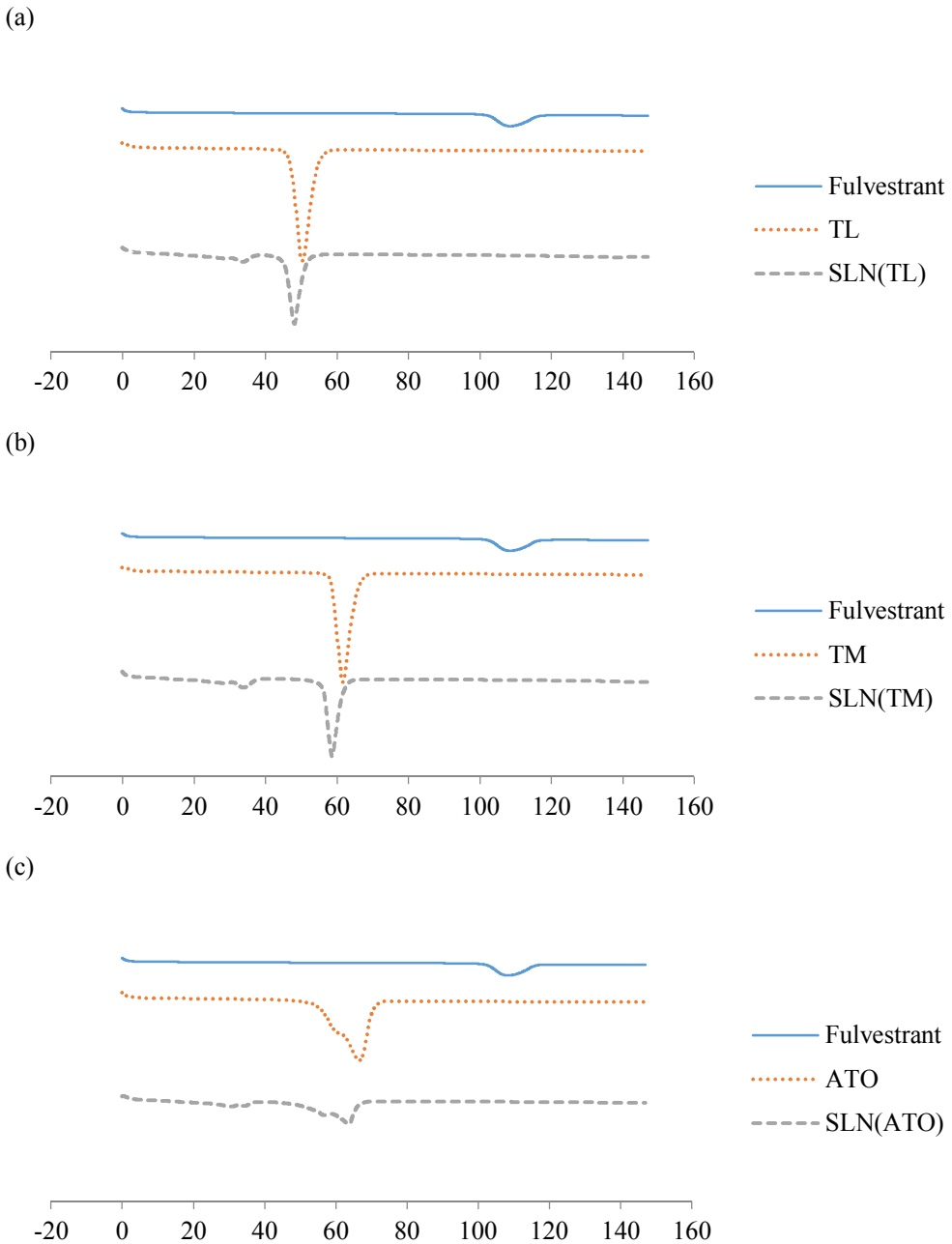

Figure 4. DSC data of FLV-loaded SLNs. (a) : SLN(TL), (b) : SLN(TM) and (c) : SLN(ATO). The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5.

