공학석사 학위논문

Enhancement of Topical Delivery and Photostability of Orobol-loaded Microemulsion and Nanostructured Lipid Carriers

마이크로에멀전과 나노구조지질담체 제조를 통한
오로볼의 피부흡수력 및 광안정성 향상 연구

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서울대학교 대학원

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Abstract

Enhancement of Topical Delivery and Photostability of Orobol-loaded Microemulsion and Nanostructured Lipid Carriers

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Isoflavone is a phytochemical mainly found in soybeans and is attracting attention due to its antioxidant and anticancer effect. In particular, orobol, which is the metabolite of genistein, has been found to show excellent efficacy against skin diseases compared with other isoflavones. Orobol was hard to find in the nature, but in recent years, it succeeded in mass production, which enabled production with an affordable price. Therefore, it is attracting attention as a functional cosmetic material of the future. However, there are two major problems in commercialization of orobol. First, orobol has poor photostability. Orobol reacts with organic solvents and causes discoloration when exposed to sunlight. In addition, since it is hydrophilic (log K_{ow} = 2.36), the skin absorption rate is low. For these reasons, formulations overcoming defaults are necessary to enhance the performance of the orobol.

In this study, microemulsion and nanostructured lipid carrier were used to formulate nanoparticles to solve the problems of orobol and to maximize its functionality. Microemulsion formulations were prepared by selecting Capmul MCM as an oil phase, Transcutol as a surfactant, and Labrasol as a cosurfactant. Nanostructured lipid carrier was selected from cocoa butter as a solid lipid, Capmul MCM as an oil phase, Tween 20 and Transcutol were used as surfactant. Each particle size and polydispersity were measured and the image of the formulations was observed by TEM. In vitro experiments using Franz diffusion cell at 37 ℃ were performed to assess the extent of skin deposition of the orobol-loaded formulations. Both ME and NLC showed an increase in the amount of skin deposition compared to the standard formulation, and NLC showed up to 6 times higher deposition amount due to the occlusion effect than ME. After exposing sunlight for 5 days to analyze the photostability, ME showed discoloration, but NLC retained color. In addition, the encapsulation efficiency of orobol in NLC is better than that of ME. This indicates that the NLC formulation exhibits more suitable vehicle as a cosmetic formulation of orobol.

Keywords: Orobol, Microemulsion, Nanostructured lipid carrier, Skin delivery, Photostability

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1. Introduction

1.1. Skin health benefits of orobol

Isoflavone, one of the phytochemical found mainly in soybeans, has attracted attention because of its antioxidant and anticancer properties. Recent studies have shown that isoflavone play an important role not only in cancer, obesity, and cardiovascular but also in skin diseases [1]. In particular, genistein and daidzein, which are the core classes of isoflavone, have shown inhibition oxidative events induced by ultraviolet when applied to topical skin [2-4]. These studies suggest that isoflavone have potential to be used as treatments in skin wrinkles and skin cancers [5]. However, there are only a few studies about formulations of isoflavone, which can maximize the efficiency.

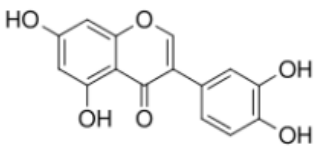


Figure 1. Chemical structure of orobol.

Orobol (5, 7, 3, 4-tetrahydroxyisoflavone) is a metabolite of Genistein (**Figure 1**), which exists in fermented soybean in nature or liver microsomes after soybean ingestion [6, 7]. Furthermore, orobol could be converted from genistein via o-hydroxylation using tyrosinase [8]. In recent studies, orobol has strong effect on skin aging and atopic dermatitis due to its antioxidant effect, which is twice as strong as other isoflavones. Therefore, orobol is attracting attention as a next-generation cosmetic and pharmaceutical ingredient. However, there have been no studies about topical delivery of orobol so far. Orobol has a low aqueous solubility that could be a difficulty in reaching the dermal layer through stratum corneum. Moreover, orobol reacts with organic solvents and turns yellow when exposed to sunlight (**Figure 2**), Therefore, the study about a suitable formulation for orobol is required to improve its photostability and skin permeability.

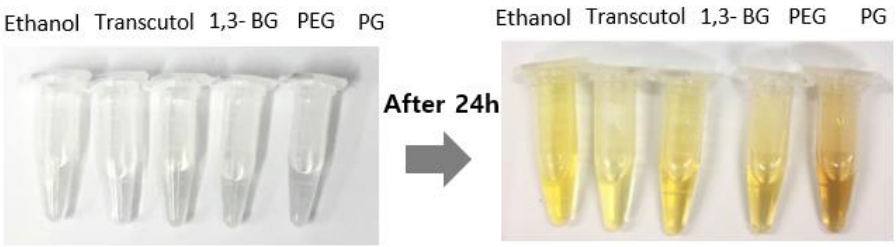


Figure 2. Discoloration of Orobol by sun light.

1.2. Nanocarriers for topical delivery

1.2.1. Theory of topical delivery

The skin is consists of stratum corneum, epidermis, dermis and subcutaneous fat. In particular, the outermost layer is stratum corneum, called skin barrier, which prevents absorbing harmful substances from outside. Therefore, passing through the stratum corneum is necessary in order to permeation active ingredient into the skin. The stratum corneum is composed of dead keratinocyte and interstitial lipid layer [9]. The permeation route is expected to be an appendage route, a transcellular route, and an intercellular route. Especially, an intercellular route for skin permeation has been studied as most effective pathway [10].

$$J = \frac{dm}{dt} = \frac{DC_oK}{\mathrm{h}} \qquad \text{(Eq. 1)}$$

Steady state flux equation (Eq. 1) is used when considering factors about drug permeation rate passed through stratum corneum [11]. In the equation, dm/dt is the steady state flux. D is the diffusion

coefficient: Low molecular mass (<500Da) and viscosity of vehicle affects this factor. C_0 is the concentration of drug, and higher values can contribute to permeation. K is the partition coefficient of the drug, and intermediate value (log K octanol/water of 1-3) can contribute to permeation. h is the thickness of stratum corneum [12]. Therefore, many researches have been studied to change the structure of drugs or formulations by Fick's law in order to increase the skin permeation rate of drugs.

