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Study on development of lipid matrix based long acting injectable formulation of donepezil

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Donepezil 의 지질 매트릭스 기반
장기 지속형 주사제 개발에 대한 연구

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이 원 영
지 도 교 수 : 지 준 필
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Donepezil 은 1996 년 FDA 에 의해 승인된 2 세대 cholinesterase inhibitor 로써 알츠하이머 환자의 인지 기능을 개선하는 목적으로 사용된다. Donepezil 은 현재 경구 투여 제형만이 시판 되고 있으며 일반적으로 1 일 1 회 복용하게끔 되어있다. 알츠하이머 환자의 특성상 이러한 용법은 복약순응도가 낮을 가능성이 매우 크다. 따라서 이러한 문제점을 개선하기 위해, donepezil 의 약효가 장기간 유지될 수 있는 장기 지속형 주사제 개발에 대한 연구를 수행하였다.

최종 제형은 castor oil (45%), cottonseed oil (34%), span 80 (13%) 및 benzyl alcohol (8%)로 구성된 지질 기반 매트릭스 (Fluid lipid matrix, FLM)에 donepezil free base 를 로딩시켜준 1 세대 제형 DP-FLM, donepezil free base 를 Trilaurin (15%), TPGS (5.625%), Solutol (5.625%), DW (73.75%)로 구성된 고형 지질 나노 입자 (Solid lipid nanoparticle, SLN)에 봉입해 동결건조 한 뒤 지질 기반 매트릭스에 분산시켜준 2 세대 제형 DP-SLN-FLM 두가지이다. FLM 의 조성에 따른 viscosity, syringeability, release test 등을 거쳐 최종 FLM 조성을

결정했다. 그리고, 고형 지질과 계면활성제의 비율에 따른 stability 및 particle diameter 와 zeta potential, PDI, 값을 토대로 SLN 의 최종 조성비를 결정하였다.

최적화된 제형에 대하여 약물의 봉입률 측정, 광학 현미경 및 편광 현미경을 통한 내부 구조 관찰, 점도 변화 측정 등의 특성 평가를 진행하였다. 또한, 4 주간 방출 경향 평가를 통해 두 제형 모두 donepezil 을 3 주 가량 방출함을 확인하였다. 제시된 결과들은 개발된 제형이 donepezil 을 장기간 방출하기에 적합한 장기 지속형 주사제제로서의 가능성이 있음을 보여주었다.

2. Introduction

Alzheimer's disease (AD) is a chronic, progressive, neurodegenerative brain disorder characterized by memory and cognitive deficits [1]. Symptom progression may be due to the loss of neuronal function and synaptic connections, accompanied by neuronal cell death in different brain regions [2]. Broad measures of dementia prevalence in developed countries suggest an overall rate of 4–8% over the age of 65 [3].

Cholinesterase inhibitors are the first choice for the treatment of AD; both the American Academy of Neurology and the UK National Institute for Clinical Excellence suggest that cholinesterase inhibitors should be considered as the standard of care for mild to moderate AD [4]. Commercially available donepezil preparations include film-coated tablets, orally disintegrating tablets and orally disintegrating films that are taken once daily [5]. This once-daily regimen may be convenient for some patients, but it is difficult to follow AD patients with memory loss. In addition, donepezil also showed the gastrointestinal side effects such as diarrhea, nausea, anorexia and muscle convulsion etc. Donepezil therefore requires the use of sustained-release formulations administered by routes other than oral administration [6]. One solution to this problem is to apply long-acting injectable formulations.

Long-acting injectable formulations (LAIFs) in recent decades have received considerable attention. The most significant advantage of LAIF is the adjustable controlled and continuous release of the drug over a period of up to several months within the intended therapeutic range of the concentration of the drug in the blood [7, 8, 9]. In addition, LAIFs can minimize systemic side effects caused by fluctuating drug concentrations in the blood by repeated administration of the typical drug regimen and deliver the drug locally for site-specific action [10, 11]. These properties also improve patient compliance by reducing the number of applications. Although the pre-shaped parenteral depot system also has these advantages, the application of LAIFs is more patient friendly as it is less invasive and less painful compared to the depot system [12].

Therefore, In order to apply these long-acting injection formulations to donepezil, we selected an organogel-based fluidal lipid matrix (FLM) formulation. FLM has a simple preparation process, can control the release rate of drugs, and also has a good

encapsulation rate of hydrophobic drugs like donepezil. FLM with these advantages can serve as a long-acting injection method. These benefits may be demonstrated by the drug release mechanism of the FLM (Fig. 1).

In addition, solid lipid nanoparticle (SLN) formulation was applied to the FLM formulation for the purpose of additional sustained release of donepezil. SLN is a lipid based drug delivery system that has no biotoxicity, allows controlled drug release and increases drug stability [13].

In this study, FLMs were developed with a combination of oils, surfactant and organic solvent. The composition ratios of FLM were determined by in vitro release test, viscosity test and syreangeability test. And we evaluated the internal structure of the final formulation of FLM to predict whether the formulation could maintain the gel form in the body environment. In addition, the final composition ratio of SLN was determined based on the stability, particle diameter, zeta potential, PDI, and values according to the ratio of solid lipid and surfactant. Release tests of the final formulations were conducted to evaluate their suitability for long-term release formulations of donepezil.

3. Materials and methods

3.1. Materials

Donepezil HCl and donepezil free base were purchased from Sigma-Aldrich (Oakville, Canada). Span 80 (Sorbitan monooleate) and tween 80 (Polysorbate 80) were purchased from Daejung (Gyeonggi-do, South Korea). Castor oil, cottonseed oil, benzyl alcohol and ethanol were purchased from Sigma-Aldrich (Oakville, Canada). Molecularporous membrane tubing was purchased from Spectrum Laboratories (Rancho Dominguez, USA). Trilaurin (Glyceryl tridodecanoate) and trimyristin (Glyceryl tritetradecanoate) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Kolliphor TPGS (Vitamin E Polyethylene Glycol Succinate) and Solutol HS 15 (Polyoxyl 15 hydroxystearate) were obtained from BASF (Ludwigshafen, Germany). 1% Methylene Blue solution was purchased from Daejung (Gyeonggi-do, South Korea). 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. Preparation

3.2.1. Preparation of donepezil free base (DP)

Donepezil HCl (DP-HCl) 2 g was dissolved completely in 50 ml distilled water, in which 4% NaOH was dropwise added under agitation. After the pH of the solution reached about 11, the synthetic was extracted twice with 50 ml of ethyl acetate, and a sufficient quantity of anhydrous magnesium sulfate was added to the separated organic phase and was shaken for 1 min to remove the residual water. With the evaporation of ethyl acetate at 54 ℃ water bath, the product was collected. And then Vacuum dry for 2 days.

3.2.2. Preparation of donepezil-loaded FLMs

Castor oil, cottonseed oil, span 80 and organic solvent (ethanol or benzyl alcohol) were mixed over-night in an appropriate ratio to prepare FLM using hot plate with magnetic stirring (37 ℃, 200 rpm). After preparation of FLM, donepezil was dissolved

in FLM at a concentration of 50 mg/ml, and after 90 min in water bath sonication, the mixture was stirred for 6 hr (25 ℃, 200 rpm). Propylene glycol was used instead of FLM as a control.

3.2.3. Preparation of donepezil-loaded SLNs

DP loaded SLNs were prepared by hot-melting sonication method with different composition ratios. Lipid (trilaurin or trimyristin), TPGS and solutol were mixed and heated at 65 ℃ for 20 min in a water bath. And then DP was added and mixed 1 hr. Purified water, heated to the same degree, was added to the mixture and mixed at 65 ℃. Homogenization was performed using a probe sonicator (VCX-500, Sonics & Materials, Inc., USA) for 10 min at 65 ℃. The obtained SLNs dispersion was filtered through 0.8 μm pore size syringe filter and cooled down for 12 hr in three ways: room temperature, -4 ℃ and, -20 ℃. Blank SLNs were prepared in a same process without DP.

