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Preparation and Evaluation of Pluronic Lecithin Organogels in Cosmetics

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Synopsis

This study was performed to investigate the application of pluronic lecithin organogel (PLO gel) in cosmetics as a topical drug delivery system. PLO gel was known as transdermal drug delivery systems. It has a very interesting system, owing to their biocompatibility; their amphiphilic nature, facilitating dissolution of various drug classes; and their permeation enhancement properties. We realized that PLO gel has a critical shortcoming of flowability at low temperatures to be used as a cosmetic ingredient. To improve this drawback, this study aimed to find an appropriate quantity of three main compositions of PLO gel, including aqueous phase (poloxamer 407 and water), polyol phase (PEG-400), and oil phase (lecithin and oil), and applied an experimental design using the response surface methodology (RSM). We assessed the elapsed time change by temperature in each PLO gel formulation, observed the morphology of PLO gel using field emission scanning electron microscope (FE-SEM), and determined the gelation point by using differential scanning calorimetry (DSC). Rheology measurements to assess viscoelastic properties were determined by using a rheometer, and skin permeation efficiency was assessed by diffusion system. It was confirmed that three main factors (hydrogenated lecithin, PEG-400, and poloxamer 407) of PLO gel should be balanced without flowability even in cold temperature. Through the RSM, it was assumed that the most effective ingredient was PEG-400 at PLO gel formulation and physical properties. The PLO gel formulation (hydrogenated lecithin 5.0%, PEG-400 20.0%, and poloxamer 407 15.0%) was evaluated as the most suitable formulation for use in cosmetics due to its viscosity and elasticity results. The shape was observed through FE-SEM, and it was confirmed that the PLO gel forms a polymeric bicontinuous microemulsion structure. Regarding the applicability of PLO gel in cosmetics, we verified that PLO gel can be used in a delivery system for active substances. The study findings suggest that PLO gel can be used as one of the ingredients in cosmetic formulations.

INTRODUCTION

Topical and transdermal drug delivery systems (TDDSs) are widely used in the field of drug delivery systems (DDSs). These DDSs are most commonly designed to deliver the drugs through the skin. The skin is composed of three main layers: the epidermis, the dermis, and the subcutaneous fat tissue (1). A variety of advanced DDSs has been formulated to facilitate drug delivery through the epidermis and dermis, but do not allow successful drug deliver *via* the epidermis layer. The special characteristic of TDDSs is to

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achieve the balance to deliver hydrophilic and hydrophobic drugs through the epidermis (2). The stratum corneum is the major barrier in drug permeation across the skin. To overcome the limitation, chemical penetration enhancers (CPEs) are commonly used in TDDSs. However, CPEs could destroy the fat layer of the skin, and its long-term use may cause irritation and sensitivity to the skin (3–5).

TDDSs responsible for the transport of drugs through the skin have gained much attention and have been continuously studied in the pharmaceutical and cosmetic industries because these systems can maintain consistent drug plasma concentration and immediately provide the desired therapeutic efficacy by not being metabolized in the liver or the stomach. Because skin as a delivery route for drugs has increasingly attracted a great attention from many researchers, TDDSs have emerged as an attractive alternative for oral delivery of drugs as the reduction of gastrointestinal problems by drug delivery through the skin has been reported (6).

Poloxamer 407 and lecithin are most commonly used substances in topical delivery of drugs. Most of the studies have demonstrated that pluronic lecithin organogel (PLO) gel has the unique capacity to transport drugs across the skin. Drug permeability of PLO gel has been improved by lecithin and organic solvent used for its preparation. Lecithin increases drug permeation by temporarily opening skin pores and making epidermal structures more flexible (2,7). Through this process, lecithin enables topical drug delivery through the skin without skin irritation. Topical drug treatment aims at providing high concentration of drugs at the desired site to avoid systemic side effects associated with oral administration of drugs. To facilitate transdermal drug delivery after topical application, these agents are formulated in a matrix type (8).

PLO gel is a soya lecithin–based yellow-colored, odorless, and nontransparent gel that is characterized by rapid absorption. Because of the unique physical nature, it is commonly used as a drug delivery vehicle including poloxamer 407, a viscosity-enhancing agent with surfactant properties that facilitate oil-in-water preparations. Other common ingredients of PLO gel include lecithin, isopropyl palmitate, isopropyl myristate, polyethylene glycol, sorbic acid, and potassium sorbate (9).

