약학석사 학위논문

Formulation of Nanostructured Lipid Carriers(NLCs) of 20(S)-Protopanaxadiol(PPD) by Box-Behnken design

Box-Behnken Design을 이용한

20(S)-Protopanaxaiol(PPD)를 함유하는

NLC의 제형화

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김 민 환

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지도교수 김 대 덕

이 논문을 약학석사학위논문으로 제출함

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ABSTRACT

Formulation of Nanostructured Lipid Carriers(NLCs) of 20(S)-Protopanaxadiol(PPD) by Box-Behnken Design

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20(S)-Protopanaxadiol (PPD) is a deglycosylated metabolite of ginseng saponins like Compound K and Rb1. It was reported that PPD has a better anti-wrinkle effect than the other glycon forms of PPD including 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (GPPD) and ginsenoside Rb1 in a human skin equivalent model and human keratinocytes (HaCaT). Despite its good anti-wrinkle effect in cell models, PPD has a low aqueous solubility because of its aglycon state and large molecular weight. Thus, it is expected be difficult to penetrate the stratum corneum (SC) layer which is the rate limiting step of topical delivery. The purpose of this study was to optimize nanostructured lipid carrier

(NLC) of PPD by Box-Behnken Design and to evaluate the deposition of PPD in Strat-M™, a model human skin. Drug amount (X1), volume of oil (X2) and surfactant amount (X3) were set as independent variables, while particle size (Y1), polydispersity index (PDI) (Y2) and entrapment efficiency (EE) (Y3) were set as dependent factors. After NLC was prepared based on the optimized formulation, its particle size, PDI and EE were actually measured. Difference between the predicted and the observed values was less than 5%, indicating the validity of the optimization. X-ray diffraction (XRD) indicates that PPD encapsulated in NLC formulation is in amorphous state. *In vitro* deposition of PPD after 3 h and 6 h after applying NLC on the Strat-M® artificial membrane was significantly higher than that of PPD suspension and oil solution. Thus, it can be concluded that the formulation of NLC was successfully optimized for the topical delivery of PPD.

Keywords: Box-Behnken design, 20(S)-protopanaxadiol, nanostructured lipid carrier (NLC), Strat-M™, topical delivery

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1. Introduction

Ginsenoside is one of the principal active components among the extracted compounds of Panax ginseng. One type of the ginsenosides is dammarane-type and it can be divided into protopanaxadiol (PPD) or protopanxatriol (PPT) types [1]. Because of its various effects like anti-cancer, anti- fatigue and whitening effect, many types of ginsenosides are widely used in Korea and China as a traditional herb and cosmetics. Nowadays algycon form of the ginsenosides has given attention to reserchers. 20(S)-Protopanaxadiol (PPD) (Fig. 1) is a one of the ginsenosides which is a deglycosylated metabolite of ginseng saponins like compound K and Rb1 from the ginseng. PPD has a lot of pharmacological potentials like anti-depressant, anti-inflammatory and colon cancer [2-4]. Recently a study elucidated that PPD has a better anti-wrinkle effect than other glycon form of PPD including 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (GPPD) and ginsenoside Rb1 in a human skin equivalent model and human keratinocytes (HaCaT). MMP-1 is one of the key factor to attenuate the collagen degradation, so that anti-wrinkle can be represented for preventing photoaging. It shows that PPD has a better suppressing UV-induced MMP-1 expression than GPPD and ginsenoside Rb1 [5]. There are few researchers of PPD in topical delivery. Inspite of its good efficacy on cell model, PPD has a low solubility in water because of the aglycon form and large molecular weight, so that it is hard to penetrate the stratum corneum(SC) which is the rate-limiting step of topical delivery.

Colloidal systems have obtained popularity as a promising topical drug delivery

carrier. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are developed to deliver lipophilic drugs [6]. The SLN were developed by substituting solid lipid for the liquid lipid(oil) which is inside of emulsions. Because of the drug mobility compared to liquid lipid, the replacement of oil with solid lipid is a very attractive to overcome the drawback of traditional formulation like emulsion. And NLC is composed of mixture of solid lipid and liquid lipid to overcome the problems associated with SLNs, such as low drug loading, drug expulsion during storage. Putting oil into the solid lipid makes polymorphic transition, so that it makes higher loading and controlled drug release [7]. Because of NLC having its unique lipid matrix for improving efficacy, lipid colloidal systems are used for many administration routes of the active compounds, like topical application [8]. Advantages of NLC over conventional topical drug delivery systems like liposomes and emulsions are skin occlusion, sustain drug or cosmetic release, increase of skin hydration, drug targeting [9] and potentials of UV blocking effects [10]. Cosmetically active compound should locate in the skin and should not permeation to minimize the systemic effects. For example, prednicarbate which is one of the topical corticosteroid drug retains more in the upper site of skin compared to an emulsion-based formulation [11].

To optimize formulation, there is a traditional approach called one factor at a time (OFAT). This approach is difficult, time consuming and hard to know the interaction effect between the factors. Recently, many statistical experimental designs are used in optimizing a formulation with less experiment and estimating

the relative significance among variables [12]. Statistical experimental design like Response Surface Methodology (RSM) has been revealed that it can be efficient to estimate the relationship between independent and dependent factors in a formulation when interactions among variable are complicated. Several studies have shown that RSM is useful for optimizing formulation in many sorts of drug delivery systems including lipid nanoparticles. There are various types of RSM which is available for statistical optimization: Central Composite Design (CCD), Box-Behnken Design (BBD) and D-optimal designs [13]. In Box-Behnken Design which has a 3-factor experimental design, three levels of the factors are located at the midpoints and the edges of the process space. This design can make less experimental runs and time. For this reason, BBD is considered a cost- and labor-effective approach than traditional optimizing processes of formulation. In this study, one of the RSM design, BBD, is used for optimizing the lipid formulation.

In recent years, there are many interests in finding alternative of animal experiment. Strat-M™ was recently launched and is now commercially available as a skin-mimic artificial membrane. This membrane is composed of three layers of polyester sulfone making similar morphology to human skin. The thickness of each layer increased from the top of the membrane [14]. There are several researches that Strat-M™ have a possibility of alternative membrane for estimating skin permeation so that the membrane can be used as an alternative membrane model for human skin experiments. But there are not much researches of estimating skin deposition test. One article shows that in-vitro deposition of PPD between hairless

mouse and Strat-M™ membrane had been investigated. Thus Strat-M™ has a potential to be an alternative of hairless mouse skin to check the deposition amount of PPD. The purpose of this study was to investigate the potential of applying topical delivery of PPD using NLC formulation. The PPD-loaded NLC formulations were prepared and analyzed with regard to in vitro particle-size distribution, encapsulation efficiency, morphology, and XRD data. Then, the in vitro deposition properties were carried out using Strat-M™ membranes as an alternative of hairless skin.