1.2.2. Nanocarriers: Microemulsions

Among the nano-carrier systems for skin delivery, microemulsions (ME) are being studied extensively because of their simple manufacturing method, thermodynamically stability, and advantage in increasing solubility and permeability of drug [13, 14]. MEs, which consists of oil, water and several types of surfactants, have 10-200nm size of droplet. Depending on the type and ratio of surfactants, MEs can be manufactured into various types such as oil

in water (O/W) microemulsion and water in oil (W/O) microemulsion, so that it can be applicable to both hydrophilic and hydrophobic drugs [15, 16]. Moreover, several components of the ME can contribute to overcome stratum corneum by acting as permeation enhancer [17]. Kitagawa reported that skin permeation of genistein and other two isoflavones is enhanced by microemulsions [18].

1.2.3. Nanocarriers: Nanostructured lipid carriers

Another novel skin delivery system, Nanostructured lipid carriers (NLC), have attracted attention recently. NLC is the second edition of Solid lipid nanoparticle (SLN). SLNs have been developed from early 1990s as alternatives to liposomes and emulsions, which are conventional colloidal drug delivery systems [19]. SLNs can be maintained solid nanoparticle structure at room temperature by replacing the liquid lipid in the emulsion with a solid lipid [20]. SLNs prolonged thermal stability and photo stability of

the drugs and stability of the formulations, by using solid lipids [21, 22]. However, due to the crystallization of solid lipids, the rate of drug inclusion decreased over time. To solve this problem, NLC was developed in the early 2000s using oil mixed with solid lipids. NLC have more advantages than SLN such as formulation stability and drug entrapment efficiency. Moreover, films of NLCs which yield occlusion effect are formed on the skin, so that it can contribute to skin permeation of drugs [23]. For example, retinol loaded NLC could capture 5 times higher amount of retinol compared to SLN made of only solid lipid compritol 888 ATO [24]. Due to these advantages, NLCs have been widely applied in the field of cosmetics and pharmaceuticals for the past 10 years.

1.3. Purpose of this study

Therefore, the purpose of this study is to investigate the feasibility of nanocarriers technology to the topical delivery of orobol using ME and NLC in terms of improving photostability and increasing skin permeation. The orobol-loaded ME and NLC were prepared based on the construction of a pseudo ternary phase diagram and solubility test. Optimized orobol-loaded ME and NLC characterized in vitro in terms of particle size, polydiversity index (PDI), and morphology using TEM. Then, in vitro skin deposition properties were studied using Strat-M membranes, an artificial skin membrane, and photostability was investigated by observing the change of color.

2. Materials and methods

2.1. Materials

Orobol (purity ≥ 95.0 %) was provided from Prof. Byung-Gee Kim's laboratory in Seoul National University. Refined Shea butter and Cocoa butter were purchased from DAMI CHEMICAL Co.(Seoul, Korea). Capmul MCM EP was gifted by ABITEC Co. (Peterborough, UK). Labrafac CC, Labrasol (PEG-8 caprylic/capric glycerides), and Transcutol HP were gifted by Gattefossé Co. (Saint Priest, Cedex, France). Tween 20, Tween 80, polyethylene glycol 400 (PEG 400) and Sodium dodecyl surfate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Phosphate buffered saline was purchased from Lonza, Ltd. (Basel, Switzerland). HPLC-grade methanol and acetonitrile were purchased from Thermo Fisher Scientific Co. (Pittsburgh, PA, USA).

2.2. Preparation of orobol-loaded ME and NLC

2.2.1. Solubility test of orobol

The solubility of orobol in various solvents was determined by adding an excessive amount of orobol into a tube containing 1ml of solvent. The mixture of orobol and solvents were allowed to approach an equilibrium state in water bath at 37 °C for 72 h. The samples were centrifuged for 5 min at 16,000 *g*. The supernatant of samples was passed through a 0.20-μm syringe filter to remove orobol undissolved. Finally, the concentration of orobol in the filtered solution was quantified by HPLC after dilution with methanol.

2.2.2. Construction of the pseudo-ternary diagram

Based on the solubility test, oil and surfactant candidates with the highest solubility of orobol were selected. Capmul MCM EP was

selected as the oil phase, whereas Transcutol, Labrasol and LAS were selected as the surfactant phase candidates. To prepare $\mathbf{S}_{mix}$ (surfactant mixture), two of the three surfactant were selected (Transcutol and Labrasol, Transcutol and LAS, Labrasol and LAS) and mixed at various ratios (1:1, 2:1, and 3:1, w/w). Then, the oil phase and $\mathbf{S}_{mix}$ were mixed at 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). Distilled water (DW) was dropped to each combination of oil and $\mathbf{S}_{mix}$ at room temperature while stirring. The mixtures were be seen transparent after equilibrium. The points that combination of oil and $\mathbf{S}_{mix}$ turns turbid state are presented in.

2.2.3. Encapsulation efficiency

The encapsulation efficiency (E.E.) of orobol into NLC and ME was determined by ultrafiltration method using centrifugal filter tubes which is a 30 kDa molecular weight cut-off. The amount of encapsulated orobol was calculated by difference in total amount of orobol and free amount of orobol remaining in the aqueous phase.

The amount of orobol was quantitatively analyzed using HPLC, and E.E. is finally expressed in percent.

2.3. Preparation of Orobol-loaded ME and NLC formulations

2.3.1. Orobol-loaded ME

At the region where the MEs could be formed stably in pseudo-ternary diagram, three ME formulations (F1-F3) minimizing the surfactant ratio (**Figure 4**) were selected. Which for the preparation of MEs with 0.05 % (w/w) orobol, the exact amount of orobol was first added into the Capmul MCM EP and dissolved using vortex-mixer. The mixture of surfactant and cosurfactant (Transcutol and Labrasol, Transcutol and LAS) were subsequently added to the oil solution with dissolved orobol and then mixed using vortex-mixer at room temperature. Then, orobol is completely dissolved in mixture by tip sonication for 1 min. Distilled water was

added dropwise into the above mixture under the same conditions with vortexing.