PLO gel is a very interesting TDDS characterized by unique properties including biocompatibility, amphiphilic nature, dissolution of various active substances, and permeation enhancement (10). PLO gel was first developed as a topical drug vehicle by an American pharmacist in the early 1990s and is currently of a great attention in the pharmaceutical sector. Sudaxshina Murdan, a pharmacist, suggests, "Based on the greater aqueous component of the gel one could say that PLO gel is a hydrogel" (11). The hydrogel system, a three-dimensional network of hydrophilic polymers, is capable of absorbing large quantities of water or biological fluids. Atrophic homopolymers or copolymers form an atrophic network *via* cross-bridge binding. Physical crosslinking includes molecular entanglement or crystallization that contributes to the formation and physical integrity of the network, and chemical crosslinking can be considered as the binding point and bonding (12–14). Because hydrogels are easily washed away, but adhere well to the mucosa or the skin by existing in wet forms due to cellular fluid, the hydrogel system is generally applied to damaged skin and eyes (15). PLO gel is mainly made up of two phases, aqueous phase (poloxamer 407) and oil phase (lecithin). Poloxamer 407 is an ABA-type triblock copolymer composed of 70% polyoxyethylene with the average molecular weight of 12,500 Da (10). Poloxamer 407 is a nontoxic polymer

that exists in a liquid state with flowability and low viscosity at low temperatures (less than 4°C) and forms a gel at elevated temperatures (body temperature) (16).

PLO gel is physical organogel that is typically formulated by undergoing the heating–cooling process. These organogels consist of lecithin-based phospholipids and polymeric surfactant molecules by forming a three-dimensional network of polymers. Because PLO gel was formed by adding droplets of water into a system, this type of matrix can be formulated as a reverse micellar–based organogel system by combining hydrophilic linkers (17).

Lecithin is a mixture of phospholipids containing phosphatidylcholine and a naturally occurring biocompatible substance that can form diverse types of supramolecular structures in collaboration with water (18,19). In the preparation of PLO gel, lecithin assembles into reversed polymer-like micelles when water is added, and initial micelles gradually tangle together into a three-dimensional network in the bulk phase when added to oil by dissolving trace amounts of water.

This study was performed to develop PLO gel into cosmetic formulations as PLO gel has drawn much attention as a TDDS.

In the previous literature, the poloxamer 407 solution at a certain concentration had liquidity at room temperature or below, and micelles turned into PLO gel at body temperature in the formation of ordered cuboidal structures due to the dehydration of the micellar core at elevated temperature (20). This process has the advantage of cost-effectiveness by efficiently concentrating a specific drug at the right time and place and increasing drug safety (8). However, this also presents a limitation to be used in cosmetics that requires cosmetic shelf life and stability at low temperature.

To improve the flowability and phase separation of PLO gel at low temperatures to be applied in cosmetic formulations, this study intends to propose PLO gel formulation suitable for cosmetics through the measurement of time-elapsed change at different temperatures, field emission scanning electron microscope (FE-SEM), differential scanning calorimetry (DSC), rheology, and skin permeation efficiency using the response surface methodology (RSM).

MATERIALS AND METHODS

MATERIALS

In the preparation of PLO gel, poloxamer 407 (Pluronic F127 NF, BASF, Ludwigshafen, Germany), cetyl ethylhexanoate (CEH, Kokyu Alcohol Kogyo Co., Chiba, Japan), PEG-400 (SFC Co., Ltd., Seoul, Korea), 1,2-hexanediol (Twinchem Inc., Gwangju, Korea), butylene glycol (1,3-butylene glycol, Daicel, Hiroshima, Japan), dipropylene glycol (Dipropylene glycol care, BASF), pentylene glycol (Hydrolite-5, Symrise, Holzminden, Germany), phenoxyethanol (Phenoxyethanol, Galaxy, Mumbai, India), and hydrogenated lecithin containing 75% phosphatidylcholine were used in the present study. In general, purified water (DI-water) used in cosmetics was prepared using a water distillation apparatus (pure RO 130, Human Co., Seoul, Korea).

To assess skin permeation efficiency, niacinamide (Western Drug, Mumbai, India) was used as an indicator substance. For mixing, agi-mixer (overhead stirrer, SL4000, Global Lab, Siheung, Korea) and a hot plate (hot plate stirrer, HS-20, LK Lab Korea, Namyangju, Korea)

were used. All ethyl alcohol (99.5%, Sigma-Aldrich, Darmstadt, Germany) and distilled water used in the assay were HPLC grade.

METHOD OF PREPARATION

PLO gel formed is liquid (a sol phase) at low temperatures (around 10°C) and undergoes a phase transition (a gel phase) when the temperature is elevated. Thermo-responsive polymers are macromolecular gels that undergo a sol–gel phase transition or volume phase transition depending on the outside temperature, and this reversible phase transition can detect temperature change (21). A sol–gel phase transition is known to exhibit a reversible phase change as a system that physically forms hydrogels in response to temperature change (Figure 1A). Volume phase transition is the phenomenon of swelling–shrinking of gels depending on temperature change without being dissolved in water (Figure 1B). A critical solution temperature is the temperature at which a phase transition occurs. Lower critical solution temperature (LCST) is the minimum temperature of phase transition in the concentration–temperature diagram and is the temperature at which phase separation occurs in the homogeneous phase with increasing temperature. Upper critical solution temperature (UCST) is the highest temperature at which an opposite phase transition occurs (22,23). Since the UCST system has been limited by relatively high temperatures that could affect the properties of drugs or physiologically active substances, the LCST system has attracted much attention in drug delivery (24). Therefore, PLO gel exhibits the LCST behavior, and this is attributable to the unique characteristic of poloxamer 407 that shows flowability at low temperatures.