2. Materials and Methods

2.1. Materials

20(S)-Protopanaxadiol (PPD) (purity>98.0%) was obtained from Xian Plant Bio-Engineering Co.,Ltd. (Shaan xi, China). Labrafac CC, Lauoroglycol CC, were obtained from Gattefosse Co. (Saint Priest, Cedex, France). Capmul MCM EP was obtained from ARITEC co. (Columbus, OH, USA). Miglyol 812 N was gifted from OLEO chemicals (Witten, Germany). Poloxamer 188 was gifted from BASF Co. (Ludwigshafen, Germany). Tween 20, Tween 80, isopropyl myristate, limonene, polyethylene glycol 400 (PEG400) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Strat-M™ was purchased from Merck Millipore (Billerica, MA, U.S.A.). HPLC grade acetonitrile, water and methanol were purchased from Thermo Fisher Scientific Co. (Pittsburgh, PA, USA).

2.2. HPLC analysis

To measure entrapment efficiency of PPD, high-performance liquid chromatography (HPLC)-UV system was used. Samples were injected into a reverse-phase C18 column (Kinetex C18; 250×4.6 mm2, 5μm; Phenomenex). HPLC-UV system was equipped with a pump (Waters 1515; Waters Company,

Milford, Massachusetts), a UV/Vis detector (Waters 2487), and an automatic injector (Waters 717 plus). The mobile phase consisted of acetonitrile and DDW (5:95, v/v). The flow rate was 1.0 mL/min, and the detection wavelength and the injection volume were 210nm and 20 μL, respectively. The lower limit of quantitation (LLOQ) of PPD was 1 μg/mL.

The deposition amount of 20S-PPD in Strat-M™ was determined by LC-MS/MS analysis using an Agilent LC-MS/MS system (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent Technologies 1260 Infinity HPLC system and Agilent Technologies 6430 Triple Quad LC-MS system. The analysis was achieved using a Hypersil BDS C18 column (50 mm 4.6 mm, 5mm; Thermo Fisher Scientific Co). The flow rate was 0.37 mL/min and the mobile phase consisted of 93% acetonitrile and 7% water containing 0.2 formic acid (v/v). The volume of injection was 10 μl. PPD was determined in the multiple reaction-monitoring mode with positive electrospray ionization. The ESI parameters were set as follows: gas flow- 9 L/min, gas temperature - 120℃, capillary voltage - 6,000 V, nebulizer pressure - 25 psi. The MRM was used to monitor the m/z 461.4 → m/z 425.5 for PPD analysis. The collision energy, fragment voltage, cell accelerator voltage and retention time of PPD were 4 eV, 111 V, and 1 V, 1.09 min, respectively. All LC-MS/MS data were carried out using the MassHunter Workstation Software Quantitative Analysis (vB.05.00; Agilent Technologies). Stock solution was made with methanol and the standard curve samples were diluted with methanol from the stock solution. The response of the detector was linear in the concentration range and the mean correlation coefficient (r^2) for the calibration curve was more than

0.999. The lower limit of quantitation (LLOQ) of PPD was 2 ng/mL.

2.3. Selecting liquid lipid and surfactant

The solubility of PPD in various oil and surfactant was analyzed by putting an excessive amount of PPD into a 2.0 ml tube containing 1mL of each vehicle. The vortex shaker (Vortex-Genie 2; Scientific Industries, Inc., Bohemia, NY, USA) was carried out to make mixtures approach an equilibrium state at 50 rpm at 25℃ for 72 h. After shaking the samples, they were centrifuged at 16,000g for 5 min, and the supernatant was filtered with 0.20-mm syringe filter to get rid of excess amount of PPD. Finally, the PPD was analyzed by HPLC-UV after appropriate dilution with methanol.

2.4. Preparation of APPD-loaded NLCs

PPD loaded NLC were prepared using previously described method with slight modification [15]. Briefly, solid lipid cetyl palmitate was melted above the 10℃ higher than the melting point of solid lipid. Liquid lipid and PPD was dissolved in little amount of ethanol and put into melted phase. Distilled water containing mixtures of Tween 20 and poloxamer was added into lipid phase and ultrasonicated (sonic vibra cell) with amplitude 26% and pulse on 2 s; pulse off 3 s. and then cooled in room temperature.

2.5. Experimental design

Box-Behnken design was used to statistically optimize the formulation and evaluate the main effects, interaction and quadratic effects of the formulation. To optimize formulation, a Box-Behnken design of experiment (DOE) was created using Minitab® 17 software (Minitab, Inc.) by varying the independent variables of the design. The Box-Behnken design was selected because it requires minimum number of experiments than a CCD design in cases of three independent variables [16]. This design generate the set of points lying at the midpoint of each edge of a multidimensional cube and central point in triplicate. The non-linear quadratic model generated by the design is of the form:

$$Y = B1 + B2*X1 + B3*X2 + B4*X3 + B5*X1*X2 + B6* X2*X3 + B7*X1*X3 + B8*X1*X1 + B9*X2*X2 + B10*X3*X3$$

The independent variables for optimization were the amount of drug (X1), volume of oil (X2), and amount of surfactant (X3), using the low, medium, and high level described in Table. 1. The obtained P-value less than 0.05 is considered statistically significant. ANOVA was also applied to determine the significance of the model.

2.5.1. Size and PDI

The particle size, polydispersity index (PDI), and intensity distribution of the formulation were analyzed in triplicate by an electrophoretic light-scattering (ELS) spectrophotometer (ELS 8000; Otsuka Electronics Co. Ltd., Tokyo, Japan). The samples were prepared in a quartz cuvette, and all measurements were carried out at 25℃.

2.5.2. Entrapment efficiency

Entrapment efficiency of PPD-NLC was calculated as described earlier with slight modification [17]. Briefly, NLC solution was obtained before the filtration process. The amount of entrapped PPD was measured by eliminating crystallized forms of PPD by filtration (Wattman syringe filter; nylon; 0.45 μm). The crude solution was put into methanol to disrupt the formulation. And then it was centrifuged at 12000 rpm for 15 min. Supernatant solution was analyzed using a HPLC system for calculating the amount of PPD in the NLC formulation. The encapsulation efficiency was calculated using following equation:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{actual weight of PPD in NLC}}{total\ weight\ of\ PPD\ added} \times 100$$

2.5.3. TEM analysis

The morphology of 20(S)-PPD-loaded NLC was absorbed by an energy-filtering transmission electron microscopy (TEM) (LIBRA 120, Carl Zeiss, Germany) at 80kV. A drop of PPD-NLC was loaded on a carbon-coated copper grid. And then it was negatively stained by 2% phosphotungstic acid. Stained copper grid was left to dry at room temperature.

2.5.4. Power X-ray diffraction (XRD)

To assess the crystallinity of drugs and other materials, powder X-ray diffraction was obtained (BRUCKER, German) using a Cu-Kα source. Samples were scanned between 2θ values (3 and 45°). Samples of the optimized NLC formulations, prepared with and without PPD and their components were run.