3.2.4. Freeze drying

As a cryoprotectant, sucrose and HP-β-CD were selected. The cryoprotectant was mixed in two ways. The first way is to add cryoprotectant in the SLN preparation step. The second method is the SLNs dispersions were diluted (9:1) with cryoprotectant solutions and frozen at -20 ℃ overnight. Both methods contained a cryoprotectant of 5% of the final mass. The freeze-drying process was carried out at – 80 ℃ at a pressure of 50 mTorr during 24 hr.

3.2.5. Preparation of donepezil-loaded SLN-FLM

In the prepared FLM formulation, lyophilized DP-SLN powder is added in an amount corresponding to 50 mg/ml and dispersed using hot plate with magnetic stirring for 12 hr (37 ℃, 200 rpm).

3.3. Characterizations

3.3.1. Differential scanning calorimetry (DSC)

Thermal analysis of DP-HCl, donepezil free base, desalted DP-HCl, DP loaded and blank form of SLNs were carried out with Universal V4.5A Instruments.

3.3.2. Viscosity test

The viscosity of the prepared FLM formulations was measured using a viscometer (DV-I, Brookfield, USA). Sufficient amounts (about 7 ml) of samples were measured at 25 ℃, 10 rpm (Cone Spindle CPA-42Z, Brookfield, USA). Samples were recorded after 10 min to stabilize temperature and rotating state of the samples in the chamber.

3.3.3. Quantitative analysis

For HPLC (Azura, Germany) analysis, a reversed-phase Phenomenex, Luna C-18 column (250 x 4.6 mm, Merck, India) was used. The mobile phase was consisting of phosphate buffer: MeOH (55:45, v/v%). One liter of phosphate buffer was composed of 1.361g potassium phosphate monobasic and adjusted to pH 3.0 with phosphoric acid. The injection volume was 20 µl. The flow rate was adjusted to 1.0 ml/min (Azura P 6.1L HPLC pump) and the wavelength was set to 268 nm (Azura DAD 2.1L UV/VIS detector). Samples were properly diluted in the mobile phase prior to analysis. Diluted samples were injected through an autosampler (Azura AS 6.1L sampler). The chromatograms were analyzed using the ClarityChrom® software (version 6.1.0.130, Knauer, Germany).

3.3.4. Optical microscopy

Optical microscope (DS-12, Nikon, Japan) was used to investigate the structure of the FLM. The samples for microscopy were prepared by dissolving a water-soluble dye (methylene blue; 0.1% w/w) in PBS. First, 0.5 ml of FLM is dropped onto 10 ml of PBS. After 24 hr, 20 µl of FLM in the upper layer of PBS was dropped and spread on the glass slide. And, the cover glass was placed on the glass slide to prevent air bubbles forming.

As a control photographs, FLM without benzyl alcohol and FLM not exposed to PBS were taken.

3.3.5. Polarized microscopy

A polarized optical microscope (Eclipse LV100, Nikon, Japan) equipped with a heating system (TMS 94, Linkam, UK) was used to observe the anisotropic texture. FLMs were prepared between the slide glass and the cover glass and were examined using a polarized optical microscope (POM) at 25 ℃. As a control photographs, FLM without benzyl alcohol and FLM not exposed to PBS were taken.

3.3.6. Particle diameter, polydispersity index, zeta potential

The mean particle diameter, polydispersity index (PDI) and zeta potential of SLNs were measured by Zeta potential & Particle size Analyzer ELSZ-2000 series (Otsuka Electronics Co., Ltd, Japan). The dispersions of the SLN were diluted with purified water in order to achieve a concentration to be measured. All analysis was carried out at room temperature.

3.3.7. Encapsulation efficiency (EE), loading capacity (LC)

To calculate EE and LC, concentrations of DP encapsulated in SLNs were analyzed. In brief, 100 μl of DP-SLNs was mixed with 900 μl methanol and sonicated using bath sonicator for 30 min. The obtained solution was centrifuged at 12,000 g for 15 min at 4℃. DP in the supernatant was analyzed using high performance liquid chromatographic system (Azura, Germany).

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

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

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

3.4. In vitro release study

3.4.1. In vitro release test

The release medium was prepared by adding 0.02% (w/v) sodium azide and 2% (w/v) tween 80 to PBS. The formulations of 1 ml and the release medium of 2 ml were transferred into dialysis tubes (Standard RC Tubing, MWCO: 12-14 Kd, Spectrum Labs, USA). The bags, sealed with clamps to prevent leakage, were then immersed into 200 ml of the release medium in a dissolution tester (DST-600A, Fine scientific instruments, South Korea) respectively. The entire system was kept at 37 ℃ with continuous stirring at 50 or 100 rpm. One milliliter of the solutions was taken at determined time to measure the drug concentrations in the dialysate with HPLC. Then, 1 ml fresh solution was added after sampling to keep the constant volume of the solution as 200 ml. The release medium was replaced daily to maintain the sink condition of the release medium. The cumulative release of donepezil was calculated as a percentage of the cumulative amount of the drug released from the membrane relative to the amount of the drug in the initial membrane bag. As a control formulation, propylene glycol was used instead of FLM to disperse DP at the same concentration.

4. Results and discussion

4.1. Preparation of DP

In pilot studies, DP-HCl was difficult to dissolve in FLM formulations (no experimental data). So we decided to use a DP form desalted with DP-HCl. Results were evaluated via DSC (Fig. 2). DP-HCl had a melt endotherm near 141.5 ℃ and 226.5 ℃, which demonstrated the melting point (Tm) of DP-HCl. However, Desalted DP-HCl had an endotherm of melting around 94 ℃, which is almost the same position as the peak of DP free base. DSC data showed that DP-HCl was successfully converted to DP.

4.2. Preparation of DP-FLMs

4.2.1. Determination of the organic solvent ratio of FLM

The composition ratio of the FLM formulation selected in the previous studies (Castor oil : cottonseed oil : span 80 : ethanol = 33 : 46: 13: 8) were adjusted to meet the purpose of this study. In addition, the organic solvent of FLM was changed to benzyl alcohol instead of ethanol. Benzyl alcohol has a local anesthetic effect, so it can reduce pain during intramuscular injections as a pain relief agent [14].

First, the viscosity of FLM according to the ratio of ethanol and benzyl alcohol was measured (Fig. 3). It was found that benzyl alcohol had a higher viscosity than that of ethanol in FLMs having an organic solvent ratio of 4-8%. Viscosity is known to affect slow release, so it could be expected that benzyl alcohol would be more suitable for slow release formulations than for ethanol. Therefore, the release tendency according to the proportion of benzyl alcohol was tested (fig. 4A).

We previously predicted that the higher viscosity of benzyl alcohol would result in longer sustained release on release. However, FLMs using benzyl alcohol showed no significant difference in release profile compared to FLMs using ethanol at the same concentration (Fig. 4B). It was considered that the initial viscosity due to the organic solvent does not have a significant effect on the sustained release due to the FLM characteristics in which the gelation occurs after the organic solvent is removed.

4.2.2. Determination of the oils ratio of FLM

In order to determine the ratio of oils, 5 types of FLM formulations were prepared in which 8% benzyl alcohol determined in the release test according to the ratio of the organic solvent was fixed and the ratio of the oils was changed (Table 1).

First, the viscosity and syringeability of the formulations were measured (Table 2). As the ratio of castor oil increased, the viscosity of FLM increased. All formulations measuring syringeability were able to pass 18 and 20 gauge needles, while 23 gauge needles could pass through FLM #3 with a viscosity of 151.5 cP.

In vitro release tests of FLM formulations were performed to observe the release tendency of FLM formulations according to oils ratio (Fig. 5). As a result of the release test, all FLM formulations showed release results corresponding to 68.4 ~ 56.8% compared to propylene glycol as a control at 7 days. In particular, FLM #3 showed 58.2% release over 7 days, releasing DP the slowest of the FLM formulations.

Based on the above results, the final FLM composition was selected as the FLM #3 formulation having the best sustained release characteristics and capable of passing a 23 gauge needle.