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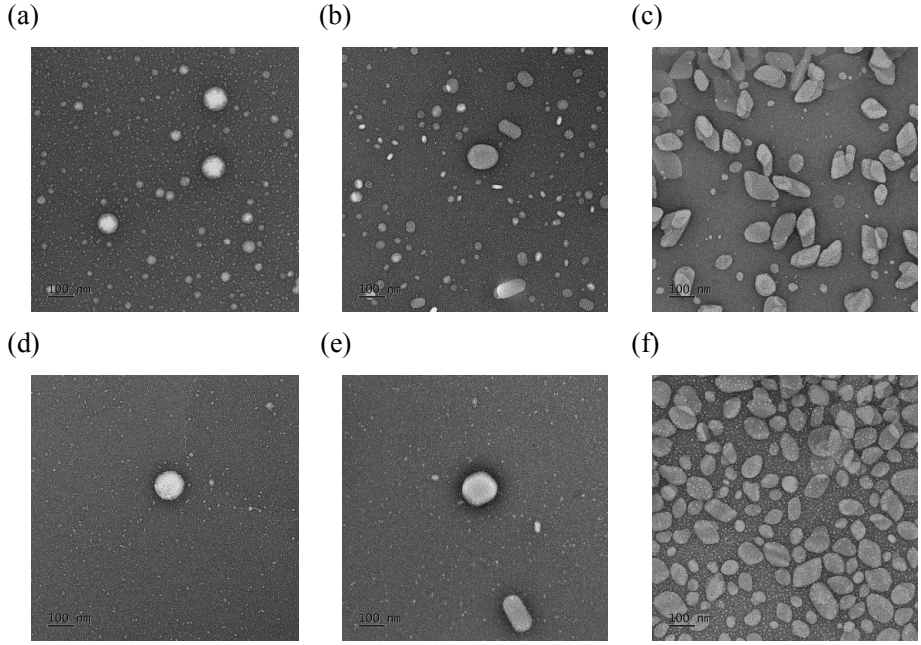

Figure 5. TEM images of SLNs. (a) : SLN(TL), (b) : SLN(TM) and (c) : SLN(ATO) before lyophilizaion. (d) : SLN(TL), (e) : SLN(TM) and (f) : SLN(ATO) after lyophilizaion. The optimal ratio of each SLNs is lipid : TPGS : Solutol : water = 10 : 3.75 : 3.75 : 82.5.

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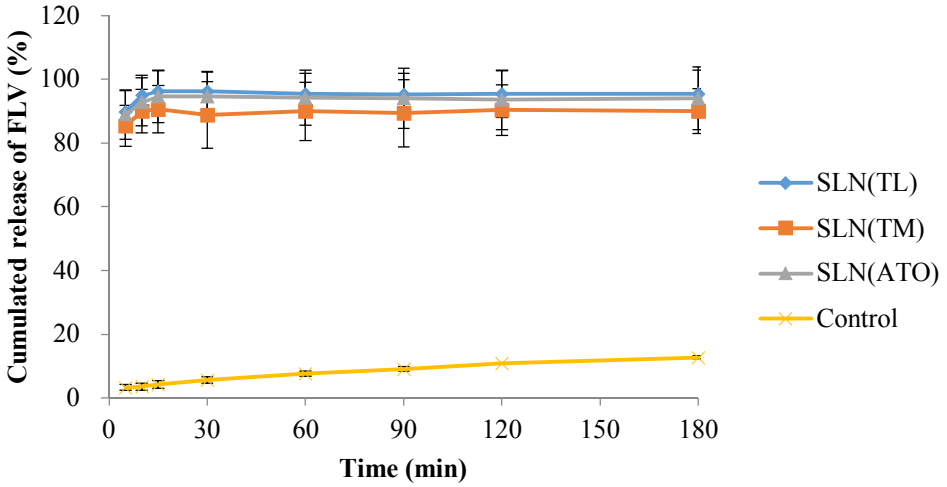

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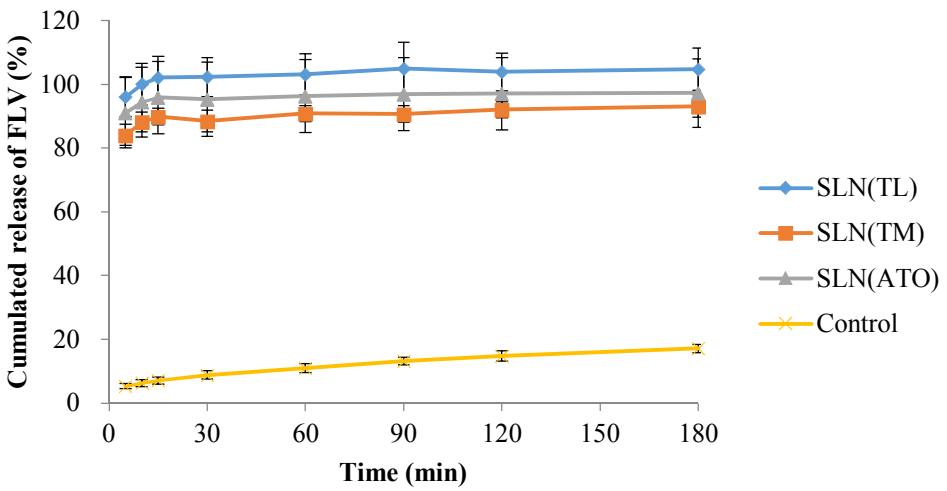