2.6. In vitro deposition test in Strat-M™ membrane

Measurement of in vitro deposition amount of PPD in Strat-M™ membrane was investigated by using previously described method with slight modification [18]. The Keshary-Chien diffusion cells have a 1.77 cm^2 of surface area and the receptor chamber was filled with 0.5% tween 80 solution. Shiny side

of Strat-M™ membrane was mounted over the donor part of Keshary-Chien cells. NLC formulation and oil solution was filled into the donor compartment and was covered with parafilm to avoid the evaporation of samples. The Strat-M™ membrane was taken from the difusion cells at 3 hr and 6 hr after putting samples into the donor compartment and washed out with methanol 3 times. Then, membranes were put into the tube with acetone: MeOH (7:3,v/v) and shaken for 3 hr. The tube was put into the centrifuge for 5.0 min at 16000g. Then, an 1.0mL aliqout of the supernatant was evaporated by a nitrogen gas stream. It was reconstituted with methanol. Amount of PPD was analyzed using LC-MS/MS as described above.

2.7. Statistical analysis

All experiments in this study were carried out at least three times, and the data were presented as mean ± standard deviation (SD). Statistical analyses were performed using the two-tailed t-test or analysis of variance (ANOVA) with *post-hoc* test (IBM SPSS Statistics software, version 21.0; IBM Corp, Armonk, NY, USA), and $p < 0.05$ was considered significantly different.

3. Results and Discussion

3.1. Solubility test

Among oils tested, Miglyol 812 N showed a highest solubility in PPD. Among surfactant tested, tween20 had a highest solubility in PPD (Table. 2).

3.2. Experimental design using Box-Behnken design

Box–Behnken design was applied to optimize the NLC formulation. Based on Preliminary experiments independent variables was set as follows; amount of drug (X1), volume of oil (X2), and amount of surfactant (X3). Dependent factor was set as follows; size (Y1), PDI (Y2) and EE (Y3). 15 Experimental runs were generated using Box-Behnken Design. Table. 3 shows the 15 experimental runs carried out for optimization. Moreover, 3D response surface graph was also plotted for the influence of variables on measured responses.

3.2.1 Effect on particle size

The size varied from 143.60 to 248.50. The effect of independent variables could be explained by the following quadratic equation.

Size = 132.2 – 0.24*X1 + 1.139X2 – 60.5 X3 + 0.160*X1*X1 – 0.00137*X2*X2 + 25.99*X3*X3 – 0.0209*X1*X2 – 0.50*X1*X3 – 0.209*X2*X3

In case of particle size case, X2 and X3 has a statistically significant meaning ($p<0.05$). The most significant factor in particle size was amount of surfactant (X3) because of the value of the coefficient from the equation (Table. 6).

Figure. 2-1 shows the 2D-contour plots and 3D-response surface plots for particle size of NLCs. X2 factor showed the rising trend significantly on the NLC formulation size. This means that if the amount of lipid increase, particle size could increase. X3 factor showed a negative effect significantly on the particle size. This means that increasing volume of oil could increase the particle size and amount of surfactant could make particle size small [19]. Increasing the amount of surfactant can be attributed to reduction of the surface interfacial tension and lead to make smaller particle size.

3.2.2 Effect on PDI

In the case of PDI, PDI varied from 0.179 to 0.298. The effect and interactions of independent variables could be explained by the following quadratic equation.

PDI = 0.2460 + 0.00648*X1 – 0.001270X2 + 0.0642 X3 + 0.000005*X2*X2 –

0.00631*X3*X3 – 0.000013*X1*X2 – 0.00270*X1*X3

In PDI, X1, X2 and X3 has a statistically significant meaning ($p<0.05$). The most significant factor in PDI was amount of surfactant (X3) because of the value of the coefficient from the equation (Table. 6).

Figure 2-2. shows the 2D-contour plots and 3D-response surface plots for particle size of NLCs. X1 and X3 factor showed the rising trend significantly on PDI value. This means that if the amount of drug and surfactant increase, PDI could increase. X2 factor showed a negative effect significantly on PDI value. This means that increasing volume of oil could make PDI small. These effect can be explained that higher concentration of surfactant, drug in nanoparticle may promote aggregation due to their adhesive feature. Increasing amount of oil can decrease the PDI value.

3.2.3 Effect on EE

In the case of EE, EE varied from 40.45 to 84.45. The effect and interactions of independent variables could be explained by the following quadratic equation.

EE = 58.8 – 4.468*X1 + 0.641*X2 + 5.96*X3 – 0.003449*X2*X2 – 2.07*X3*X3 + 0.01503*X1*X2 + 0.878*X1*X3

In EE, X1, X2 and X3 has a statistically significant meaning ($p<0.05$). The most significant factor in PDI was amount of surfactant (X3) because of the value of the

coefficient from the equation (Table. 6).

Figure 2-3 shows the 2D-contour plots and 3D-response surface plots for EE of NLCs. X1 factor showed the decreasing trend significantly on the NLC EE. This means that if the amount of PPD increase, EE could decrease. X2 factor showed a positive effect significantly on the EE. This means that increasing volume of oil could increase the EE. X3 factor shows a positive effect on EE. This means that increasing surfactant make EE higher. This effect can be explained that increasing oil and surfactant might increase PPD concentration and inner matrix space because of the imperfections of inner phase of the NLC formulation [20].

3.2.4 optimization and validation

The formulation was optimized on these criteria: minimum particle size, make PDI closer to 0.250, and maximum EE (Table. 4). Optimized formulation was made with composition of 5 mg of PPD, 82.3 µl of oil and 1.05% (v/v) of surfactant. This formulation was analyzed to check the validity of the optimization. Predicted value of the optimized formulation was found to be 155.42 of particle size, 0.250 of PDI and 80.63 of EE, respectively. The optimized formulation showed 148.7 ± 1.53 of particle size, 0.257 ± 0.014 of PDI, and 78.20 3.11 of EE (Table. 5). Observed data and predicted data presented a similar value, so that validity of optimization can be supported.

3.3 Transmission electron microscopy (TEM)

Shape and size of the optimized formulation were checked by TEM. NLC

showed a circle or oval shape without aggregation. The diameter of the observed formulation from TEM images had a good agreement with the data from an electrophoretic light-scattering spectrophotometer.

3.4 X-ray diffraction study

Fig. 4. shows the XRD data of PPD, cetyl palmitate, poloxamer, physical mixture of PPD, cetyl palmitate and poloxamer, NLC formulation without PPD, NLC formulation with PPD. The crystalline peak of PPD was clearly shown in the physical mixture (PM), whereas peak of PPD was not shown in the NLCs formulation. Decreasing peak intensity of PPD might be a evidence of changing crystallinity of PPD in NLC formulation. It shows that PPD encapsulated in lipid nanoparticle is existed in an amorphous condition. Changing crystalline state of PPD to amorphous state present improved drug solubility. Intensity of PPD-NLC was stronger than that of blank NLC. This can be assumed that PPD makes the PPD-NLC more crystalline [21].