4.3. Evaluation of DP-FLMs properties

4.3.1. Changes in FLMs after PBS exposure

Optical microscopy and polarized microscopy were performed to evaluate the internal structure of selected final FLM formulations (Fig. 6). FLM formulations undergo internal structural changes as organic solvents escape upon exposure to the body environment. After 24 hr exposure of FLM to PBS, optical microscope observation revealed that 5~10 μm of vesicles were formed. The inside and outside of the vesicles were the same color, and small vesicles appeared to aggregate to form larger vesicles. The same trends were also observed in the photographs observed with a polarized microscope. These results suggest that gel formation consists of internal interconnections as the organic solvent escapes over time.

Viscosity changes in FLM support this (Fig. 7). The viscosity of the FLM increased by 38% from the initial viscosity of 151.83 cP to 209.73 cP after 24 hr exposure to

PBS. These results suggest that FLM has been converted to gel due to the escape of benzyl alcohol for 24 hr when exposed to the body environment. So we can expect that when FLM is injected into the muscle, it can come into contact with body fluids and be a high viscosity gel after the benzyl alcohol has escaped.

4.3.2. *In vitro* release profile of DP-FLM

To verify the sustained release characteristics of DP-FLM, a release test was conducted for 4 weeks at 37 ℃ and 50 rpm (Fig. 8). DP-FLMs released half of the loaded DP by week 1, and the release appears saturated at 3 weeks. DP-propylene glycol used as a control released 90.69% of the donepezil loaded, while DP-FLM released 66.99%. As a result, DP-FLM, the first generation formulation using FLM, was identified as a formulation capable of releasing DP for 3 weeks.

4.4. Preparation of DP-SLNs

4.4.1. Determination of the composition ratio of SLNs

The method of encapsulating donepezil in SLN was chosen to produce a formulation that releases DP for longer periods than the first-generation formulation, DP-FLM. To determine the optimal SLN composition, 6 types of SLN formulations were prepared and characterized (Table 3).

First, particle size, PDI, and zeta potential of SLN #1 and SLN #2 loaded with DP at 25 mg/ml were measured (Table 4). When cooled at RT, the SLNs were immediately gelled and could not be evaluated. SLNs cooled at 4 ℃ and -20 ℃ were able to measure particle size, PDI and zeta potential. However, even the SLNs dispersion gelled after one day in refrigerated conditions, showing stability problems. SLN #1 and SLN #2 had a particle size of 63.44 nm to 75.63 nm and showed zeta potential values below -9.56. In the case of SLNs cooled at -20 ℃, the PDI values were over 0.3. From the LC values, it can be assumed that the lipid ratio of SLN #1 and SLN #2 was low compared to the levels in DP content, which leads to stability problems (Fig. 9). Both SLN #1 and SLN #2 showed LC values greater than 25%.

Therefore, the particle size, PDI and zeta potential were evaluated in the blank condition to find the optimum lipid ratio among the SLN #3 ~ SLN #6 formulations with increased lipid ratio (Table 5). All of the SLNs exhibited sizes below 100 nm when cooled at room temperature and 4 ℃, but over 100 nm when cooled at -20 ℃. In addition, the stability of the SLN #3, 4 with a lipid ratio of 15% was maintained without a precipitate. However, in the case of SLN #5, 6 with a lipid ratio of 20%, precipitation occurred and gelation occurred one day after recrystallization. So we decided to evaluate the other factors by fixing the lipid ratio to 15% (Table 6). The particle size, PDI and zeta potential of SLNs loading DP at 25 mg/ml were similar to the experimental results in the blank SLNs and the properties of SLN remained stable. Based on the results of EE and LC, we decided to increase the DP content, as it could increase the DP content (Fig. 10A, B). The result was a problem with the properties under other conditions, but the SLNs dispersion showed stable appearance when the SLN #3 was cooled to 4 ℃. SLN #3 cooled at 4 ℃ had the lowest PDI and the highest zeta potential of SLN #3 (Table 6). These zeta potential values were not as large as desired but were adequate to show stability [15]. The loading capacity of SLN #3 cooled at 4 ℃ was calculated to be 15.76% (Fig. 10C, D). Thus, the final SLN composition was determined to be SLN #3 (TL 15%, TPGS 5.625%, solutol 5.625%, DW 73.75%) and the cooling condition was determined to be 4 ℃.

4.4.2. Optimization of freeze drying

Lyophilization of SLN proceeded with the purpose of increasing the stability of SLN and easy loading on FLM. The SLN using trilaurin as the lipid among the selected SLN compositions showed no significant change from 5% to 25% when the particle size was changed according to the ratio of cryoprotectant in the previous studies. Therefore, the ratio of cryoprotectant was fixed at 5% to reduce the final volume. We evaluated the changes of SLNs according to the type of cryoprotectant (sucrose and HP-β-CD) and preparation method (Fig. 11). The particle size, zeta potential, EE and LC were evaluated. DP-SLN prepared by applying HP-β-CD in the

SLN preparation step showed the most stable results. Therefore, this was selected as the final manufacturing method of DP-SLN.

4.4.3. DSC analysis of SLNs

DSC analysis of the selected final DP-SLN formulations was performed (Fig. 12). In the case of DP-SLN, there was no DP peak. This suggests that DP was well encapsulated inside SLN. DSC peaks were similar between SLNs with and without the cryoprotectant HP-b-CD. Therefore, cryoprotectant did not affect the formation of SLNs.

4.5. *In vitro* release of DP-SLN-FLM

A 4-week release test of the DP-SLN-FLM formulation with the DP-SLN dispersed in the FLM was performed (Fig. 13). DP-SLN was released rapidly and reached saturation on day 5. DP-SLN-FLM, like the first-generation formulation DP-FLM, reached saturation at the third week. However, the DP-SLN-FLM produced 57.91% of the emission, slightly lower than the DP-FLM's 66.99%. It can be inferred that this release tendency appeared through the process of DP eluted from SLN to FLM and then released from FLM to release medium. After 4 weeks of observation, DP-SLN-FLM remained DP-SLN powder inside the FLM. If lipase is activated, the degradation of lipids is expected to release the remaining DP.

5. Summary

We have successfully developed the first-generation formulation (DP-FLM) and second-generation formulation (DP-SLN-FLM) based on the manufacture of FLM through a simple manufacturing process. Both formulations showed in vitro release test release of donepezil for 3 weeks. In particular, the second-generation formulation DP-SLN-FLM showed longer sustained release characteristics than the first-generation formulation DP-SLN. As a result, our FLM-based long acting injection formulation has shown us as a platform technology that can be applied not only to donepezil but also to other CNS drugs and biopharmaceuticals.

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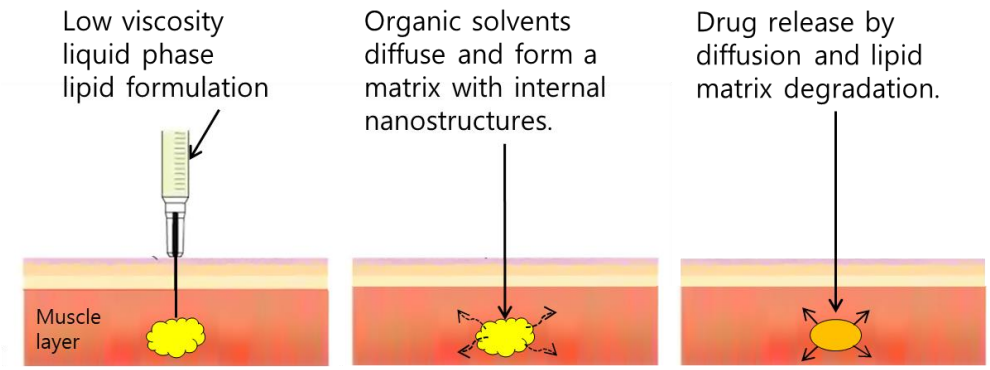


Fig. 1. Schematic illustration of the FLM.