Figure 6. In vitro release profiles of FLV-loaded SLNs. The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5. (a) is a release graph in solution 1(pH 1.2) and (b) is in solution 2(pH 6.8) for SLNs before freeze drying (n=3).

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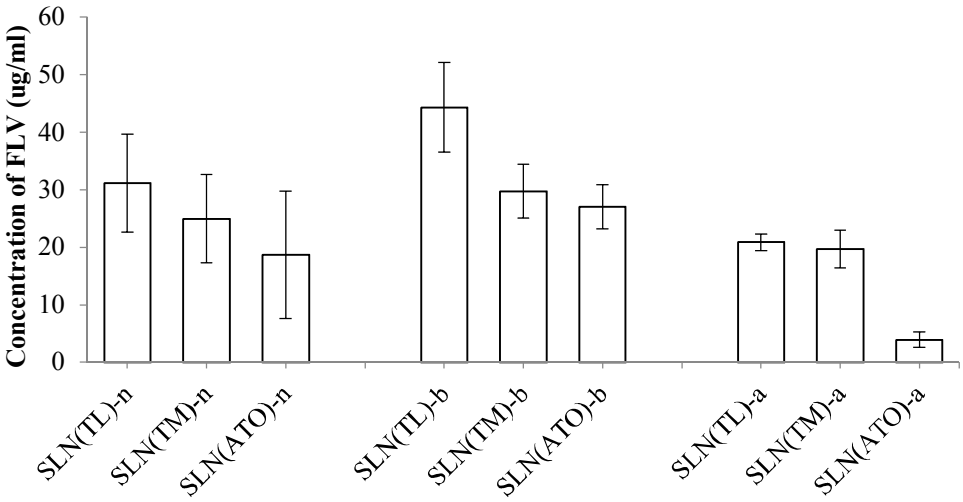

Figure 7. PAMPA assay of FLV-loaded SLNs (n=9). The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5.

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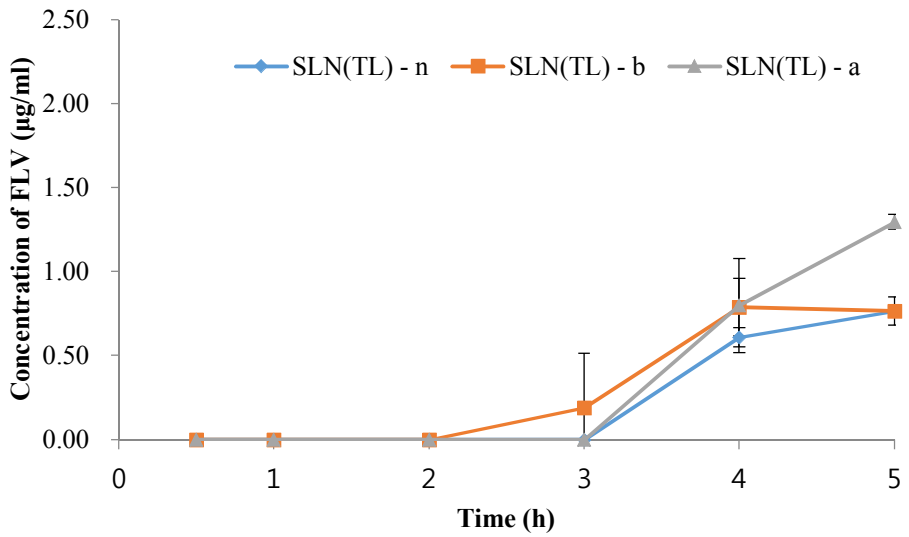