3.5 In-vitro deposition study in Strat-M™

Fig. 5. shows in vitro deposition of PPD at 3 hr and 6 hr in Strat-M™

membranes after the application of suspension, oil solution or NLC formulation of PPD. Deposition amounts of PPD were NLC formulation > oil solution > suspension. The PPD deposition at 3 hr and 6 hr on the Strat-M™ membrane was higher in NLC formulation and oil solution than in suspension ($p < 0.01$). NLC formulation had a higher deposition amount in Strat-M™ membrane than oil solution of PPD ($p < 0.01$). Based on known mechanisms that explain the increasing deposition amount of NLCs on topical drug delivery, it can be suggested that the NLC formulation enhances the deposition amount of PPD through the mechanisms described below:

(1) From Fick's law, the concentration gradient is one of the dependent factor of the permeation flux [22]. As the lipophilicity of the lipids like oil can encapsulate more PPD in the formulation, concentration gradient is made higher and deliver PPD through the stratum corneum (SC). It can be supposed that using oil can make topical delivery better [23]. And (2) The hydration of the skin by the formulation is due to the water loss reduction caused by occlusion [9]. Hydration via skin occlusion can increase hydration in the stratum corneum and influence percutaneous absorption [24]. And (3) The surfactants can act as a chemical enhancer by irritating the lipid layers of SC or increasing the solubility of the drug in the formulation. These factors were involved in increasing the concentration of PPD in the skin [25,26].

4. Conclusion

In this study, PPD-loaded NLCs were optimized and developed by 3-factor, 3-level Box-Behnken design by using in the Minitab software. Based on the data from the Box-Behnken design, the amount of surfactant and PPD and oil were important factors for their physical properties of PPD-loaded NLCs. XRD analysis shows that the PPD had entrapped efficiently in amorphous state in the NLCs. In addition, PPD-loaded NLCs might be further incorporated into a gel-like formulation, so that it can enhance skin contact with easy application [27].

Results suggested that PPD-loaded NLCs enhanced drug deposition in the Strat-M™ membrane compared to oil solution and suspension. From this data, PPD loaded NLCs is a potential tool for topical delivery to suppress MMP-1 expression than the control. Further studies could be focused on the evaluation of the efficacy of the optimized formulation in clinical test.

References

[1] SM Park , EH Jung, JK Kim , KH Jegal , CA Park , IJ Cho, SC Kim. 20S-Protopanaxadiol, an aglycosylated ginsenoside metabolite, induces hepatic stellate cell apoptosis through liver kinase B1-AMP-activated protein kinase activation. Journal of Ginseng Research. 2017;41:392-402.

[2] CJ Xu, JJ Teng, WD Chen, QG, ZQ Yang, CY Yu, ZR Yang, W Jia. 20(S)-protopanaxadiol, an active ginseng metabolite, exhibits strong antidepressant-like effects in animal tests. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2010 ;34:1402-1411.

[3] YY Yang, JS Lee, MH Rhee, T Yu, KS Baek, NY Sung, Y Kim, KJj Yoon, JH Kim, YS Kwak, SY Hong, JH Kim, JY Cho. Molecular mechanism of protopanaxadiol saponin fraction-mediated anti-inflammatory actions. Journal of Ginseng Research. 2015 ;39: 61-68.

[4] Zhang Z, Li Z, Wu X, Zhang C. F, Calway, T, He T. C, Du W, Chen J.J, Wang C.Z, Yuan C.S. TRAIL pathway is associated with inhibition of colon cancer by protopanaxadiol. Journal of Pharmacological Sciences. 2015;127: 83-91.

[5] S Han, TG Lim, JE Kim, H Yang, DK Oh, Y Park, HJ Kim, YK Rhee, KW Lee, The Ginsenoside Derivative 20 (S)□Protopanaxadiol Inhibits Solar Ultraviolet Light□Induced Matrix Metalloproteinase□1 Expression. Journal of Cellular Biochemistry. 2017;118:3756–3764.

[6] SP Balguri , GR Adelli, S Majumdar. Topical ophthalmic lipid nanoparticle formulations (SLN, NLC) of indomethacin for delivery to the posterior segment

ocular tissues. European Journal of Pharmaceutics and Biopharmaceutics. 2016;109:224–235.

[7] PO Nnamani , S Hansen , M Windbergs , Claus-Michael Lehr. Development of artemether-loaded nanostructured lipid carrier (NLC) formulation for topical application, International Journal of Pharmaceutics. 2014;477:208–217.

[8] E Lasoń, E Sikora, M Miastkowska, P Socha, J Ogonowski NLC delivery systems for alpha lipoic acid: Physicochemical characteristics and release study. Colloids and Surfaces A. 2017;532:57-62.

[9] J Pardeike, A Hommoss, RH Müller. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. International Journal of Pharmaceutics. 2009;366:170-184.

[10] SS Salunkhe, NM. Bhatia, VB. Pokharkar, JD. Thorat, Manish S. Bhatia. Topical delivery of Idebenone using nanostructured lipid carriers: evaluations of sun-protection and anti-oxidant effects. Journal of Pharmaceutical Investigation. 2013;43:287-303.

[11] RH Müller, RD Petersen, A Hommoss, J Pardeike. Nanostructured lipid carriers (NLC) in cosmetic dermal products. Advanced Drug Delivery Reviews. 2007;59:522-530.

[12] J Hao, X Fang, Y Zhou, J Wang, F Guo, F Li, X Peng. Development and optimization of solid lipid nanoparticle formulation for ophthalmic delivery of chloramphenicol using a Box-Behnken design. International Journal of Nanomedicine. 2011;6:683-692.

[13] AA Abdelbary, MHH AbouGhaly. Design and optimization of topical

methotrexate loaded niosomes for enhanced management of psoriasis: Application of Box–Behnken design, in-vitro evaluation and in-vivo skin deposition study. International Journal of Pharmaceutics 2015;485:235-243.

[14] T Uchida, WR. Kadhum, S Kanai, H Todo, T Oshizaka, K Sugibayashi. Prediction of skin permeation by chemical compounds using the artificial membrane, Strat-M™. European Journal of Pharmaceutical Sciences. 2015;67:113-118.

[15] SS Salunkhe , NM Bhatia, VB Pokharkar, JD Thorat, MS Bhatia. Topical delivery of Idebenone using nanostructured lipid carriers: evaluations of sun-protection and anti-oxidant effects. Journal of Pharmaceutical Investigation 2013;43:287-303.

[16] J das Neves, B Sarmento. Precise engineering of dapivirine-loaded nanoparticles for the development of anti-HIV vaginal microbicides. Acta Biomaterialia. 2015;18:77-87.

[17] SE Lee, JK Lee, WS Jang, TH Kim, A Tunsirikongkona,b,c, JS Choi, JS Park. Enhancement of stability and controlled drug release of lipidnanoparticles by modified solvent-evaporation method. Colloids and Surfaces A. 2016;508:294-300.

[18] KT Kim, MH Kim, JH Park, JY Lee , HJ Cho, IS Yoon, DD Kim. Microemulsion-based hydrogels for enhancing epidermal/dermal deposition of topically administered 20(S)-protopanaxadiol: in vitro and in vivo evaluation studies. Journal of Ginseng Research. In press.

[19] M Ferreira, LL. Chaves, SACosta Lima, S Reis. Optimization of nanostructured lipid carriers loaded with methotrexate: A tool for inflammatory and

cancer therapy. International Journal of Pharmaceutics. 2015;492:65-72.