For the preparation of the water phase, poloxamer 407 was slowly isolated at 1,500 rpm with agitator in 3°C purified water and kept at 3°C for 12 h. The oil phase was prepared by completely dissolving poloxamer 407 using a hot plate set at 75°C at 250 rpm and keeping at room temperature for 12 h.

Last, the polyol phase was performed. The aqueous phase at cold temperature was slowly added to the oil phase and stirred with agi-mixer at 1,800 rpm for 10 min. The polyol phase was gradually added to the mixture of the oil and water phases at 1,800 rpm for 10 min.

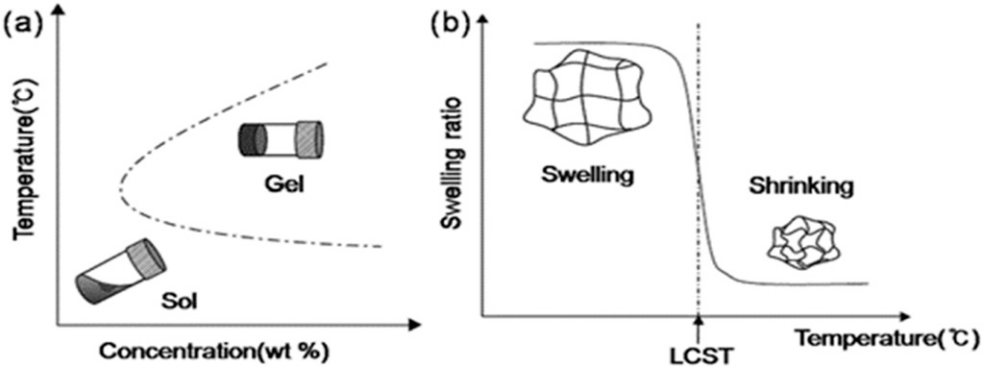


Figure 1. Phase diagram of thermo-responsive polymers: (A) sol–gel transition and (B) volume transition (25).

In conventional PLO gel formulations, isopropyl palmitate (298.5 g/mol) or isopropyl myristate (270.5 g/mol) was used to dissolve hydrogenated lecithin in the oil phase. In the present study, CEH (368.6 g/mol) with higher molecular weight was used.

Various polyols were tested in the preliminary experiment (Table I). Polyols are used as moisturizer because of their humectant function, and can serve as cosolvents of active substances and act as cosurfactants through hydrogen bonding with water molecules (25). Polyol candidates used in the experiment are all commonly used as cosmetic ingredients, including butylene glycol, dipropylene glycol, pentylene glycol, 1,2-hexanediol, and PEG-400, and tests were performed for each substance at a concentration of 18%.

RANGES OF THE INDEPENDENT VARIABLES IN RSM

In this study, RSM was used to design the experiments to determine the effects of hydrogenated lecithin, the main constituent of the oil phase; PEG-400 of the polyol phase; and poloxamer 407 of the water phase on PLO gel formulation by choosing PEG-400 as a representative polyol based on the results of preliminary experiments. The concentrations of hydrogenated lecithin, PEG-400, and poloxamer 407 were selected as independent variables. The range of independent variables is shown in Table II.

For experiment design, Design-Expert Version 11 Software (Stat-Ease Inc., Minneapolis, MN) was used, and Box–Behnken design, an RSM design, was used in the experiment. The Box–Behnken design involves IBFact, center points, and factors located at the same distance from the center of the cube. Figure 3 represents the structure of the Box–Behnken design with three factors (26). Figure 3 and Table III depict a 17-run Box–Behnken design.

Using the data collected from the experiment design, the relationship between response variables (Y) and independent variables (X) can be expressed as a mathematical formula, and this equation is called a model (14). In this study, a response surface model by RSM can be represented as the following equation.

$$Y_i = a_0 + a_1A + a_2B + a_3C + a_{12}AB + a_{13}AC + a_{23}BC + a_{11}A^2 + a_{22}B^2 + a_{33}C^2,$$

where *Yi* presents the estimated value of the response variable, and *A*, *B*, and *C* are independent variables. The value of a_0 is a constant, and a_i, $a_{ii,}$ and a_{ij} are the linear, quadratic, and interactive coefficients, respectively.