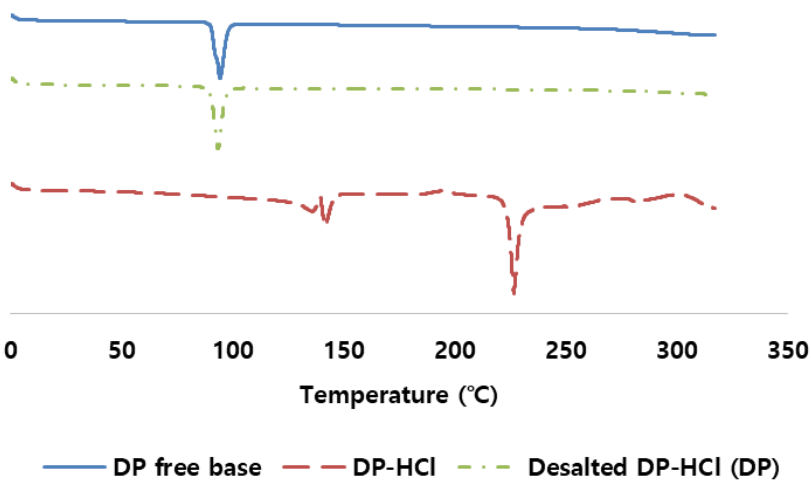


Fig. 2. DSC analysis of DPs

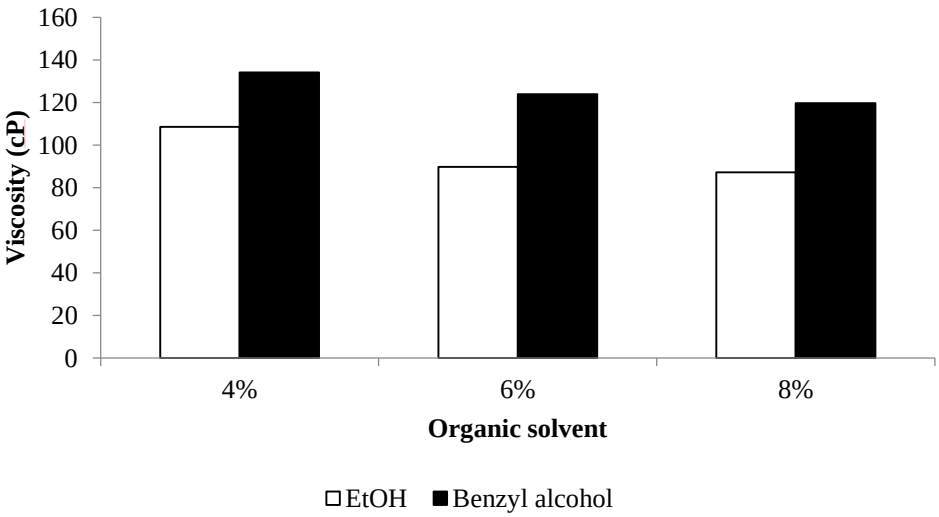
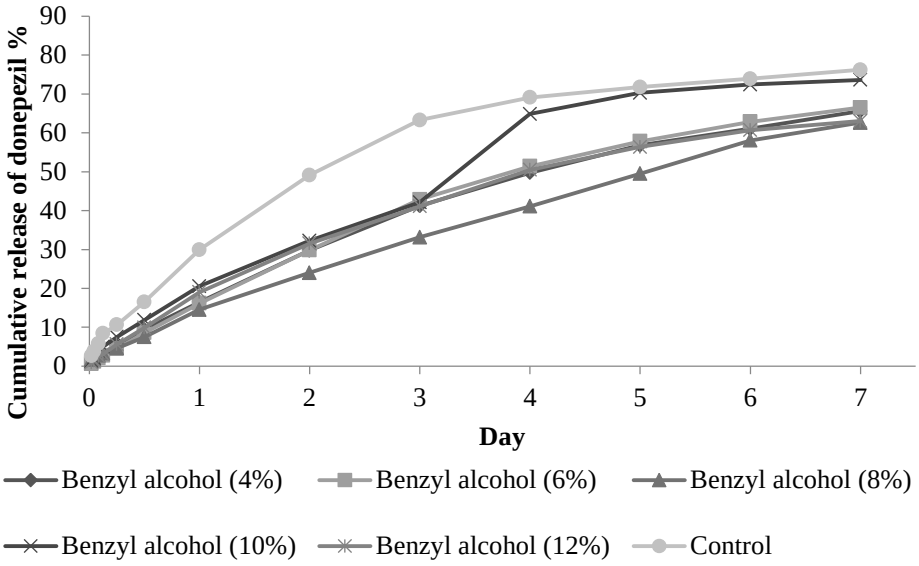


Fig. 3. Viscosity of FLMs from different ratio of organic solvent (25 ℃, 10 rpm). As the proportion of organic solvent increased, the viscosity of FLM decreased and the viscosity of FLM using Benzyl alcohol was higher than that of ethanol.

(A)



(B)

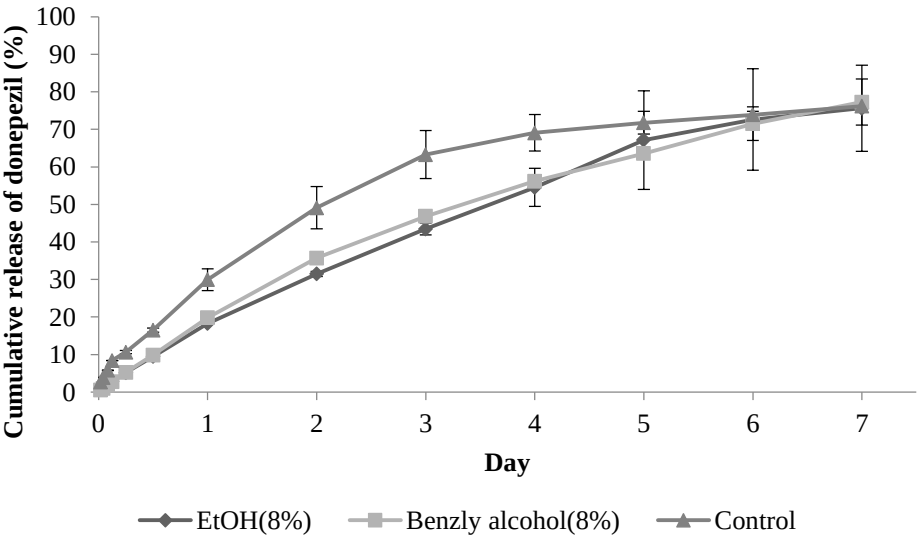


Fig. 4. One-week release profile of donepezil for organic solvent determination (37 ℃, 100 rpm). (A) Release according to benzyl alcohol ratio; (B) Comparative release profile of donepezil from benzyl alcohol and ethanol. Results are expressed as mean ± standard deviation (SD) n = 3.

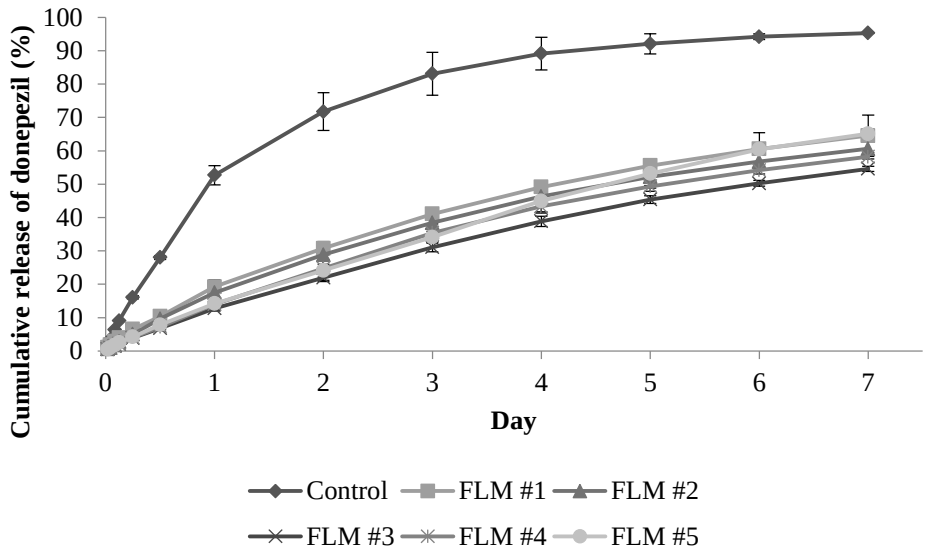


Fig. 5. Release profile of donepezil from different ratio of oils (37 ℃, 100 rpm). Results are expressed as mean ± standard deviation (SD) n = 3.