[20] V Teeranachaideekul, EB Souto, VB Junyaprasert, & RH Müller. Cetyl palmitate-based NLC for topical delivery of Coenzyme Q 10–Development, physicochemical characterization and in vitro release studies. European Journal of Pharmaceutics and Biopharmaceutics, 2007;67 : 141-148.

[21] Y Chen, X Yang, L Zhao, L Almásy, VM Garamus, R Willumeit, & A Zou. Preparation and characterization of a nanostructured lipid carrier for a poorly soluble drug. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2014; 455: 36-43.

[22] K Moser, L Kriwet, A Naik, YN Kalia, & RH Guy. Passive skin penetration enhancement and its quantification in vitro. European Journal of Pharmaceutics and Biopharmaceutics. 2001;52 :103-112.

[23] S Jain, N Patel 2, MK Shah , P Khatri , N Vora. Recent advances in lipid-based vesicles and particulate carriers for topical and transdermal application. Journal of Pharmaceutical Sciences. 2017;106:423-445.

[24] H Zhai, HI Maibach, Effects of skin occlusion on percutaneous absorption: An overview. Skin Pharmacology and Physiology. 2001;14:1-10.

[25] S Moghassemi, A Hadjizadeh. Nano-niosomes as nanoscale drug delivery systems: an illustrated review. Journal of Controlled Release. 2014;185:22-36.

[26] ME Lane. Skin penetration enhancers. International Journal of Pharmaceutics. 2013;447 :12-21.

[27] KA Shah, AA Date, MD Joshi, & VB Patravale. Solid lipid nanoparticles

(SLN) of tretinoin: potential in topical delivery. International Journal of Pharmaceutics. 2007;345 : 163-171.

Table 1. Independent variables and their levels in Box-Behnken design

Independent variables	Levels	
	Low	High
X1 = PPD amount (mg)	2	10
X2 = oil amount (µl)	0	100
X3 = Tween20 (%,v/v)	0	2

Table 2. Solubility of PPD in various liquid lipids and surfactants.

Solubility of liquid lipid(mg/ml)	Miglyol 812 N	12.64 ± 1.38
	Capmul MCM	10.60 ± 1.44
	L.A.S	8.06 ± 1.59
	Lauroglycol CC	6.87 ± 0.38
	Labrafac CC	3.80 ± 0.82
Solubility of Surfactant(mg/ml)	Tween 20	5.37 ± 0.89
	Tween 80	3.55 ± 0.64
	Isopropyl myristate	2.53 ± 0.73
	Limonene	1.54 ± 0.26
	PEG 400	0.02 ± 0.01

Each value in the mean ± SD (n=3)

Table 3. Size (Y1), PDI (Y2), EE (Y3) of optimized PPD-NLC formulations.

Run	Independent variables			Observed data		
	x1	x2	x3	Y1	Y2	Y3
1	-1	0	-1	232 ± 2.17	0.18 ± 0.037	70.28 ± 1.458
	1	-1	0	151 ± 2.44	0.29 ± 0.008	39.79 ± 0.929
3	0	0	0	167 ± 3.01	0.250 ± 0.007	70.22 ± 2.417
4	0	0	0	165 ± 1.30	0.257 0.001	72.57 ± 1.200
5	1	0	1	153 ± 1.21	0.29 ± 0.009	75.41 ± 2.688
6	0	0	0	162 ± 2.19	0.252 ± 0.022	75.16 ± 0.543
7	0	1	-1	248 ± 2.30	0.22 ± 0.03	56.73 ± 1.356
8	0	1	1	180 ± 2.88	0.25 ± 0.013	77.30 ± 1.787
9	-1	1	0	190 ± 2.55	0.25 ± 0.004	76.46 ± 5.799
10	-1	0	1	148 ± 3.10	0.272 ± 0.012	81.22 ± 4.560
11	1	0	-1	247.23 ± 1.55	0.249 ± 0.010	46.87 ± 2.251
12	0	-1	-1	174.2 ± 1.21	0.262 ± 0.010	43.94 ± 1.522
13	-1	-1	0	144 ± 1.79	0.28 ± 0.001	74.47 ± 1.891
14	0	-1	1	147.2 ± 0.62	0.298 ± 0.013	68.64 ± 0.913
15	1	1	0	176.8 ± 1.35	0.250 ± 0.013	56.82 ± 0.529

Table 4. Goal of the dependent factors.

Dependent factors	Goals
Y1 = Particle size(nm)	Minimize
Y2 = PDI	0.250
Y3 = Entrapment Efficiency	Maximize

Table 5. Comparision between the observed and predicted value of Y1, Y2, Y3 in optimized formulation

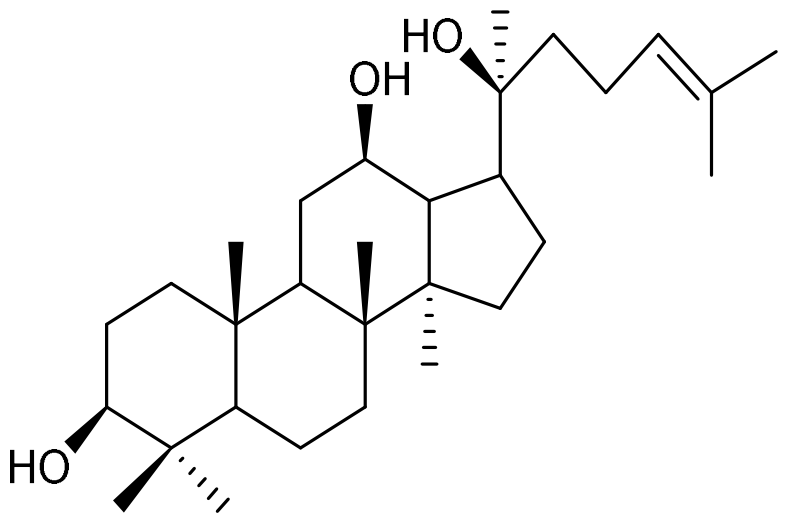
Optimal factor settings			Optimal response			Validation results		
PPD (X1)	Oil (X2)	Tween20 (X3)	Size (nm)	PDI	EE (%)	Size (nm)	PDI	EE (%)
5	82.3	1.05	155.43	0.250	80.63	148.7 ± 1.53	0.250 ± 0.014	78.20 ± 3.11

Each unit of X1, X2, X3 is mg, ul, and %(v/v)

Table 6. Results of regression analysis for Y1, Y2, Y3

Parameter	Size(Y1)		PDI(Y2)		EE(Y3)	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Intercept	132.2	0.000	0.2460	0.000	58.8	0.000
X1	-0.24	0.740	0.00648	0.041	-4.468	0.000
X1^X1	0.160	0.629	-	-	-	-
X2	1.139	0.008	-0.001270	0.008	0.641	0.000
X2^X2	-0.00137	0.679	0.000005	0.118	-0.003449	0.005
X3	-60.5	0.008	0.0642	0.002	5.96	0.001
X3^X3	25.99	0.020	0.00631	0.414	-2.07	0.358
X1X2	-0.0209	0.515	0.000013	0.657	0.01503	0.106
X1X3	-0.50	0.753	0.00270	0.047	0.878	0.067
X2X3	-0.209	0.221	-	-	-	-
R^2	0.934		0.882		0.954	