2.3.2. Orobol-loaded NLC

Orobol-loaded NLC was prepared by hot homogenization followed by sonication technique. Orobol, Cocoa butter, Capmul MCM and Transcutol were dissolved in 15 ml conical tube and melted by heating 70 ℃. An aqueous phase was prepared by dissolving tween 20 (2 % w/v) in distilled water and heated to same temperature of oil phase. Hot aqueous phase was added to oil phase using vortex-mixer for 1 min. Then the mixture received energy through the tip sonication for 15 min. Orobol loaded NLC were formed by cooling down at room temperature.

2.4. Characterization of orobol-loaded ME and NLC formulations

2.4.1. Particle size and PDI

The mean particle size and polydispersity index (PDI), intensity distribution of particle size, of orobol loaded MEs and orobol loaded NLCs were measured in triplicate by an electrophoretic light-scattering (ELS) spectrophotometer (ELS 8000; Otsuka Electronics Co. Ltd., Tokyo, Japan). The samples were filled in a standard quartz cuvette, and all measurements were performed at 25°C.

2.4.2. Morphology detection using transmission electron microscopy (TEM)

The particle morphologies of the orobol-loaded ME and NLC were observed by an energy-filtering transmission electron

microscopy (TEM; LIBRA 120; Carl Zeiss, Jena, Germany) at 80 kV. 5 µl of the samples were placed on a copper grid and then negatively stained for 10 sec with 2 % sodium phosphotungstic acid (PTA). Copper grid with samples were washed twice with distilled water and dried in the air at room temperature prior to the operation.

2.5. In vitro deposition studies using artificial membrane

In vitro deposition of orobol into a Strat-M membrane was evaluated using Keshary–Chien diffusion cells at 32 °C, which have a surface area of 1.77 cm^2. The receptor cells were filled with phosphate buffered saline (PBS) containing 0.05 % w/v sodium dodecyl sulfate (13.0 mL). Strat-M membrane (2.5 cm diameter) was placed between the receptor cell and donor cell, with the shiny side up. Then, orobol in various vehicles (0.05 %, w/w), i.e., ME, NLC, distilled water and oil solution (Capmul MCM EP), were applied to the donor cell side and sealed by para film to prevent evaporation of the samples. The Strat-M membranes were separated from the

diffusion cells after 3 h, 6 h and 9 h. The membranes were washed out with methanol and distilled water. Then, they were divided into several pieces and placed into a 2.0-mL tube containing mixture of acetone and methanol (70:30 v/v %, 1.5mL). The tube was shaken for 3 h using vortex shaker for the extraction of orobol from the Strat-M membranes, and then centrifuged for 5.0 min at 16,000 *g*. A 1.2-mL aliquot of the supernatant was evaporated using a nitrogen gas stream at 60 °C and reconstituted with 0.4 mL methanol. Finally, the amount of orobol in the Strat-M membranes at 3 h, 6 h and 9 h was analyzed using HPLC system. The deposited amount value of orobol was normalized by the membrane surface area, with a dimension of μg/cm^2.

2.6. Photostability test

After the formulation containing orobol was prepared, a photo-stability test was carried out. The change of color were observed at 24 h immediately after leaving the formulation in the

sunny place.

2.7. HPLC analysis of Orobol

The amount of orobol was determined by HPLC analysis. The HPLC analysis equipment was Thermo Ultimate 3000 HPLC (USA) and using C18 (250x4.6 mm, 5 u) column at room temperature. The mobile phase used 0.3% TFA of water and acetonitrile (8:2), and the sample was used after filtration with 0.20 μm membrane filter. The sample was injected with 10 μl and the mobile phase was flowed at a flow rate of 0.8 mL / min, and orobol was detected at 261 nm.

2.8. Statistical analysis

All data were presented as mean ± standard deviation (SD). Significance of difference was evaluated using Student's *t*-test at the

probability level of 0.05.

3. Results and discussion

3.1. Design of orobol-loaded nanocarriers

3.1.1. Preparation of ME and NLC formulations

Table 1. Solubility test of orobol in various vehicles.

Phase	Vehicle	Solubility (mg/mL)
Oil	Capmul MCM	9.51
	Olive oil	1.19
	Miglyol	1.05
	MCT	0.97
	Labrafac CC	0.74
Surfactant	Transcutol	88.93
	Labrasol	51.22
	LAS	50.89
	PEG	31.42
	Tween 80	28.65
	Propanediol	16.82
	Tween 20	13.69