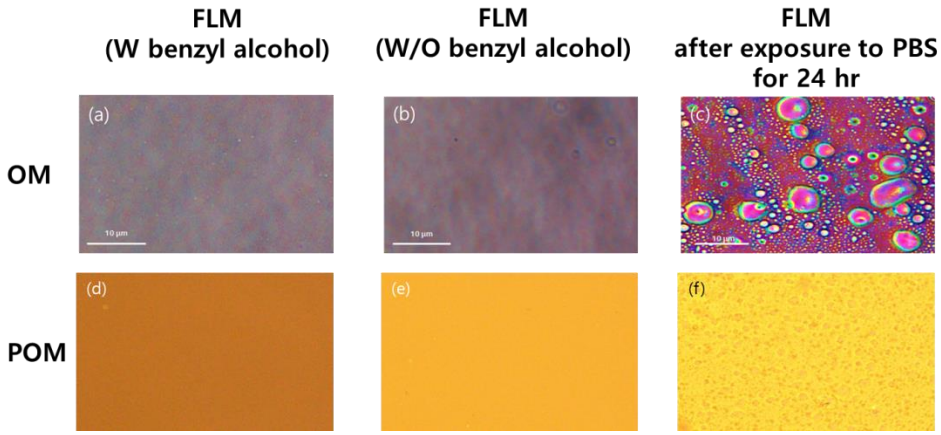


Fig. 6. Internal structure change after PBS exposure of FLM.

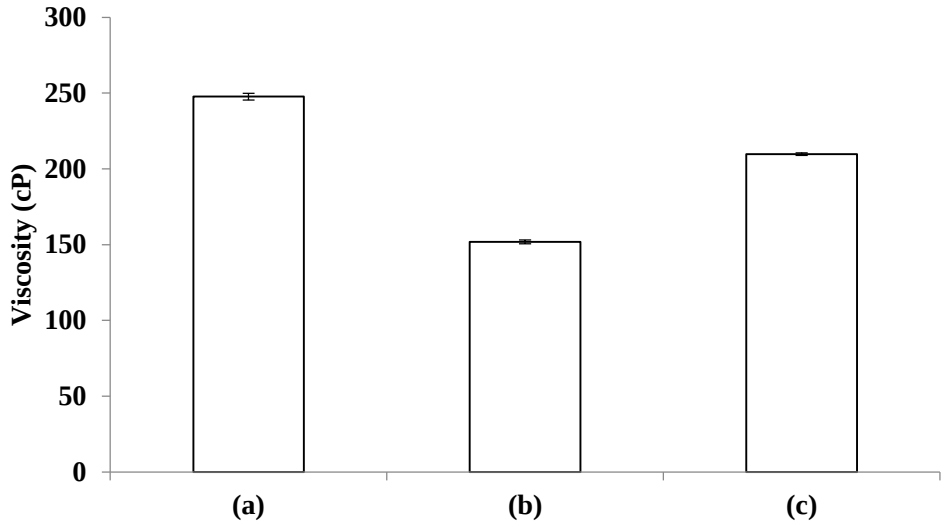


Fig. 7. Viscosity change of FLMs. (a) The initial viscosity of FLM without organic solvent, (b) The initial viscosity of FLM, (c) The viscosity of FLM after exposure to PBS for 24 hr. The viscosity of FLM was increased after the organic solvent had spread into PBS. Results are expressed as mean ± standard deviation (SD) n = 3.

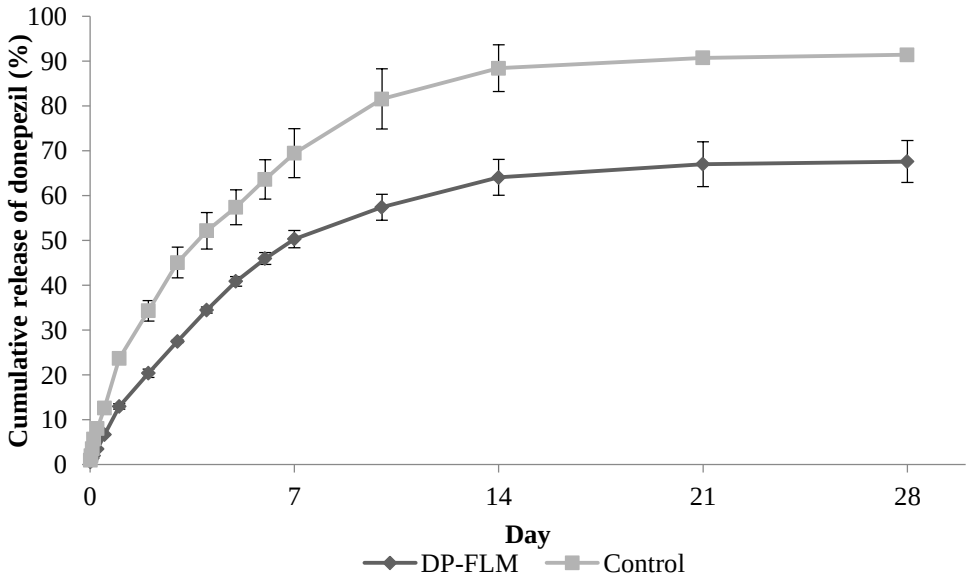
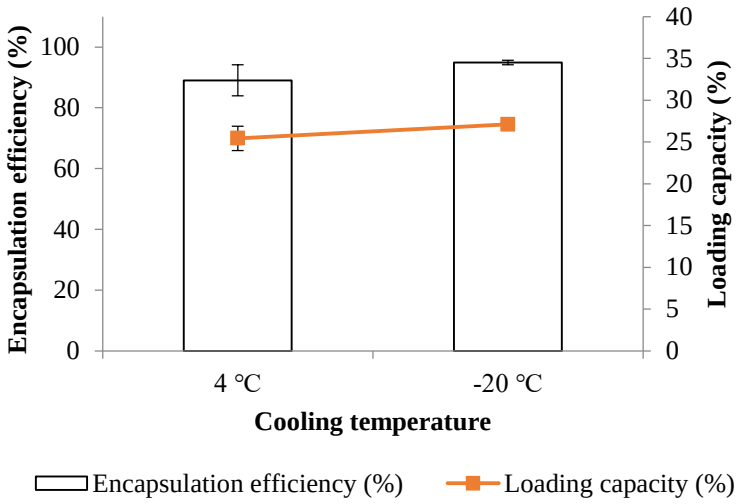


Fig. 8. In vitro release profile of DP-FLM for 4 weeks (37 ℃, 50 rpm). Results are expressed as mean ± standard deviation (SD) n = 3.