Table I
Basic Formula for PLO Gel Preparation with Different Three-Phase Compositions

	Ingredients	#1–1	#1–2	#1–3	#1–4	#1–5
Oil phase	Hydrogenated lecithin	1.8	1.8	1.8	1.8	1.8
	CEH	10.2	10.2	10.2	10.2	10.2
Polyol phase	1,2-Hexanediol	18.0	-	-	-	-
	Butylene glycol	-	18.0	-	-	-
	Dipropylene glycol	-	-	18.0	-	-
	Pentylene glycol	-	-	-	18.0	-
	PEG-400	-	-	-	-	18.0
Water phase	DI-water	To 100	To 100	To 100	To 100	To 100
	Poloxamer 407	20.0	20.0	20.0	20.0	20.0
	Phenoxyethanol	0.3	0.3	0.3	0.3	0.3

Table II
Independent Variable Range of Preliminary Experiment

	Level		
Independent variables	Low (−1)	Middle (0)	High (+1)
A: hydrogenated lecithin (wt%)	1.0	3.0	5.0
B: PEG-400 (wt%)	15.0	20.0	25.0
C: poloxamer 407 (wt%)	15.0	20.0	25.0

STABILITY TEST

This study performed the sensory evaluation of time-elapsed change made over a month by date in the formulated PLO gel stored at low temperature (4°C), room temperature (25°C), and constant temperature (40°C). To determine the flowability and separation of formulations, the cycling test was conducted to test physical stability of formulations by storing them at –15°C, 40°C, and 25°C each for 24 h.

FE-SEM OBSERVATION

FE-SEM (Quanta 3D FEG, FEI Company) was used to observe the morphological characteristics and properties of PLO gel. Unlike conventional electron microscopy using a thermal emission gun, FE-SEM is a field emission scanning electron microscope that

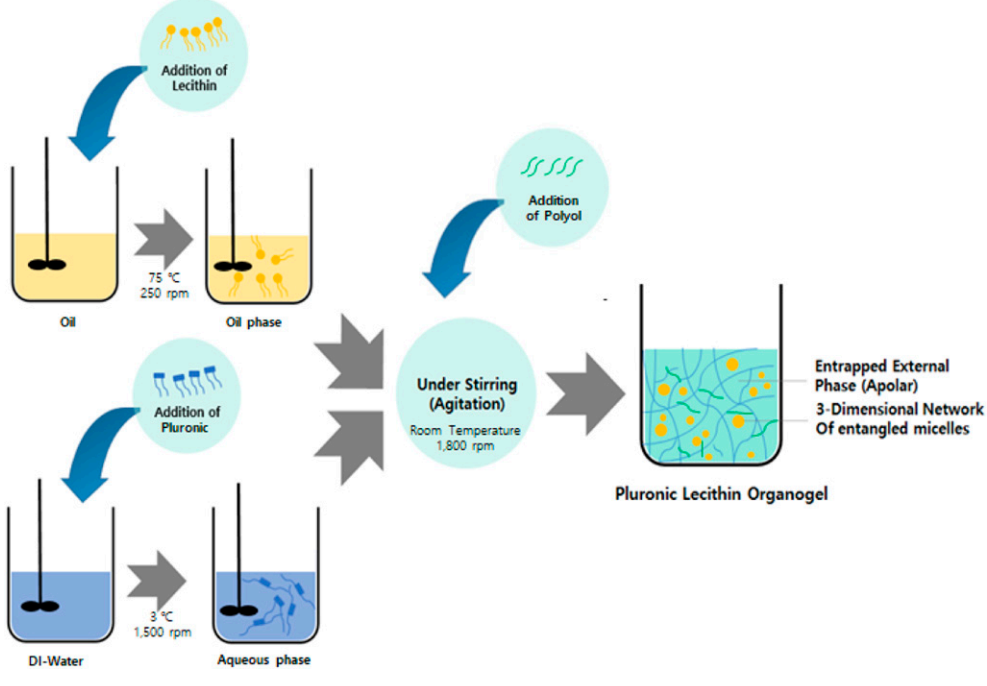
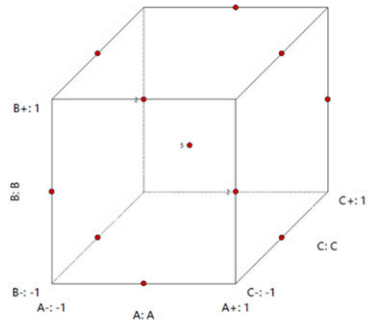


Figure 2. Preparation method of PLO gel.



	A	B	C
IBFact	-1	0	-1
	-1	0	+1
	+1	0	-1
	+1	0	+1
	0	-1	-1
	0	-1	+1
	0	+1	-1
	0	+1	+1
	-1	-1	0
	+1	-1	0
	-1	+1	0
	+1	+1	0
Center	0	0	0

Figure 3. Box–Behnken design. The point at the center of the cube corresponds to the center of the Box–Behnken design, and the point at the middle of each line of the cube corresponds to the IBFact.

emits electrons by generating a strong electric field on the filament metal surface as an electron source. Compared with traditional SEM, FE-SEM has the advantage of higher resolution that provides images with a higher resolution and allows observation at high magnification (up to ×100,000) using a low accelerating voltage in specimens with electron beam–induced damage (27).