(A)



(B)

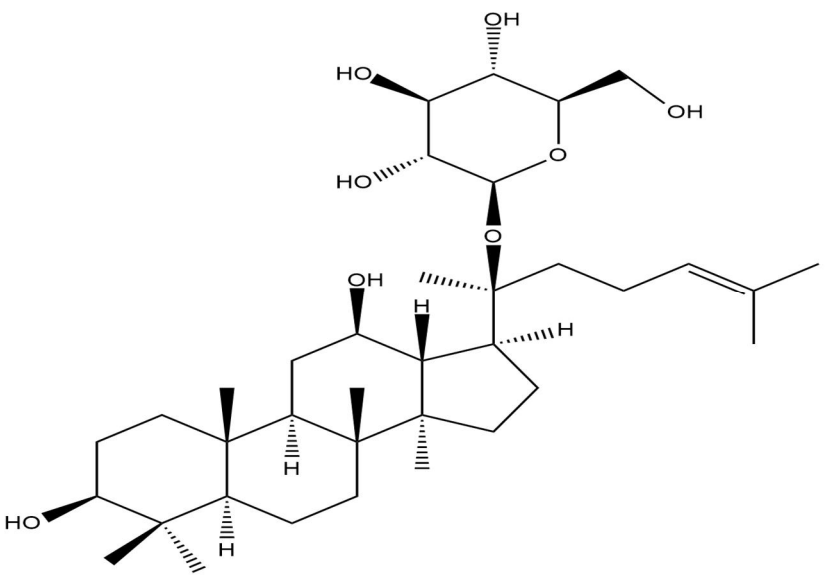


Fig 1. Structure of (A) 20(S)-Protopanaxadiol and (B) Compound K

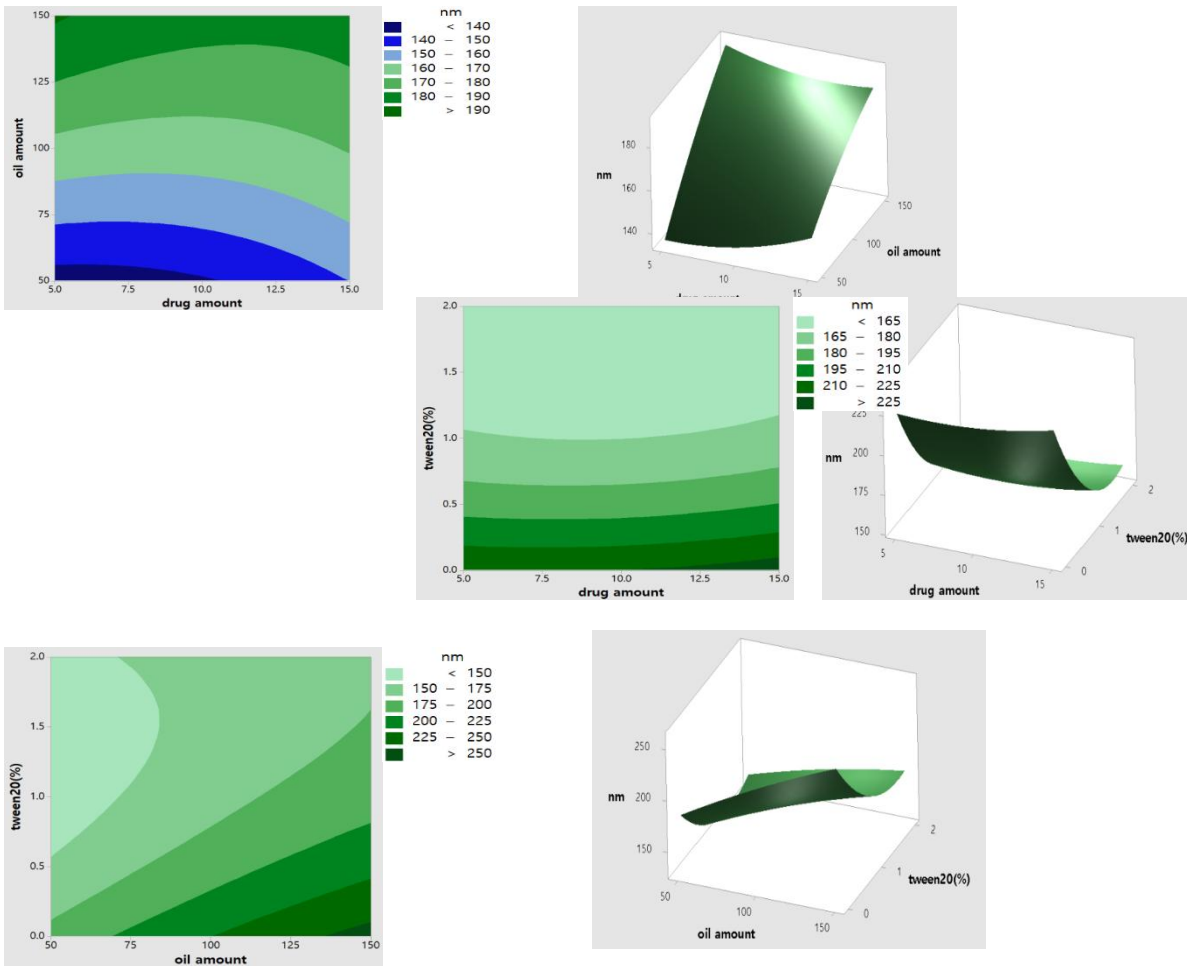
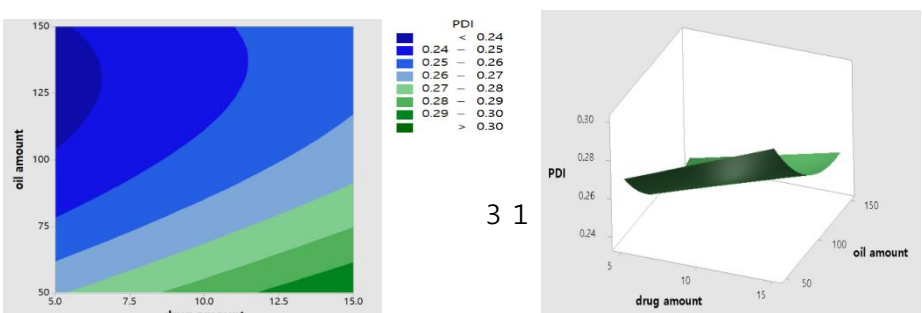


Fig 2.1 2-D contour plots and 3D response surface plots for the effect of PPD amount, volume of oil, and surfactant amount on particle size