(A)



(B)

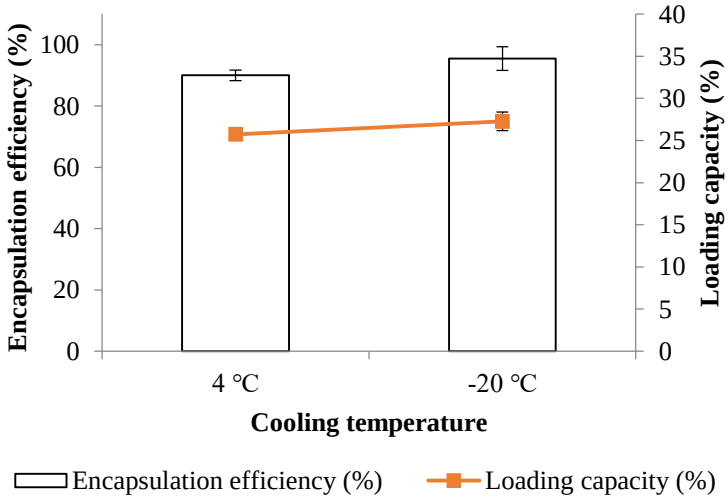
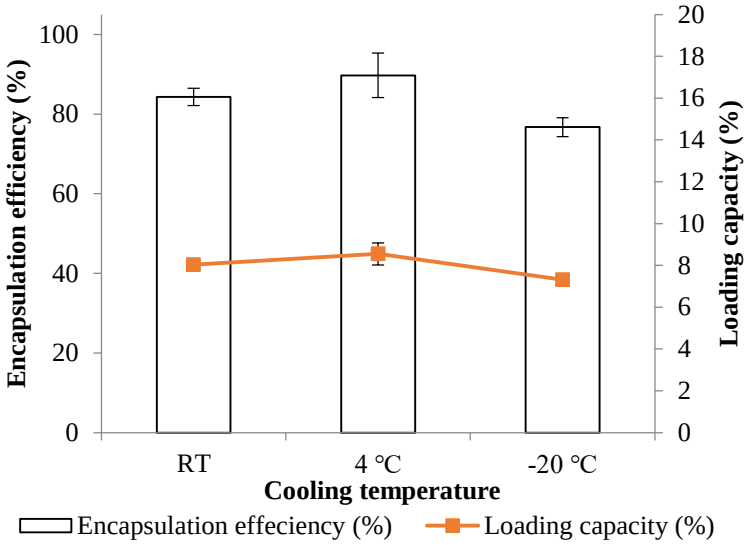
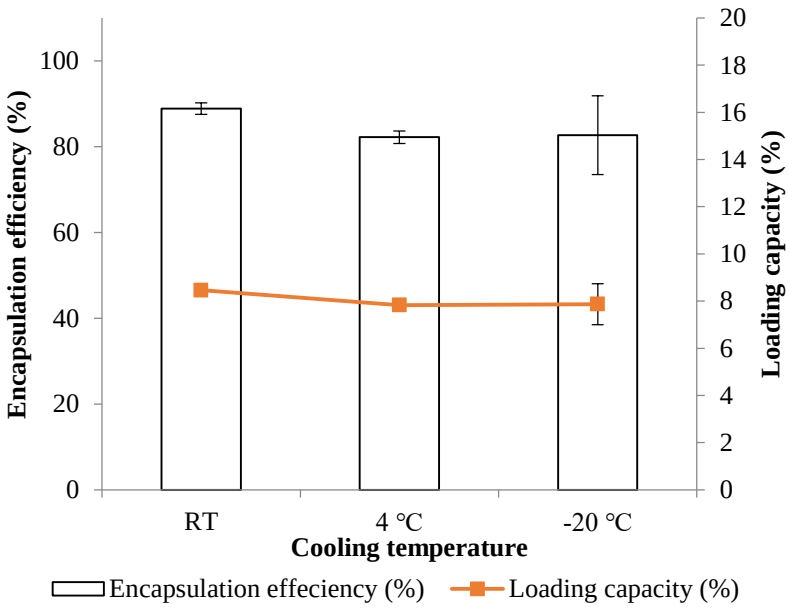


Fig. 9. Encapsulation efficiency (EE) and loading capacity of DP 25 mg/ml SLNs.
(A) SLN #1, (B) SLN #2. Results are expressed as mean ± standard deviation (SD) n = 3.

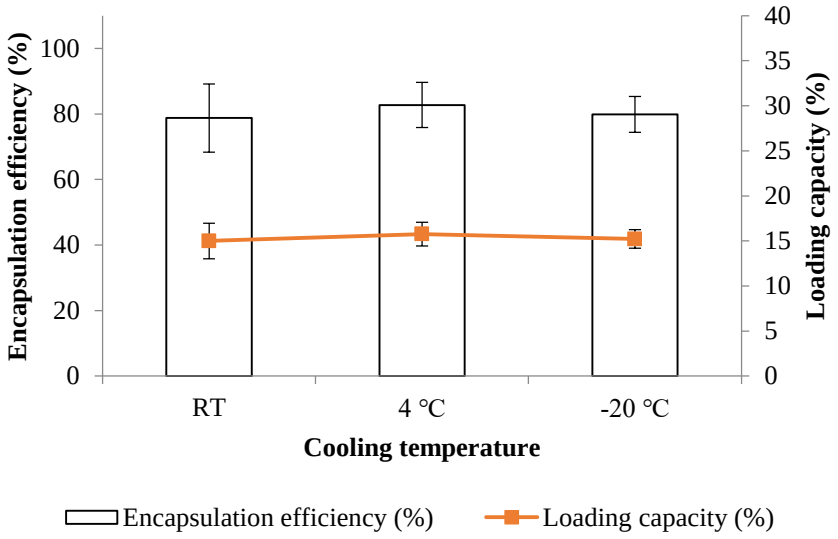
(A)



(B)



(C)



(D)

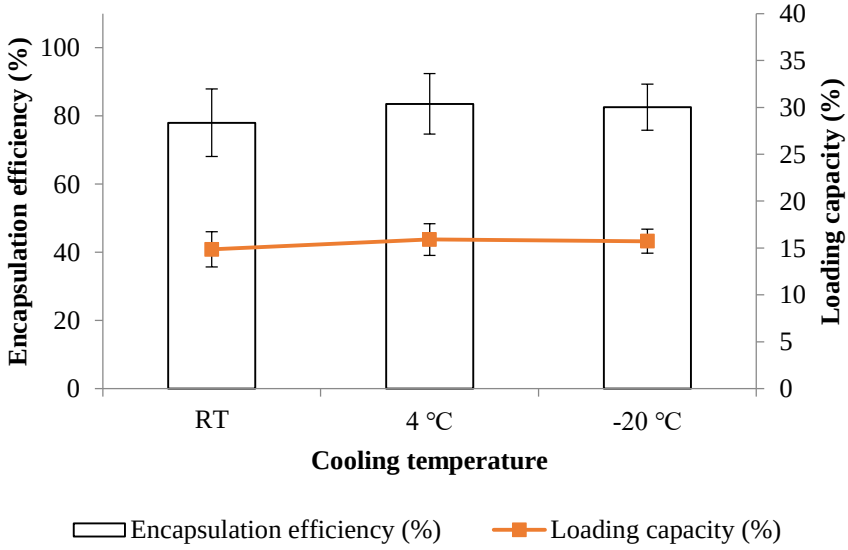
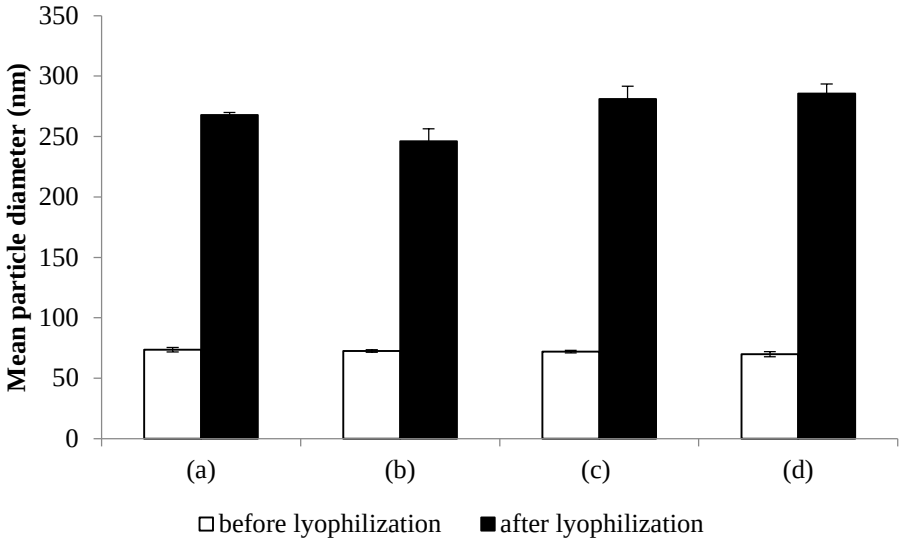
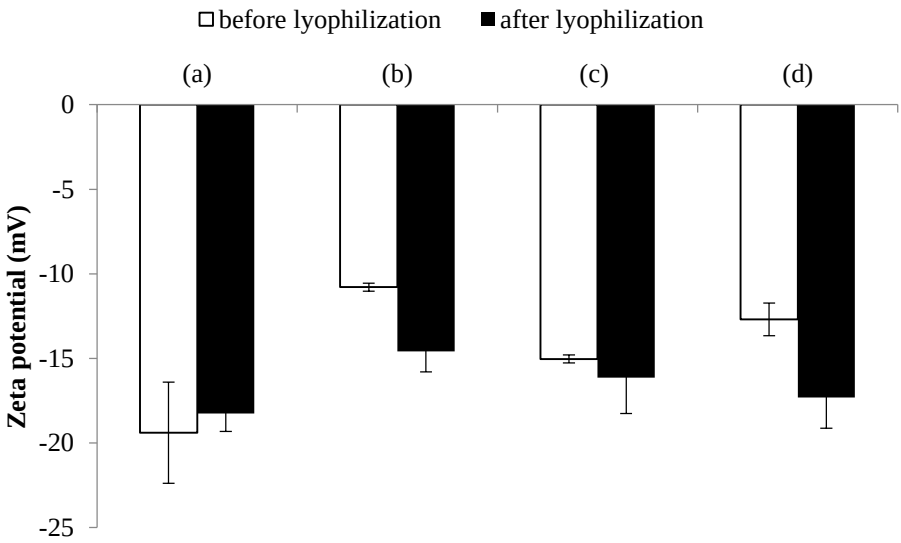