Table III
Independent Variable Range for PLO Gel Optimization Experiment

		Factor 1	Factor 2	Factor 3
		A: hydrogenated lecithin	B: PEG-400	C: poloxamer 407
Run	Space type	(wt%)	(wt%)	(wt%)
#2–1	Center	3.0	20.0	20.0
#2–2	IB factor	1.0	25.0	20.0
#2–3	IB factor	5.0	20.0	15.0
#2–4	IB factor	5.0	15.0	20.0
#2–5	IB factor	3.0	25.0	15.0
#2–6	IB factor	1.0	20.0	15.0
#2–7	Center	3.0	20.0	20.0
#2–8	Center	3.0	20.0	20.0
#2–9	IB factor	3.0	25.0	25.0
#2–10	IB factor	1.0	25.0	25.0
#2–11	IB factor	3.0	15.0	15.0
#2–12	IB factor	3.0	25.0	25.0
#2–13	IB factor	5.0	20.0	20.0
#2–14	Center	3.0	20.0	20.0
#2–15	IB factor	5.0	25.0	25.0
#2–16	IB factor	1.0	20.0	20.0
#2–17	Center	3.0	20.0	20.0

DSC MEASUREMENT

Differential scanning calorimetry (DSC 214 Polyma, Netzsch, Germany) was used in thermal analysis of formulated PLO gel. Temperatures ranged between –6 ~ 90°C in the first heating, 90 ~ -60°C in the first cooling, and 60 ~ 90°C in the second heating cycles at a heating rate of 10°C/min. The temperature ranges were selected to assess the change in the physical properties of PLO gel by temperature, and measurements on nitrogen were carried out.

RHEOLOGY MEASUREMENT

To assess rheological properties including viscosity and elasticity in PLO gel formulations, viscoelasticity was measured using a rheometer (Rheolaser Master, Formulaction, Toulouse, France). In general, scatterers (particles, droplets, fibers, *etc*.) are constantly in motion because of Brownian motion in samples with viscoelastic properties. This constant motion of scatterers results in deformation of speckle image by time. The speed of scatterer movement varies by viscoelastic properties and also affects the deformation speed of speckle image. Therefore, the rheological properties of the sample including viscoelasticity can be assessed by measuring the deformation speed of speckle image. The Rheolaser Master uses diffusing-wave spectroscopy (DWS), which is an optical technique derived from dynamic light scattering (DLS). The DWS method is based on microrheology (28).

The measurement of rheological properties was performed by converting the change in the mean square displacement (MSD) over a period of time into numerical values. Measurement was performed at room temperature (25°C) for 3 h.

IN VITRO SKIN PERMEATION TEST

The transdermal Franz diffusion cell system (FDC-6T, Logan, Somerset, NJ) was used to determine the skin permeation efficiency of the formulated PLO gel. An artificial membrane (Strat-M membrane) was set in the diffusion cell array system, and *in vitro* percutaneous absorption test was carried out by applying 400 µL of test solution in the donor and 50% ethanol solution in the receptor at 32 ± 1°C. The receptor fluid in the receptor compartment was collected at 2, 4, and 8 h after applying the test solution, and skin penetrant in the donor solution and all membranes was collected 8 h after applying the test solution. The collected solutions were analyzed with the HPLC system (Alliance HPLC, Waters, Santa Clara, CA) under the conditions presented in Table IV.

Table IV
HPLC Analysis Condition

Instrument	Conditions
Column	C_{18} 4.6 mm × 250 mm, 5.0 µm
Column temp.	40°C
Mobile phase	Methanol: 0.01% Trifluoreoacetic acid in Water (5:95)
Detector	PDA Detector (261 nm)
Flow rate	1.0 mL/min
Injection volume	10 µL

STATISTICAL ANALYSIS

Experiments were repeated three times, and data were presented as means ± SD. Significance in difference was tested by Student's *t*-test. Differences were considered significant at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

RESULTS AND DISCUSSION

STABILITY EVALUATION

Stability evaluation was conducted at low temperature (4°C), room temperature (25°C), and constant temperature (45°C) each. PLO gel remained the same and stable at room (25°C) and constant (45°C) temperatures, but separated and turned to liquid at low temperature (4°C), showing that the gels were not suitable ingredients for cosmetic formulations.

In the polyol selection experiment, stability evaluation by temperature revealed that the all-formulated organogels were stable at room (25°C) and constant (45°C) temperatures. Excluding PEG-400, phase separation began to occur from day 7 at low temperature (4°C), and complete separation occurred 3 weeks after starting observation of time-elapsed change (Figure 4). Based on these findings, PEG-400 was considered as the most appropriate PLO gel, and the experiment was additionally carried out by choosing PEG-400 as a polyol. In the cycling test, phase separation also occurred in all PLO gel, except for PEG-400 (data not shown).

When PEG (polyethylene glycol) is adsorbed to the colloidal surface, the colloidal activities are substantially reduced, and the growth rate of the colloids is limited in certain aspects. In the experiment, the addition of PEG changes the kinetics of the growth process by inducing the rapid growth of nucleation and the aggregation of nanoparticles. Therefore, the addition of PEG can promote the crystallinity of samples and change the product morphology (29). The present study confirmed that the addition of PEG-400 maintains the stability of PLO gel at low temperature.