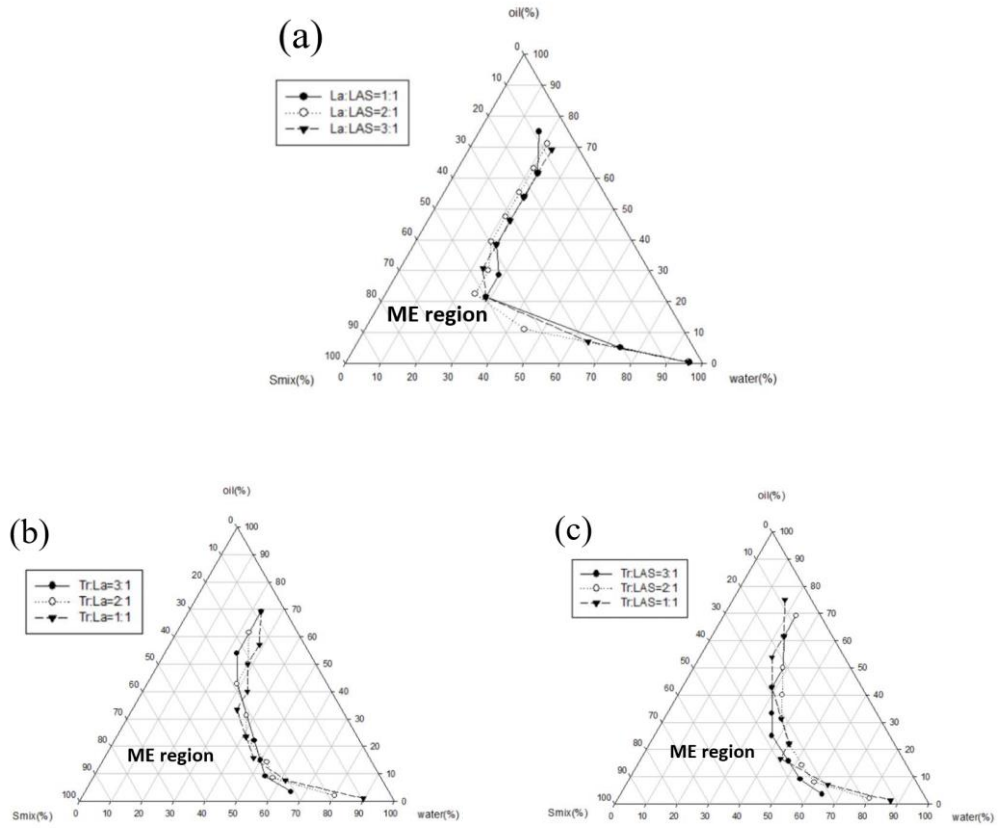


Figure 3. Pseudo-ternary phase diagrams of microemulsions. (a) Labrasol and LAS, surfactant and cosurfactant respectively, as ration of 3:1, 2:1, 1:1 (w/w). (b) Transcutol and Labrasol, surfactant and cosurfactant respectively, as ration of 3:1, 2:1, 1:1 (w/w). (c) Transcutol and LAS, surfactant and cosurfactant respectively, as ration of 3:1, 2:1, 1:1 (w/w). Capmul MCM was used as the oil phase for all microemulsion systems.

Due to the low solubility of orobol in water (0.53 mg/mL), solubility in various oil and surfactant were investigated to select more appropriate vehicle. According to **Table 1**, the order of decreasing the solubility of orobol in oil is as follows: Capmul MCM > Olive oil > Miglyol > MCT> Labrafac CC. The solubility in surfactant is as follows: Transcutol > Labrasol > LAS > PEG > Tween 80 > Propanediol > Tween 20. According to Fick's law (Eq. 1.), high drug concentration contribute to skin permeation of drug. Therefore, by selecting oils and surfactants that shows high solubility for orobol, it is possible to design a formulation that can increase the skin permeation more. As a result of the lipid screening test, Capmul MCM as the oil to be used in the microemulsion, and Trasncutol, Labrasol, LAS as the surfactant. Selecting several surfactants is necessary to maintain the thermodynamic stability of the microemulsion. The major difference between microemulsion and emulsion is thermodynamic stability, which is determined by the type and amount of surfactant. The emulsion is formed using a small amount of surfactant, but coalescence is occurred by gravity over time. In other words, one surfactant is insufficient to maintain

thermodynamic of emulsion system. This is because the critical micelle concentration is reached before reaching the concentration for spontaneous microemulsion formation. Therefore, by using various surfactants, the interfacial energy is lowered, which indicates that a cosurfactant is essential in the production of microemulsion [25]. The microemulsion will be prepared by selecting two kinds of surfactant, among Transcutol, Labrasol and LAS, which have the highest solubility of orobol.

Figure 3 shows a pseudo-ternary phase diagram consisting of oil, $\mathbf{S}_{mix}$, and water, and $\mathbf{S}_{mix}$ is a mixture of the main surfactant and the co-surfactant in three different ratios (1:1, 2:1, 3:1 w/w). Each of the three surfactant combinations is shown on graph, and the "ME region" represents the point where a transparent, highly stable microemulsion is formed.

The pseudo ternary diagram was constructed to investigate the stable formation region of microemulsion and the effect of surfactant combination. Capmul MCM was selected as oil, and the combination of two surfactant (Transcutol and Labrasol, Transcutol and LAS, Labrasol and LAS) were selected among three candidates.

First, it can be confirmed that the regions where the microemulsions are stably formed depend on the combination of the surfactants. In particular, the region of microemulsions in the combination of Labrasol and LAS was smaller than others (**Figure 3(a)**). The difference in the area of the region can be attributed to the difference in the structure of the surfactants. The structure of the surfactant can be considered to be divided into a hydrophilic part and a lipophilic part, which can expressed as hydrophilic liphophic balance (HLB) values. The HLB value is inherent depending on the kind of the surfactant, and should match to required HLB value of oil to form stable microemulsion. The HLB value of Labrasol is about 14 and the value of LAS is 13~15. These value shows two surfactants have more hydrophilic part than lipophilic part. However, the HLB value of Transcutol is 4. The graph (**Figure 3(b), 3(c)**) shows that the microemulsion is formed at a low surfactant ratio when using Transcutol as a main surfactant, and the required HLB value of Capmul MCM is lower than combination value of Labrasol and LAS Therefore, Transcutol was selected as a main surfactant and Labrasol and LAS as co-surfactant.

3.1.2. Physicochemical characterization of orobol-loaded ME and NLC formulations

3.1.2.1. Particle size and PDI

Table 2. Composition of ME and SLN formulations (% w/w).

Phase	**Vehicle**	F1 ME	F2 ME	F3 ME	F4 SLN	F5 NLC	F6 SLN	F7 NLC
Oil	Capmul MCM	20	20	20	-	0.5	-	0.5
Surfactant	Transcutol	28,7	32.25	28.7	2	2	2	2
	Labrasol	14.3	10.75	-	-	-	-	-
	LAS	-	-	14.3	-	-	-	-
	Tween 20	-	-	-	2	2	2	2
Solid lipid	Cocoa butter	-	-	-	2	1.5	-	-
	Shea butter	-	-	-	-	-	2	1.5
Water		37	37	37	93.5	93.5	93.5	93.5

Table 3. Physicochemical properties of ME and NLC.