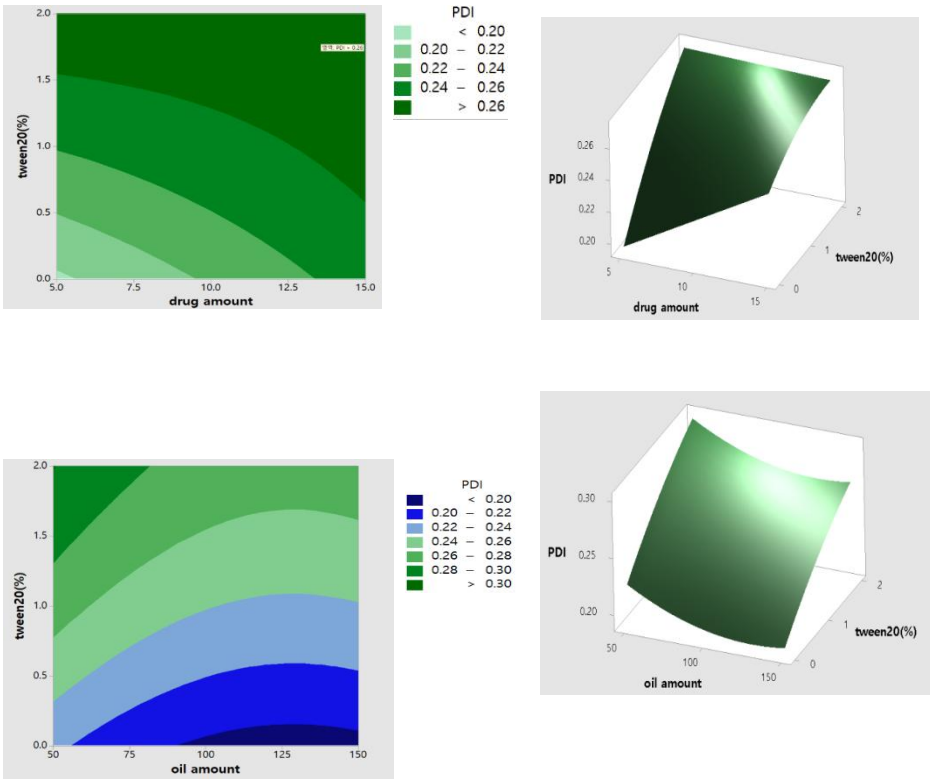
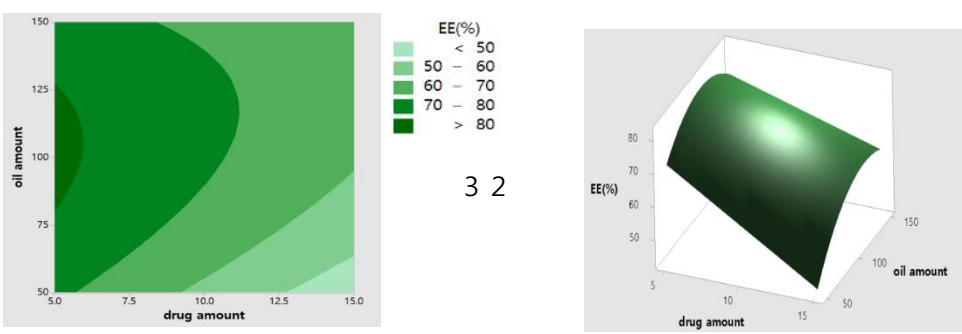


Fig 2.2 2-D contour plots and 3D response surface plots for the effect of PPD amount, volume of oil, and surfactant amount on PDI



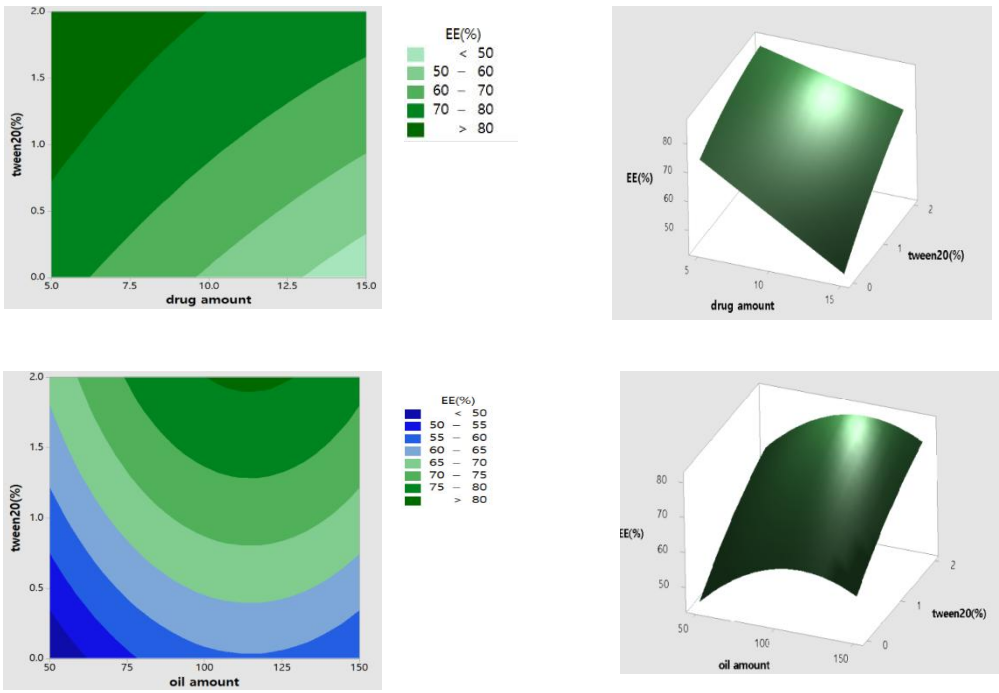


Fig 2.3 2-D contour plots and 3D response surface plots for the effect of PPD amount, volume of oil, and surfactant amount on EE

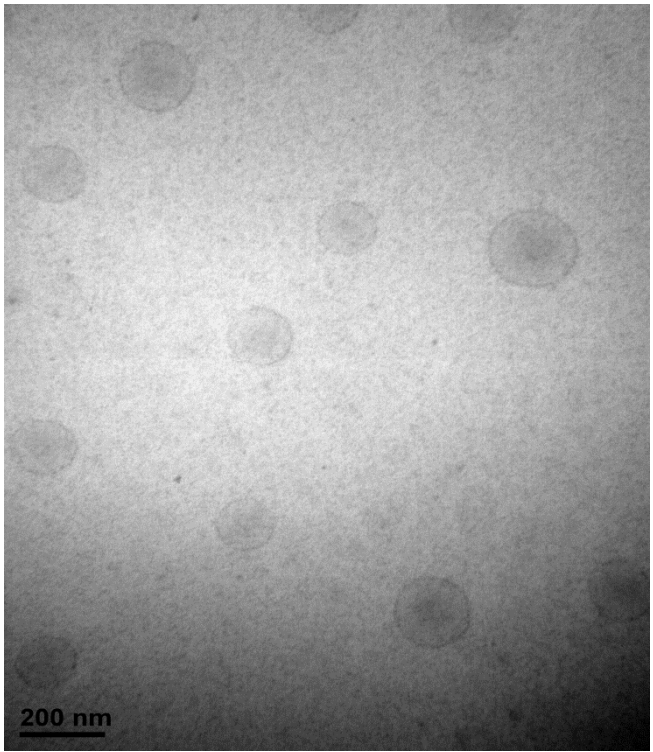


Fig 3. TEM images of NLC formulation.