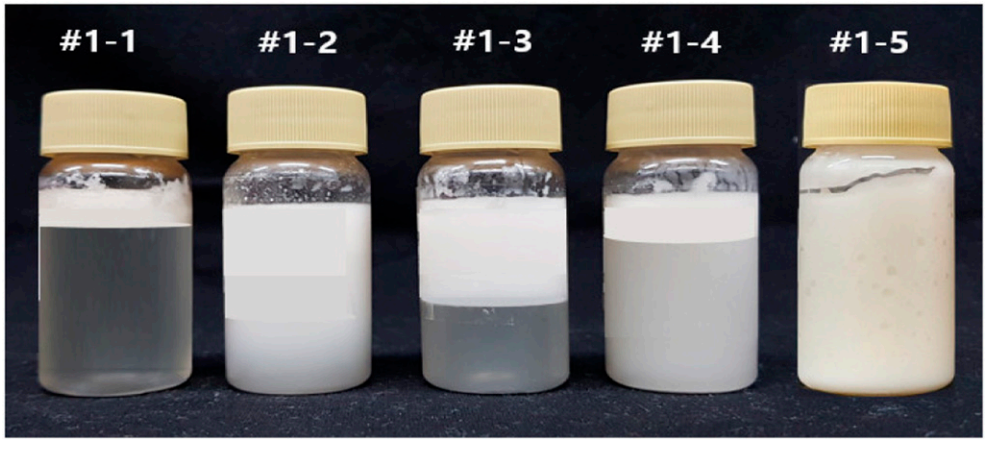


Figure 4. After 3 weeks' stability at low temperature (4°C) (PLO gel #1).

After choosing PEG-400 as a polyol, the experiments on PLO gel formulations at different ratios of each composition were performed using RSM. Stability was displayed in all formulations at room temperature (25°C) and constant temperature (45°C), but the formulated gels #2–6 and #2–11 showed separation and flowability at low temperature (4°C) from day 7, which indicated a gradual phase separation with time. Complete phase separation occurred 3 weeks after starting observation of time-elapsed change. All other formulations showed stability even after 6 months (Figure 5). In the cycling test, complete phase separation occurred in #2–6 in the first cycle and #2–11 in the second cycle. Excluding these two formulated gels, phase separation did not occur in all PLO gel formulations. Formulation #2–6 appeared to be unstable due to relative smaller amounts of poloxamer 407 and hydrogenated lecithin. Formulation #2–11 seemed to be unstable due to relatively smaller concentrations of poloxamer 407 and PEG-400. Of all testing formulations #2–3, #2–5, #2–6, and #2–11 with a poloxamer 407 concentration of 15.0%, phase separation occurred in #2–6 and #2–11. This outcome is thought to be attributable to relatively smaller amounts of PEG-400 and hydrogenated lecithin. Of all testing formulations #2–2, #2–6, #2–10, and #2–16 with a hydrogenated lecithin concentration of 1.0%, phase separation occurred in #2–6 alone. This outcome seems to be resulting from relatively smaller content of poloxamer 407 at 15.0%. Of #2–4 and #2–11 with PEG-400 concentration of 15.0%, phase separation only occurred in #2–11. This outcome is thought to be attributable to relatively smaller amounts of poloxamer 407 and hydrogenated lecithin. The outcomes of stability evaluation revealed that all three phases of PLO gel had significant effects on stability.

MORPHOLOGY OF PLO GEL

For observation of PLO gel morphology, FE-SEM was used to examine the morphology of stable PLO gel #2–3. After pretreatment with cryo-system, the structure of PLO gel

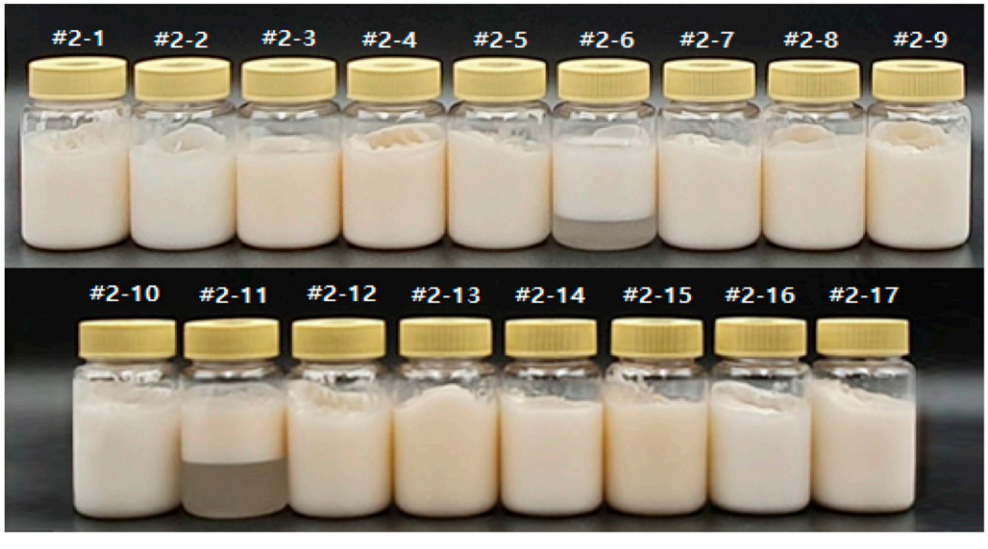


Figure 5. After 3 weeks' stability at low temperature (4°C) (PLO gel #2).