Formulation	Size (nm)	PDI
F1	167.6 ± 14.2	0.13 ± 0.03
F2	209.3 ± 4.8	0.20 ± 0.02
F3	196.4 ± 3.9	0.23 ± 0.01
F4	88.6 ± 4.7	0.29 ± 0.04
F5	189.7 ± 10.9	0.05 ± 0.02
F6	130.6 ± 1.4	0.25 ± 0.01
F7	233.2 ± 11.5	0.12 ± 0.03

Microemulsion was designed based on solubility test and pseudo-ternary diagram (**Table 2**). Based on the pseudo-ternary diagram, the ratio of surfactant to oil is determined as 20:43:37. The ratio of surfactant to cosurfactant was designed as 2:1 (F1) and 3:1 (F2) when using Labrasol as a cosurfactant, and 2:1 (F3) when using LAS as a cosurfactant. **Table 3** shows that the PDI is less than 0.25 in all three formulations, confirming that all formulations were formed in a uniform size. The size of F1 is smaller than F2 using the same composition. The amount of Transcutol in F2 is higher than that in F1. It can be considered that the Transcutol is used more than the amount required to form a microemulsion, thereby forming a plurality of layers. When comparing F1 and F3, the size of F3 is larger. This may be due to different structural differences from Labrasol when using LAS as a co-surfactant.

Based on solubility test, NLC was SLN was designed (**Table 2**). The total lipid content was fixed at 2 % and the ratio of solid lipid to oil was 3: 1 for NLC. 2 % of Tween 20 was used as surfactant, and Transcutol was used as the solvent of orobol. **Table 3** shows that the size of NLC (F5, F7) is larger than SLN (F4, F6) by

about 100 nm. The solid lipids and oil used are not mixed with each other, so solid lipids are first crystallized and form a layer outer of oil when cooling. Therefore, the size of NLC is larger because the oil is contained inside of the solid lipid layer. In addition, the PDI of the NLC shows a narrow particle size distribution, which is less than half of the SLN. There is a study that the PDI value is affected by the ratio of oil to lipid. In particular, the higher the ratio of oil to lipid, the lower the PDI value[26]. Finally, the sizes of F6 and F7 using Shea butter are larger than F4 and F5 using Cocoa butter. It can be seen that the particle size is affected by type of solid lipid used. Both Cocoa butter and Shea butter are composed of various fatty acids, which differ in their melting points. Shea butter is about 37 to 42 ℃ and is about 5 ℃ higher than Cocoa butter. This difference in melting point can be attributed by the difference in the structure of each solid lipid. Cocoa butter has a slightly more loose structure and is expected to contain more oil and orobol in between. Therefore, the size of lipid particles used Shea butter seems to be larger.

3.1.2.2. Morphology of orobol-loaded nanocarriers

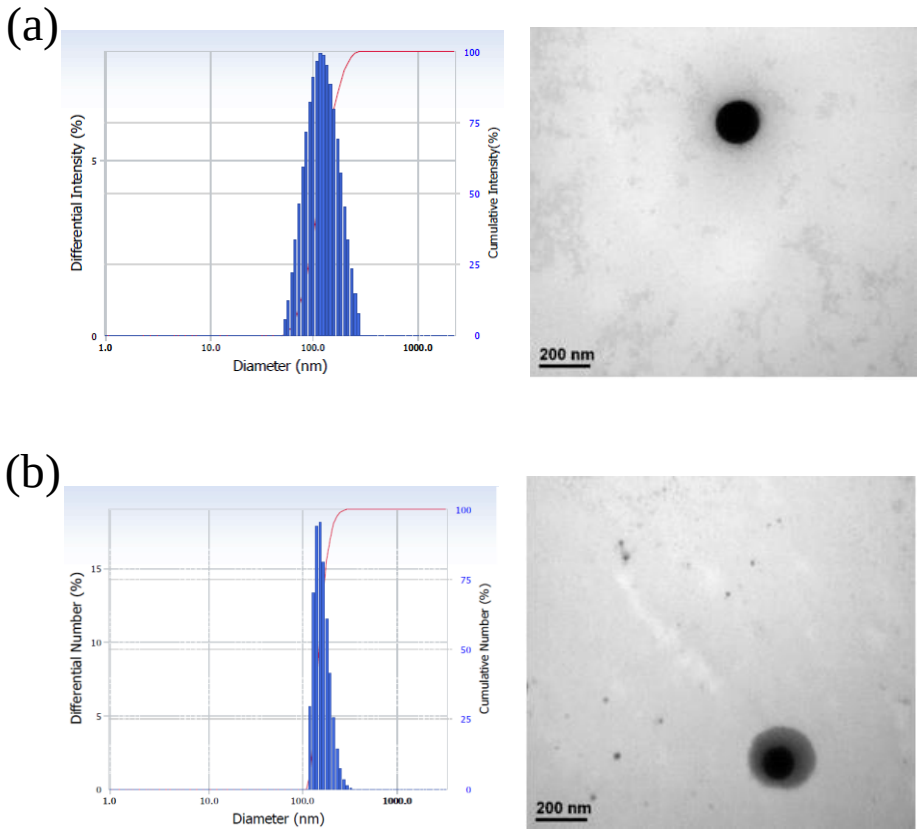
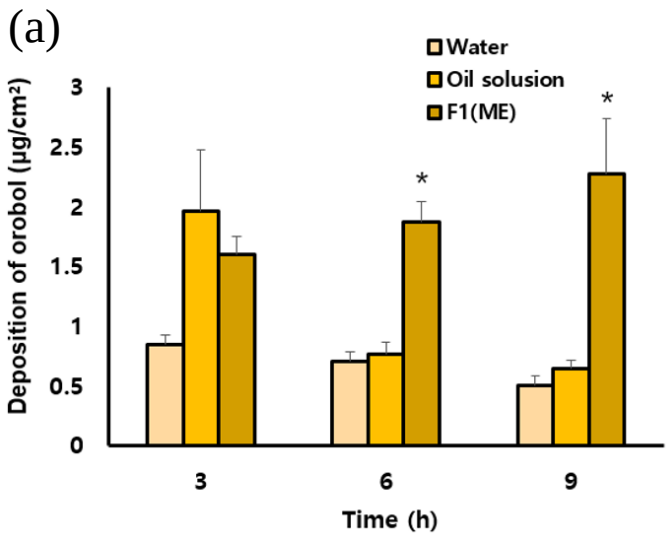


Figure 4. Morphological shapes of formulations observed by TEM and size distribution. The length of bar is 200 nm. (a) TEM images of orobol-loaded microemulsion. (b) TEM images of orobol-loaded nanostructured lipid carrier.