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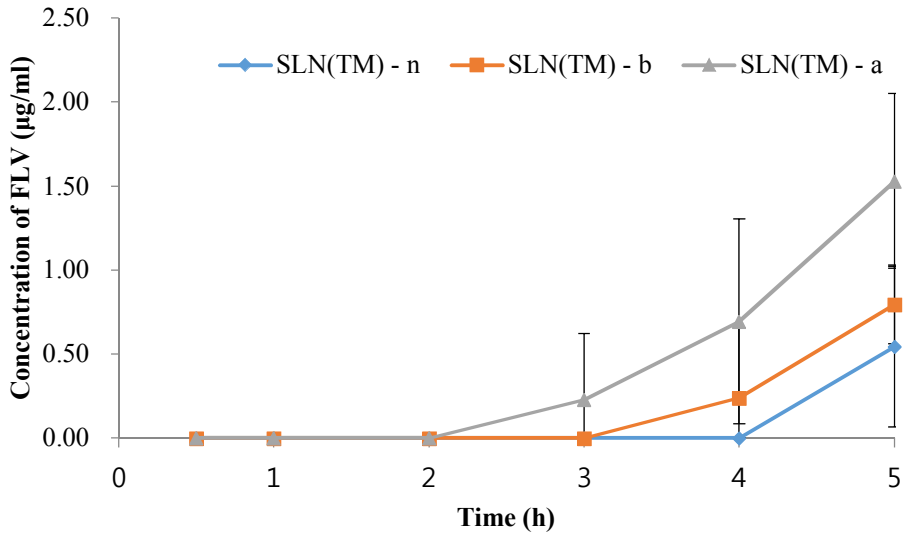

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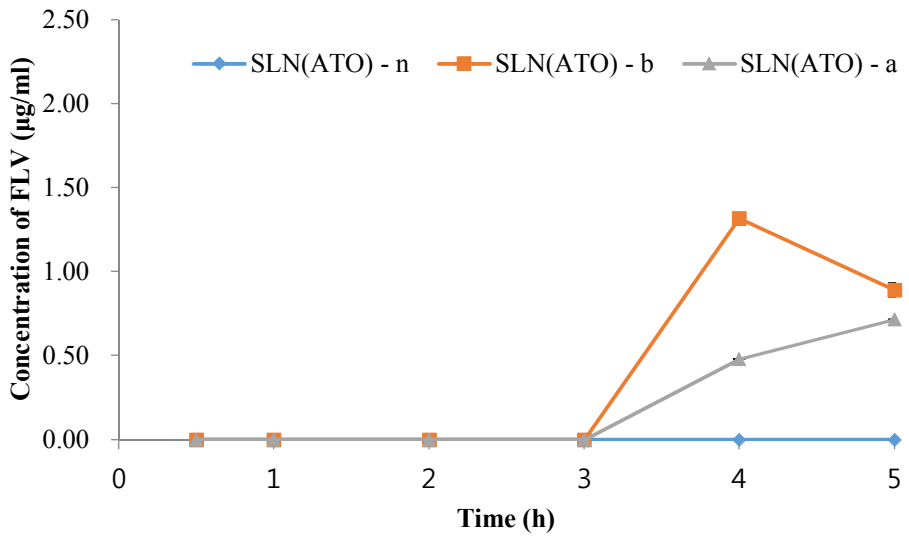

Figure 9. Permeability study of FLV-loaded SLNs. (a) : SLN(TL), (b) : SLN(TM) and (c) : SLN(ATO) (n=3). The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5.

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Table 1. The Composition of SLNs

Formulation	Lipid(%)	Surfactant		Water(%)
		TPGS(%)	Solutol(%)	
SLN #1	5	10	0	85
SLN #2	5	7.5	2.5	85
SLN #3	5	5	5	85
SLN #4	5	2.5	7.5	85
SLN #5	5	0	10	85
SLN #6	10	5	0	85
SLN #7	10	3.75	1.25	85
SLN #8	10	2.5	2.5	85
SLN #9	10	1.25	3.75	85
SLN #10	10	0	5	85
SLN #11	10	7.5	0	82.5
SLN #12	10	5.625	1.875	82.5
SLN #13	10	3.75	3.75	82.5
SLN #14	10	1.875	5.625	82.5
SLN #15	10	0	7.5	82.5
SLN #16	10	10	0	80
SLN #17	10	7.5	2.5	80
SLN #18	10	5	5	80
SLN #19	10	2.5	7.5	80
SLN #20	10	0	10	80
SLN #21	15	10	0	75
SLN #22	15	7.5	2.5	75
SLN #23	15	5	5	75
SLN #24	15	2.5	7.5	75
SLN #25	15	0	10	75

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Table 2. Mean particle diameter, zeta potential and PDI of SLN(TL), SLN(TM) and SLN(ATO) (average ± SD, n=3). The optimal ratio of each SLNs is lipid : TPGS : solutol : water = 10 : 3.75 : 3.75 : 82.5.