Fig. 10. Encapsulation efficiency (EE) and loading capacity (LC) in SLN #3 and #4 formulations at different DP concentrations. (A) SLN #3 DP 25 mg/ml , (B) SLN #4 DP 25 mg/ml, (C) SLN #3 DP 50 mg/ml, (D) SLN #4 DP 50 mg/ml. Results are expressed as mean ± standard deviation (SD) n = 3.

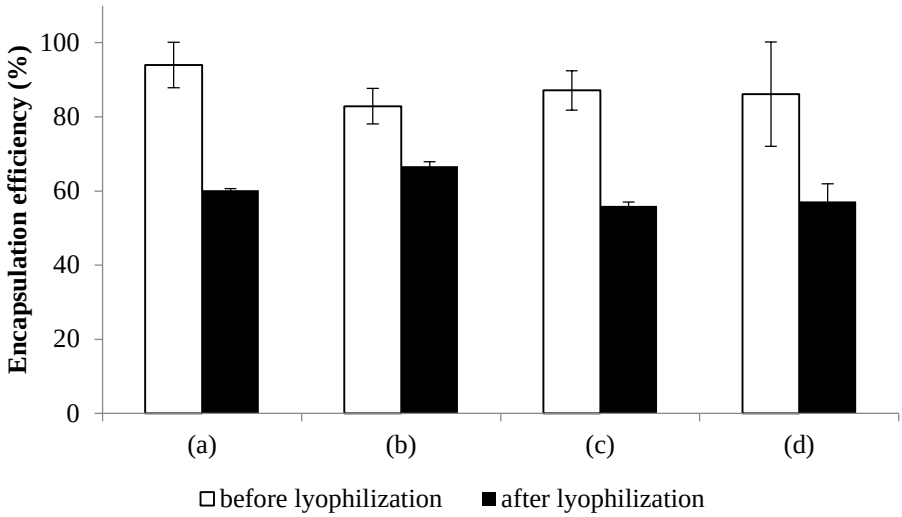
(A)



(B)



(C)



(D)

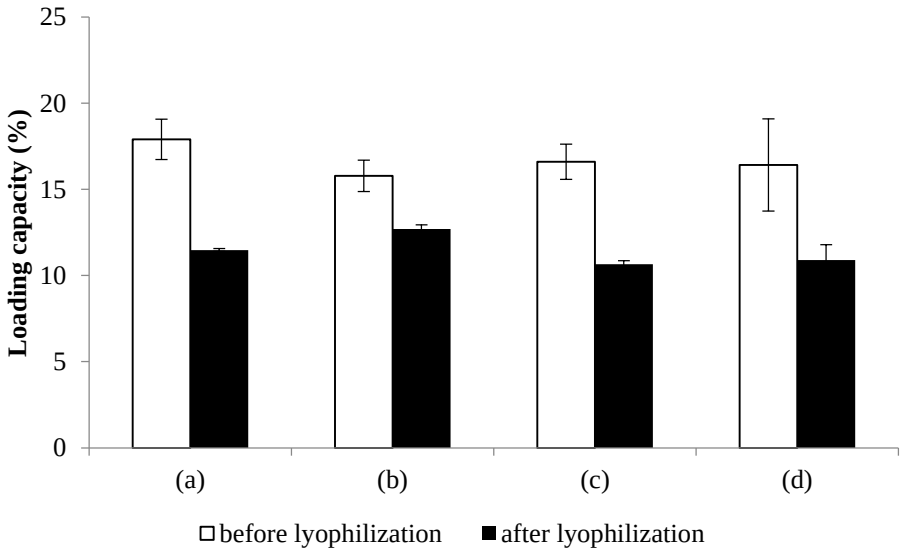


Fig. 11. Effect of cryoprotectant on (A) particle size; (B) zeta potential; (C) EE and (D) LC value of SLNs before and after freeze drying. (a) 5% HP-b-CD 9:1 diluted; (b) 5% HP-b-CD Non diluted; (c) 5% sucrose 9:1 diluted; (d) 5% sucrose non diluted. Results are expressed as mean ± standard deviation (SD) n = 3.