Figure 4 shows the TEM image and size distribution of the orobol-loaded ME and NLC. **Figure 4(a)** shows a orobol-loaded microemulsion, which is formed as spherical shape with a mean size less than around 200nm. **Figure 4(b)** shows an orobol-loaded NLC. Unlike the microemulsion, it can be seen that a thin layer is formed around the spherical shape. This is because the solid lipid and the oil are not dissolved together, so the solid lipid is first crystallized in the cooling process of NLC and the layer separation occurs. It can be confirmed that spherical particles are well formed in the form of a solid lipid enveloped on the surface.

3.2. Skin deposition capability and photostability of orobol-loaded nanocarriers

3.2.1. Effect of nanocarrers on skin deposition of orobol



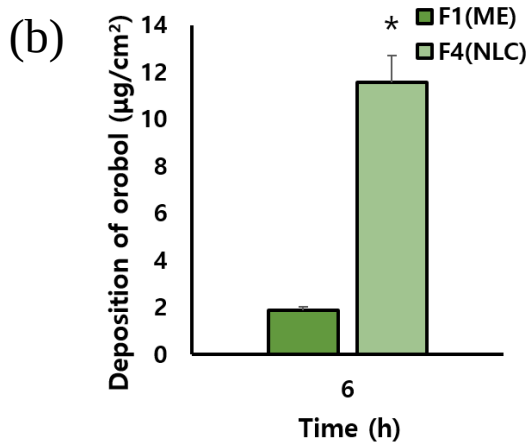


Figure 5. In vitro skin permeation of orobol. (a) Amount of orobol retained in the Strat-M at the 3 h, 6 h, 9 h in vitro deposition studies from various formulations (orobol 0.05 % w/v) (n=3). *; $p<0.05$ (significantly different from the control(water and oil)) (b) Amount of orobol retained in the Strat-M at the 6 h of in vitro deposition studies from microemulsion and nanostructured lipid carrier (orobol 0.05 % w/v) (n=3). *; $p<0.05$ (significantly different from the F1)

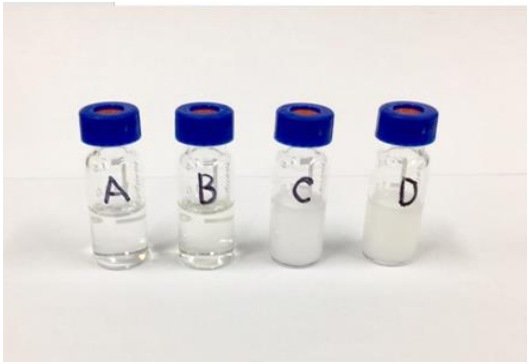
Figure 5 shows the in vitro deposition of orobol from various vehicles over time using Strat-M, an artificial membrane. In particular, **Figure 5(a)** shows the deposition of orobol in F1 for 3 h, 6 h, and 9 h compared to water and oil. After the first 3 h of absorption, the amount of orobol in F1 was higher than water, but less than that of oil. However, as the time passes to 6 h and 9 h, it can be seen that the deposition amount of orobol in F1 significantly increases more than water and oil. In the case of cosmetics, it is aimed at reaching the dermis, and it is unnecessary to transdermal delivery that permeate the blood vessels. Thus, the amount of orobol in oil was deposited in large quantities at first, but transdermal delivery progressed over time. On the other hand, since microemulsion has a higher deposition amount over time, it can be deduced that it will reach more in actual dermis. This is because the concentration of drug in microemulsion is higher than that of the conventional formulations such as oil and water. As mentioned in Fick's law, the higher the drug concentration, the higher the diffusion rate. Especially, it was affected by Transcutol and Labrasol, which are surfactants with high solubility of orobol. Secondly, there is an

influence of the components constituting the microemulsion. Labrasol, a surfactant that constitutes a microemulsion, is known as a permeation enhancer [27]. Labrasol causes disturbance in the stratum corneum so it could enhance permeation of the drug. Also, Transcutol is a permeation enhancer as reservoir of drug by enhancing solubility of drug [28]. For this reasons, it can be seen that the deposition of orobol in the microemulsions is better.

Figure 5(b) compares the deposition amount of orobol in ME and NLC after 6 h. It shows that the deposition amount of orobol was higher in the NLC. In general, nanoparticles containing solid lipids such as SLN and NLC are known to cause an occlusion effect on the skin [29]. That is, when the particles were applied the skin, the film is formed by the capillary phenomenon between the particles, and this film prevents evaporation of moisture from the skin [29]. Thus, it appears to have a hydration effect, which affects the spread of the lipid layer between the keratinocytes, allowing the drug to penetrate better. Therefore, it could be confirmed that orobol absorbed better by the occlusion effect of NLC.

3.2.2. Effect of nanocarriers on photostability of orobol

(a)



(b)

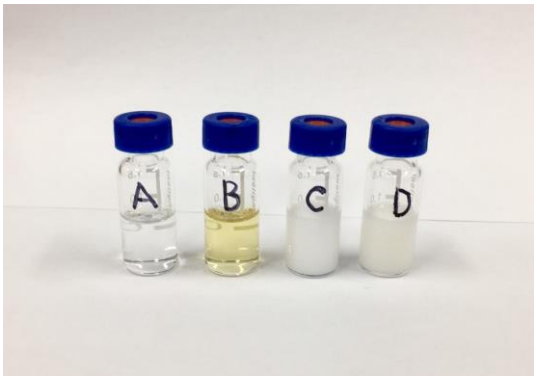


Figure 6. Color change of various orobol-loaded formulations. Sample A is an empty-ME, sample B is orobol-loaded ME, sample C is empty-NLC, sample D is orobol-loaded NLC. (a) Samples immediately after being manufactured. (b) Samples that were in the sun for 5 days.