#2–3 resembled a microemulsion-based gel (10,30) and polymeric bicontinuous microemulsion structure (31) (Figure 6). A bicontinuous microemulsion structure is formed by mixing the appropriate amounts of oil, water, and amphipathic substances, and bicontinuous microemulsion is known to form an interesting structure consisting of undulation and boundary having a mean curvature (32).

DSC MEASUREMENT

The inflection points of the DSC curves of the formulated PLO gel commonly lingered around –10°C, which corresponds to the temperature where property changes in PLO gel began to occur (Figure 7).

Phase separation or reduced viscosity (flowability) occurred at low temperature (4°C) in the formulated PLO gel #2–5, #2–6, and #2–11, with DSC values of less than 0.4 around the peak of –10°C as shown in Figure 7, and this resulted in pre-marked marks on the surface disappear.

On the contrary, PLO gel formulations with DSC values of greater than 0.4 at –10°C were mostly stable. These results indicate that there is a correlation between formulation stability and DSC value.

RHEOLOGY STUDY RESULT

Rheological characteristics were assessed using a rheometer. This instrument uses DWS, which is an optical technique derived from DLS. Because a rheometer determines rheological properties based on the Brownian motion, this instrument has the advantage of accurately identifying formulation properties compared with physical appraisal methods. With rheology measurement, particle motion speed represents viscosity and particle displacement denotes elasticity.

To present them into numerical values, index values were obtained through data processing of the change in MSD with time. Macro viscosity index (MVI) indicates viscosity.

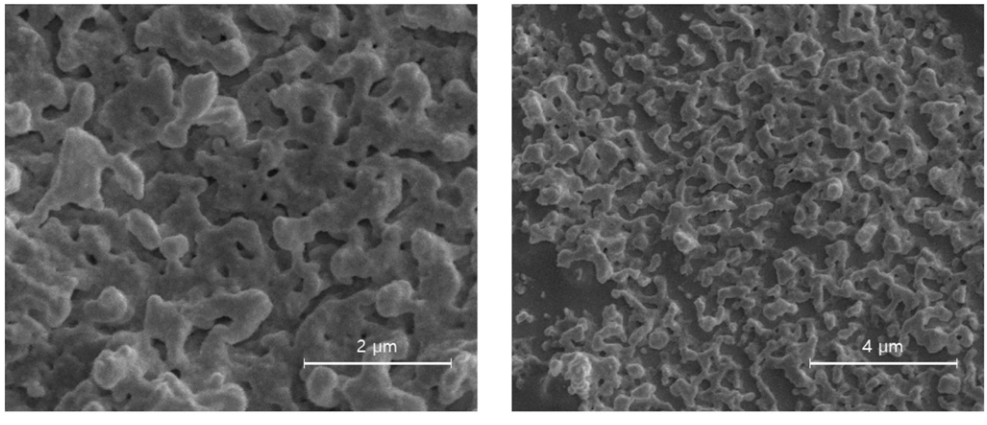


Figure 6. Cryo FE-SEM result of PLO gel #2–3.