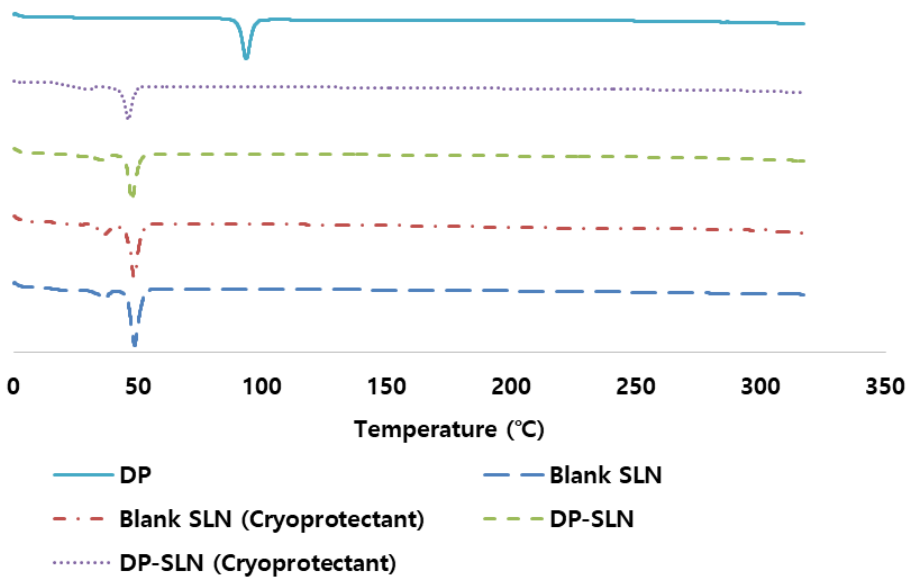


Fig. 12. DSC analysis of SLNs

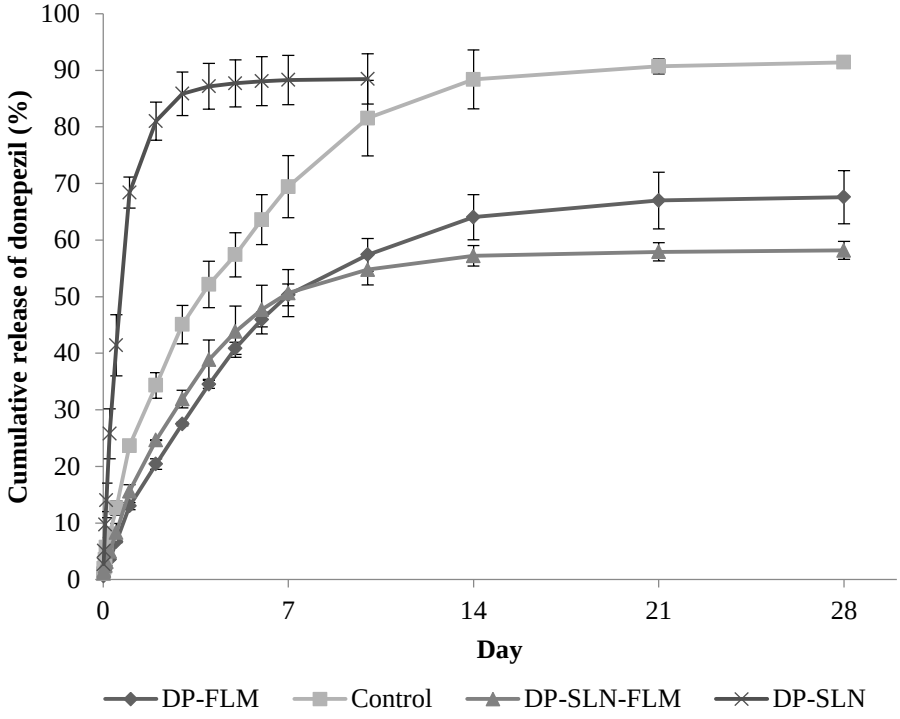


Fig. 13. 4 weeks donepezil release profile of DP-SLN-FLM formulation. DP-SLN released more than 80% of Donepezil in just two days. And the other formulations were almost complete at 3 weeks (37 ℃, 50 rpm). Results are expressed as mean ± standard deviation (SD) n = 3.

Table 1. Composition ratio of FLMs

Formulation	Castor oil (%)	Cottonseed oil (%)	Span 80 (%)	Benzyl alcohol (%)
FLM #1	35	44	13	8
FLM #2	40	39	13	8
FLM #3	45	34	13	8
FLM #4	50	29	13	8
FLM #5	55	24	13	8

Table 2. Viscosity and syringeability of the composition ratio of FLMs

Formulation	Viscosity (cP)	Syringeability		
		18G	20G	23G
FLM #1	120.3	O	O	O
FLM #2	132	O	O	O
FLM #3	151.5	O	O	O
FLM #4	173.1	O	O	X
FLM #5	193.5	O	O	X

Table 3. Composition ratio of SLNs.

Formulation	Lipid		Surfactant		Water (%)
	TL (%)	TM (%)	Solutol (%)	TPGS (%)	
SLN #1	10	-	3.75	3.75	82.5
SLN #2	-	10	3.75	3.75	82.5
SLN #3	15	-	5.625	5.625	73.75
SLN #4	-	15	5.625	5.625	73.75
SLN #5	20	-	7.5	7.5	65
SLN #6	-	20	7.5	7.5	65

Table 4. Mean particle diameter, zeta potential and PDI of DP 25 mg/ml SLNs.

Formulation	Cooling temperature	Mean particle diameter (nm)	PDI	Zeta potential (mV)
SLN #1	4 ℃	72.67±3.31	0.208±0.02	-13.14±1.59
	-20 ℃	75.63±10.43	0.302±0.08	-9.56±1.04
SLN #2	4 ℃	65.07±2.67	0.193±0.02	-12.22±3.34
	-20 ℃	63.44±2.58	0.351±0.12	-15.17±2.68

Results are expressed as mean ± standard deviation (SD) n = 3.

Table 5. Mean particle diameter, zeta potential and PDI of Blank SLNs.

Formulation	Cooling temperature	Mean particle diameter (nm)	PDI	Zeta potential (mV)
SLN #3	RT	78.78±1.75	0.216±0.013	-6.48±2.65
	4 ℃	82.32±3.75	0.217±0.012	-9.03±1.12
	-20 ℃	114.8±3.44	0.157±0.023	-8.38±1.93
SLN #4	RT	80.29±2.31	0.196±0.018	-7.99±0.88
	4 ℃	80.42±1.09	0.225±0.005	-7.67±0.41
	-20 ℃	108.63±1.46	0.127±0.016	-6.30±2.45
SLN #5	RT	72.00±4.07	0.208±0.019	-4.91±1.69
	4 ℃	76.46±2.97	0.220±0.029	-7.59±3.35
	-20 ℃	102.06±1.56	0.148±0.014	-8.33±2.32
SLN #6	RT	80.11±2.38	0.173±0.013	-7.00±3.04
	4 ℃	76.14±2.04	0.213±0.012	-7.89±1.15
	-20 ℃	99.13±5.32	0.125±0.012	-8.52±1.37

Results are expressed as mean ± standard deviation (SD) n = 3.

Table 6. Mean particle diameter, zeta potential and PDI of DP-SLNs.

Formulation	Drug concentration (mg/ml)	Cooling temperature	Mean particle diameter (nm)	PDI	Zeta potential (mV)
SLN #3	25	RT	77.10±2.40	0.216±0.012	-12.86±4.49
		4 ℃	79.32±2.72	0.201±0.008	-14.54±0.86
		-20 ℃	81.02±2.46	0.135±0.008	-10.11±2.63
SLN #4		RT	81.07±3.43	0.207±0.018	-17.63±5.06
		4 ℃	74.88±0.29	0.240±0.010	-10.42±2.31
		-20 ℃	79.20±9.52	0.160±0.036	-13.56±3.85
SLN #3	50	RT	75.04±2.47	0.205±0.015	-4.78±1.13
		4 ℃	75.40±2.43	0.199±0.010	-11.39±0.06
		-20 ℃	76.61±3.24	0.231±0.031	-9.60±2.49
SLN #4		RT	71.88±4.08	0.225±0.026	-14.61±0.82
		4 ℃	64.57±2.17	0.232±0.016	-11.76±1.85
		-20 ℃	67.30±0.33	0.222±0.037	-9.39±0.41

Results are expressed as mean ± standard deviation (SD) n = 3.

ABSTRACT

A Study on development of lipid matrix based long acting injectable formulation of donepezil

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Donepezil is a second-generation cholinesterase inhibitor approved by the FDA in 1996 and is used to improve cognitive function in Alzheimer's patients. Donepezil is currently only available in oral dosage forms and is generally intended to be taken once daily. Due to the symptoms of Alzheimer's patients, this medication is likely to have low compliance. Therefore, in order to improve this problem, the study was carried out to develop long-acting injections that can maintain the efficacy of donepezil for more than a month.

As final formulations, formulation of the first generation (DP-FLM) and formulation of the second generation (DP-SLN-FLM) were developed and evaluated for their characteristics. DP-FLM was prepared by loading the donepezil free base (DP) into the fluidal lipid matrix (FLM), and DP-SLN-FLM was prepared by encapsulating DP into solid lipid nanoparticles (SLN) and loading it into the FLM.

Based on the data of viscosity, syringeability, and release test, the final FLM composition was determined to be castor oil (45%), cottonseed oil (34%), span 80 (13%), and benzyl alcohol (8%). The final composition ratio of SLN was determined as Trilaurin (15%), TPGS (5.625%), Solutol (5.625%), and DW (73.75%) based on stability, particle size, zeta potential, and PDI values.

Evaluation of properties such as measurement of drug encapsulation rate, observation of internal structure by optical and polarized microscope, and measurement of change in

viscosity was carried out for the optimized formulation. In addition, the 4 weeks release profile showed that both formulations release donepezil for 3 weeks or longer. The results indicated that the formulations developed have potential as long-acting injections suitable for controlled release of donepezil.

Keywords: Donepezil, Alzheimer disease, Fluidal lipid matrix, Solid lipid nanoparticle, Long-acting injection