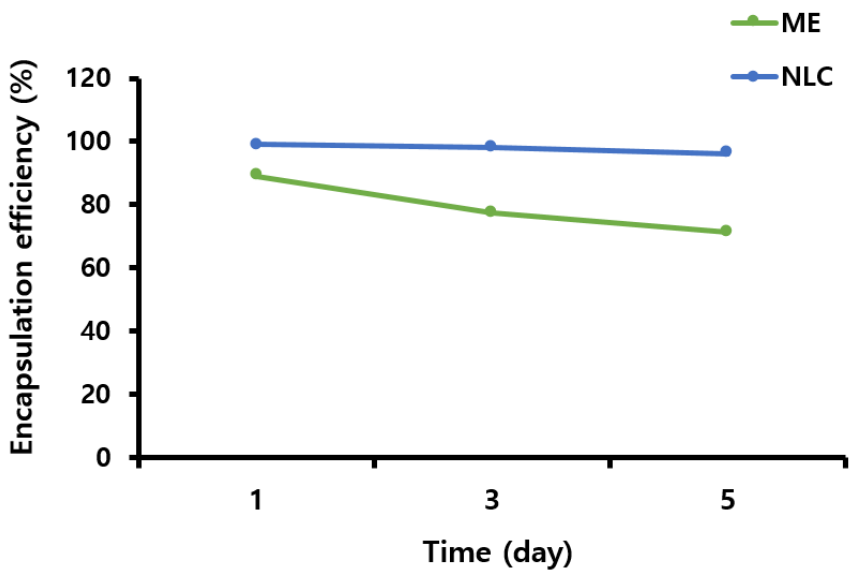


Figure 7. Encapsulation efficiency of orobol-loaded NLC and ME.

Figure 6 shows the results of the photostability test of the formulations loading orobol. Immediately after sample preparation, the microemulsion is transparent and NLC is white, regardless of whether it contained orobol. Result of placing the samples in a sunny place for 5 days, only the sample B, which is orobol-loaded microemulsion, changed to yellow and the other samples had no color change. In addition, **Figure 7** shows that the encapsulation efficiency of orobol-loaded NLC is better than that of ME during 5 days. The encapsulation efficiency of orobol-loaded ME was decreased from 89.1 % to 71.3 %, but that of orobol-loaded NLC was maintained up to 95%.

In other words, when sample A and B are compared with each other, it can be seen that the discoloration is caused by orobol. However, in the case of Sample D containing orobol, there was no color change. This can be attributed to the fact that NLC contains solid lipid. Solid lipids are solid at room temperature, scattering or absorbing the light. Especially cinnamic acid, which is contained in Shea butter and Cocoa butter absorb UV maximum at 275 nm [30]. Therefore, if orobol is encapsulated in NLC, it can be expected that

the light will not be transmitted to the orobol. On the other hand, the microemulsion penetrates the light as it is, so that the discoloration of orobol has occurred. Therefore, it can be said that NLC formulation has helped improve the photostability of orobol.

4. Conclusion

In this study, orobol-loaded ME and NLC were designed. Solubility test of orobol was used to select oils and surfactants for design of formulations. A pseudo-ternary diagram was prepared and the ratio of ME formation was determined. NLC was designed in the same way as above. Finally, in the ME, Capmul MCM was used as oil, Transcutol and Labrasol were used as surfactant and cosurfactant. Cocoa butter was used as solid lipid for NLC, and Tween 20 was used as surfactant. Droplet size, PDI, TEM images confirmed that the formulation was successfully manufactured. In vitro skin deposition studies have shown that both ME and NLC are suitable topical applications for the effective delivery of orobol to skin. Especially, the deposition amount of orobol in NLC is larger than that of ME because of occlusion effect. In the photostability test, it was confirmed that the solid lipid component of NLC inhibited the discoloration of the orobol. Thus, NLC has shown possibility as a formulation for orobol when using as cosmetics.

References

1. Sakai, T. and M. Kogiso, *Soy isoflavones and immunity.* J. Med. Invest., 2008. **55**(3-4): p. 167-73.
2. Wei, H., et al., *Inhibition of ultraviolet light-induced oxidative events in the skin and internal organs of hairless mice by isoflavone genistein.* Cancer Lett, 2002. **185**(1): p. 21-9.
3. Wang, Y., et al., *Inhibition of ultraviolet B (UVB)-induced c-fos and c-jun expression in vivo by a tyrosine kinase inhibitor genistein.* Carcinogenesis, 1998. **19**(4): p. 649-54.
4. Zhao, D., et al., *Daidzein stimulates collagen synthesis by activating the TGF-beta/smad signal pathway.* Australas J Dermatol, 2015. **56**(1): p. e7-14.
5. Barnes, S., *Effect of genistein on in vitro and in vivo models of cancer.* J Nutr, 1995. **125**(3 Suppl): p. 777S-783S.
6. Klus, K. and W. Barz, *Formation of polyhydroxylated isoflavones from the soybean seed isoflavones daidzein and*

glycitein by bacteria isolated from tempe. Arch Microbiol, 1995. **164**(6): p. 428-34.

7. Kiriakidis, S., et al., *Novel tempeh (fermented soyabean) isoflavones inhibit in vivo angiogenesis in the chicken chorioallantoic membrane assay.* Br J Nutr, 2005. **93**(3): p. 317-23.
8. Lee, S.H., et al., *Using tyrosinase as a monophenol monooxygenase: A combined strategy for effective inhibition of melanin formation.* Biotechnol Bioeng, 2016. **113**(4): p. 735-43.
9. A, w., *Transdermal and topical drug delivery : from theory to clinical practice*. 2003, Pharmaceutical Press, London.
10. Scheuplein, R.J., *Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration.* J Invest Dermatol, 1967. **48**(1): p. 79-88.
11. BW, B., *Dermatological formulations : percutaneous absorption*. 1983, Marcel Dekker, New York.
12. Maibach, H.I., *percutaneous penetration enhancers chemical*

methods in penetratin enhancement. 2015, USA: Springer.