Formulation	Mean particle diameter (nm)	PDI	Zeta potential (mV)
SLN(TL)	98.13 ± 1.97	0.267 ± 0.023	-12.89 ± 0.70
SLN(TM)	94.59 ± 2.30	0.231 ± 0.023	-12.84 ± 0.66
SLN(ATO)	63.62 ± 4.63	0.177 ± 0.034	-22.07 ± 2.14

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Table 3. Comparison of mean particle diameter before and after freeze drying of SLNs according to cryoprotectant (mean particle diameter ± SD, n=3).

	Mean particle diameter (nm)					
	Before freeze-drying			After freeze-drying		
Conc. of cryoprotectant	SLN(TL)	SLN(TM)	SLN(ATO)	SLN(TL)	SLN(TM)	SLN(ATO)
5% sucrose	94.23 ± 3.86	89.04 ± 15.24	64.70 ± 6.92	360.82 ± 27.53	339.74 ± 41.28	273.65 ± 77.92
10% sucrose	95.39 ± 1.28	90.88 ± 11.41	62.60 ± 2.23	489.50 ± 142.03	333.44 ± 32.20	221.72 ± 75.97
20% sucrose	97.79 ± 0.48	93.02 ± 5.68	63.96 ± 3.09	470.59 ± 110.66	340.31 ± 85.09	199.50 ± 21.41
5% HP-β-CD	96.81 ± 3.57	86.81 ± 5.49	66.37 ± 5.66	270.44 ± 23.39	273.08 ± 37.19	239.84 ± 102.26
10% HP-β-CD	97.94 ± 3.62	89.92 ± 2.73	66.92 ± 8.94	220.56 ± 22.08	176.83 ± 36.21	154.09 ± 31.79
20% HP-β-CD	94.08 ± 2.68	96.60 ± 7.25	65.68 ± 4.45	165.89 ± 10.18	176.42 ± 14.65	109.02 ± 4.41

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ABSTRACT

Development and characterization of fulvestrant loaded SLNs for enhanced oral bioavailability

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Advisor: Prof. Jun-Pil Jee
Department of Pharmacy
Graduate School of Chosun University

Fulvestrant(FLV) is the first-agent of the selective estrogen receptor down regulators (SERDs). Unlike tamoxifen, one of the selective estrogen receptor modulators (SERMs), it acts as an only antagonist to the estrogen receptor. FLV is more potent than tamoxifen in inhibiting the growth of human breast tumors and was effective in inhibiting the growth of tumors resistant to tamoxifen. So, FLV is recommended for hormone-positive breast cancer patients for first line treatment. However, the commercial product of FLV (Faslodex) is only administrated through intramuscular injection in every four weeks.

In this study, we developed FLV-loaded solid lipid nanoparticles (SLNs) for oral administration. SLN is absorbed into the body through the Peyer's patch pathway involved in the absorption and migration of lipid-soluble substances in the small intestine, and is circulated through the lymphatic system, thus avoiding the first pass effect of the liver. In addition, TPGS, which is used as a surfactant, has the effect of inhibiting the efflux by p-gp protein in the body and absorbing poorly soluble drugs and increasing the bioavailability.

According to the chain length of solid lipids, three different lipids (trimyristin (TM), trilaurin (TL) and precirol ATO 5 (ATO)) were selected to prepare SLNs and evaluate the properties to decide the final formulation. And, fifty formulations for each lipid with

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different proportions of composition were prepared and evaluated their properties to determine the final ratio of formulation.

The physicochemical properties of optimal FLV-loaded SLNs were investigated by analyzing particle diameter, zeta potential, PDI, and drug encapsulation efficiency. To elucidate the characteristics of drug release from SLNs, in vitro release evaluation was performed in artificial gastric and intestinal fluid. And Caco-2 cell permeation experiment and PAMPA assay were performed to evaluate the absorption of drugs by oral administration. The results suggested that FLV-SLNs can be a potential candidate formulation for oral administration of FLV.

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