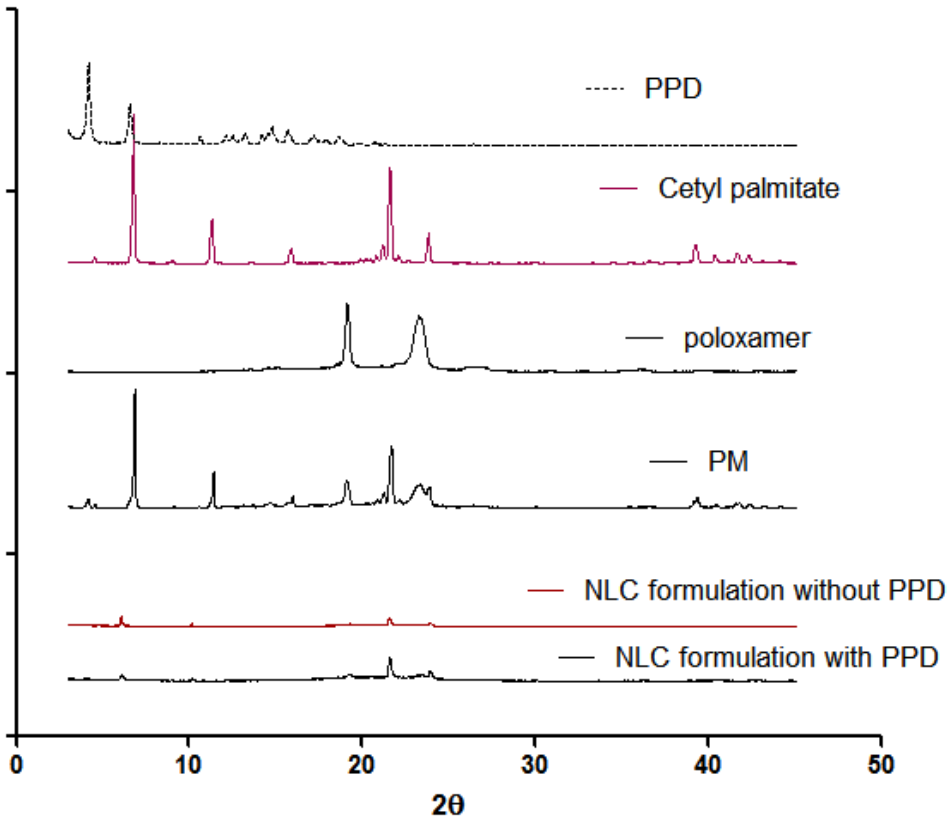


Fig 4. X-ray diffraction patterns of PPD, cetyl palmitae, poloxamer, physical mixture of PPD, cetyl plamitate, and poloxamer, NLC without PPD, NLC with PPD

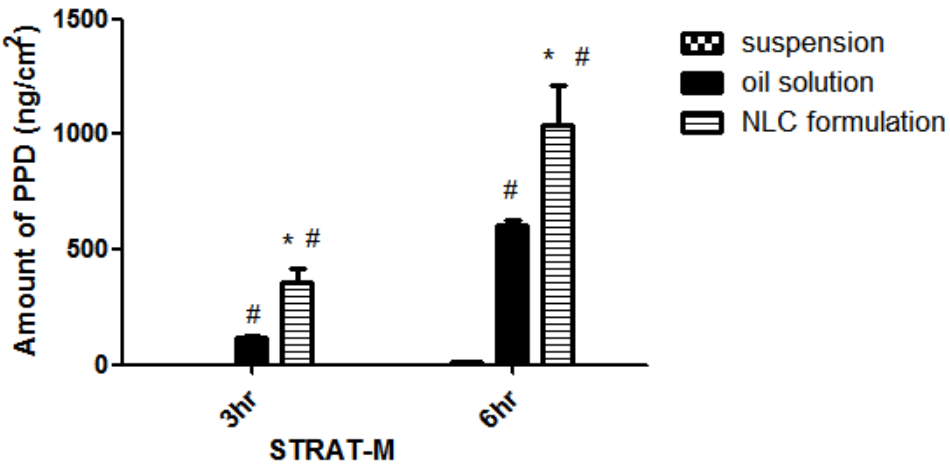


Fig 5. In-vitro deposition of PPD in Strat-M™ membrane at 3 hr and 6 hr after topical administration of optimized formulation

국문초록

20(S)-Protopanaxadiol(PPD)는 Compound K와 ginsenoside

Rb1에서 당이 떨어진 대사체로서, matrix metalloproteinase-1 (MMP-1) 활성 감소에 의한 피부노화방지 작용을 가지고 있다. 활성 성분의 피부 흡수과정에서 각질층을 통과하는 것이 가장 중요한 단계인데, PPD는 물에 잘 녹지 않고 분자량이 크기 때문에 각질층을 잘 통과하지 못하여 피부 흡수가 잘 안 될 것으로 예측이 된다. 그러므로 Fick의 확산 법칙에 근거하여, PPD의 피부 흡수를 증가시키기 위해서는 이를 가용화 할 수 있는 제형 개발이 필요하다. Lipid nanoparticle의 한 종류인 Nanostructured Lipid Carrier(NLC)는 기존에 알려진 리포좀이나 에멀젼에 비해 여러 장점을 가지고 있는데, 피부수화효과, 약물의 지속방출, 안정성 증가 등이 대표적이다. 본 연구의 목적은 반응표면법 중 하나인 Box-Behnken Design을 이용하여 지용성 물질인 PPD의 피부 흡수를 증가시키기 위한 최적의 NLC 조성을 얻는 것이다. PPD의 양(X1), 오일의 양(X2), 계면활성제의 양(X3)을 세 개의 변수로 설정하였고, 입자의 크기(Y1), polydispersity index (PDI) (Y2), 봉입율(Y3)을 바탕으로 최적화를 진행하였다. 최적 조성으로부터 얻은 예측 값과 실측 값을 비교함으로써 실제로 최적화가 되었는지를 확인 하였다. Y1, Y2, Y3 값 모두 예측 값과 실측 값의 차이가 5%이내인 것으로부터 최적의 조성이 잘 도출되었음을 확인하였다. 또한, X-ray diffraction (XRD)를 통하여 PPD가 NLC 제형에 무정형의 상태로 봉입되어있음을 알 수 있었다. 최적 조성의 NLC를 피부 대체

인공막인 Strat-M™에 적용한 후 시간에 따른 PPD의 잔류량을 평가하였다. PPD를 함유한 suspension과 oil solution에 비해 최적화된 NLC 조성을 적용하였을 때, 3시간과 6시간 후의 PDD 잔류량이 모두 유의성 있게 높은 값을 나타내었다. 이를 통해, PPD의 피부 적용을 위한 최적의 NLC 조성이 Box-Behnken design을 통해 성공적으로 도출되었음을 알 수 있었다.

주요어: Box-Behnken design, 20(S)-Protopanaxadiol, Nanostructured Lipid Carriers(NLCs), Strat-M™, topical delivery

학번: 2016-21830

APPENDIX