13. Lawrence, M.J. and G.D. Rees, *Microemulsion-based media as novel drug delivery systems.* Advanced Drug Delivery Reviews, 2000. **45**(1): p. 89-121.
14. Cavalcanti, A.L., et al., *Microemulsion for topical application of pentoxifylline: In vitro release and in vivo evaluation.* Int J Pharm, 2016. **506**(1-2): p. 351-60.
15. Winsor, P.A., *Hydrotropy, Solubilisation and Related Emulsification Processes .1. To .4.* Transactions of the Faraday Society, 1948. **44**(6): p. 376-398.
16. Lee, P.J., R. Langer, and V.P. Shastri, *Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs.* Pharm Res, 2003. **20**(2): p. 264-9.
17. Hathout, R.M., et al., *Visualization, dermatopharmacokinetic analysis and monitoring the conformational effects of a microemulsion formulation in the skin stratum corneum.* J Colloid Interface Sci, 2011. **354**(1): p. 124-30.
18. Kitagawa, S., et al., *Enhanced skin delivery of genistein and*

other two isoflavones by microemulsion and prevention against UV irradiation-induced erythema formation. Chem Pharm Bull (Tokyo), 2010. **58**(3): p. 398-401.

19. Mukherjee, S., S. Ray, and R.S. Thakur, *Solid lipid nanoparticles: a modern formulation approach in drug delivery system.* Indian J Pharm Sci, 2009. **71**(4): p. 349-58.

20. Muller, R.H., K. Mader, and S. Gohla, *Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art.* Eur J Pharm Biopharm, 2000. **50**(1): p. 161-77.

21. Heiati, H., R. Tawashi, and N.C. Phillips, *Drug retention and stability of solid lipid nanoparticles containing azidothymidine palmitate after autoclaving, storage and lyophilization.* J Microencapsul, 1998. **15**(2): p. 173-84.

22. Carlotti, M.E., et al., *Study on the photostability of octyl-p-methoxy cinnamate in SLN.* Journal of Dispersion Science and Technology, 2005. **26**(6): p. 809-816.

23. Wissing, S., A. Lippacher, and R. Muller, *Investigations on the occlusive properties of solid lipid nanoparticles (SLN).* J

Cosmet Sci, 2001. **52**(5): p. 313-24.

24. Jenning, V., et al., *Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin.* Eur J Pharm Biopharm, 2000. **49**(3): p. 211-8.

25. Hunter, R.J., *Introduction to Modern Colloid Science Oxford University Press*. 1994: Oxford.

26. Azhar Shekoufeh Bahari, L. and H. Hamishehkar, *The Impact of Variables on Particle Size of Solid Lipid Nanoparticles and Nanostructured Lipid Carriers; A Comparative Literature Review.* Adv Pharm Bull, 2016. **6**(2): p. 143-51.

27. Bejugam, N.K., H.J. Parish, and G.N. Shankar, *Influence of formulation factors on tablet formulations with liquid permeation enhancer using factorial design.* AAPS PharmSciTech, 2009. **10**(4): p. 1437-43.

28. Mura, P., et al., *Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations.* Eur J Pharm Sci, 2000. **9**(4): p. 365-72.

29. Muller, R.H., M. Radtke, and S.A. Wissing, *Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC)*

in cosmetic and dermatological preparations. Adv Drug Deliv Rev, 2002. **54 Suppl 1**: p. S131-55.

30. Uscumlic, G.S., V.V. Krstic, and M.D. Muskatirovic, *Correlation of Ultraviolet-Absorption Frequencies of Cis and Trans Substituted Cinnamic-Acids with Hammett Substituent Constants.* Journal of Molecular Structure, 1988. **174**: p. 251-254.

초 록

이소플라본은 콩에 들어있는 파이토케미칼로, 항산화효과 및 항암효과로 주목받고 있다. 특히 제니스테인의 대사체인 오로볼은 다른 이소플라본에 비해 피부 주름 및 아토피 등 피부 질환에 대하여 뛰어난 효능을 보이는 것으로 밝혀졌다. 오로볼은 자연계에 소량 존재하였으나, 최근에는 대량생산에 성공하여 저렴한 가격으로 생산이 가능해지게 되었다. 따라서 미래 천연 화장품 기능성 소재로 각광받고 있다. 그러나 오로볼을 상용화하기에는 크게 두 가지 문제점이 있다. 먼저, 광안정성이 떨어진다는 것이다. 오로볼은 다른 이소플라본과 마찬가지로 햇빛을 받으면 유기용매와 반응하여 변색을 일으킨다. 또한, 친수성(log K_{ow} = 2.36)을 띄기 때문에 피부흡수율이 떨어진다. 따라서 본 연구에서는 마이크로 에멀전과 나노구조지질담체를 이용하여 나노제형화 시킴으로써 오로볼의 문제점을 해결하고 기능성을 극대화하였다.

마이크로 에멀전 제형은 Capmul MCM을 유상으로, Transcutol을 surfactant로, Labrasol을 cosurfactant로 선정하여

제조하였으며, Nanostructured lipid carrier는 고체지질로 cocoa butter를 선정하였고, 유상은 Capmul MCM, 계면활성제로는 Tween 20과 Transcutol을 사용하였다. 각각의 입자 크기, 다분산성을 측정하였으며, TEM으로 제형의 이미지를 관찰하였다. 37℃에서 Franz diffusion cell을 이용한 in vitro 실험에서 제형별 오로볼의 피부 침적 정도를 평가했다. ME와 NLC 모두 일반 제형에 비해 피부 침적량의 증가를 보였으며, 특히 NLC는 ME에 차폐효과로 인해 최대 6배 높은 침적량을 보였다. 태양빛에 5일 동안 광안정성 평가를 진행한 결과, ME는 변색이 일어났으나 NLC는 색이 유지되었다. 또한 NLC에서 오로볼의 봉입률이 ME에 비해 높게 유지되었다. 이는 NLC 제제가 오로볼의 화장품 제형으로 더 적합한 사용 가능성을 보임을 나타내었다.

주요어 : 오로볼, 마이크로에멀전, 나노구조지질담체, 피부흡수, 광안정성

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