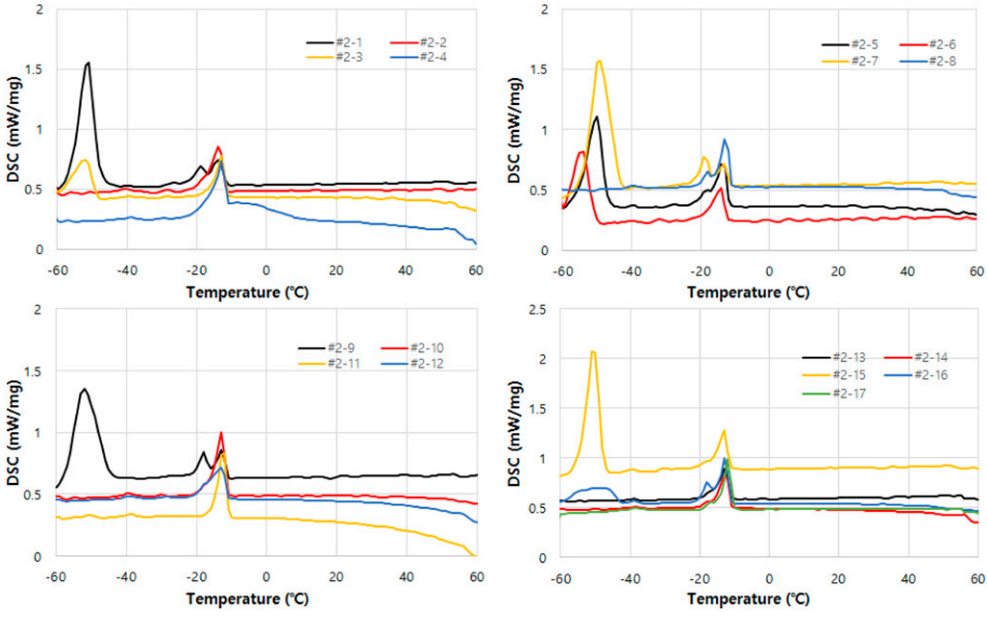


Figure 7. DSC results of PLO gels.

Because the area of the MSD increases with decreasing viscosity over time, the viscosity index was obtained as the multiplicative inverse of the MSD. The elasticity index (EI) indicates elasticity. By comparing the area of the MSD repeatedly measured 15 times at the same time, the larger the change in the area of MSD, the lower the elasticity. EI was estimated as the multiplicative inverse of the change in MSD area. The smaller the change in the area of MSD, the higher the elasticity; EI was calculated as the multiplicative inverse of the change in MSD area. As results, the MVI that indicates the viscosity grade and the EI that presents the elasticity grade were obtained. This study identified that the higher the MVI, the higher the viscosity, and the higher the EI, the higher the elasticity.

In rheology measurement at room temperature (25°C) with time and assessment of the change in the MSD, the all 17 formulated PLO gel showed a curved-shape line, implying that all of these formulations had viscoelasticity. Of these, taking #2–1, the center value, as the baseline in Figure 8, #2–6 showing flowability at low temperature had high MSD over time. The formulation #2–12 had low MSD in the beginning, but MSD showed a rapidly increasing tendency from a decorrelation time of 10^2 sec. The relatively smooth and spreadable formulation #2–3 had higher MSD than #2–1, the center value, but displayed lower MSD than #2–6 with flowability at low temperature.

Based on MSD values, data were converted to numerical index values reciprocally. Figures 9 and 10 represent viscosity and elasticity, respectively.

In the analysis of viscosity and elasticity in each PLO gel, #2–6 formulation exhibited phase separation at cold temperature and had low viscosity and elasticity. PLO gel #2–11 had lowest viscosity and low elasticity. On the contrary, PLO gel #2–2 with the lowest elasticity appeared to have no phase separation by having relatively higher viscosity.

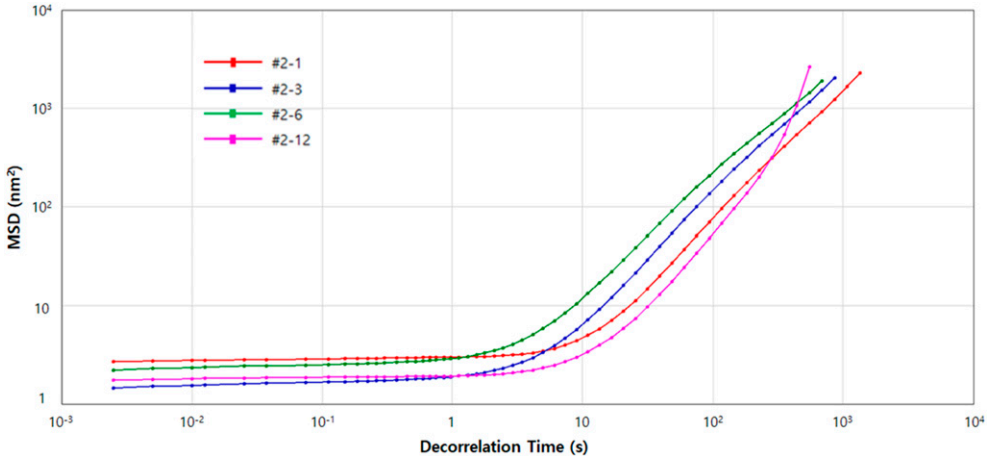


Figure 8. Visco-elasticity results of PLO gels.

These findings imply that viscosity has more significant influence on formulation stability at low temperature than elasticity.

RSM RESULT

Table V shows the data for response variables by independent variables according to the experimental design used in this study. Analysis of variance (ANOVA) was used in determining the most appropriate response surface model for the effect of independent variables. Using this response surface model, the changes in response variables including viscosity, elasticity, and DSC (mW/mg) at a gelation point of –10°C were estimated by the concentrations of poloxamer 407, hydrogenated lecithin, and PEG-400 as independent variables. Table VI represents an adequate model according to the results of the ANOVA. Interactive coefficients and *p* values represent the effect of independent variables on response variables in the model. The 3D response surface graphs (Figures 11–13) are used in interpreting the

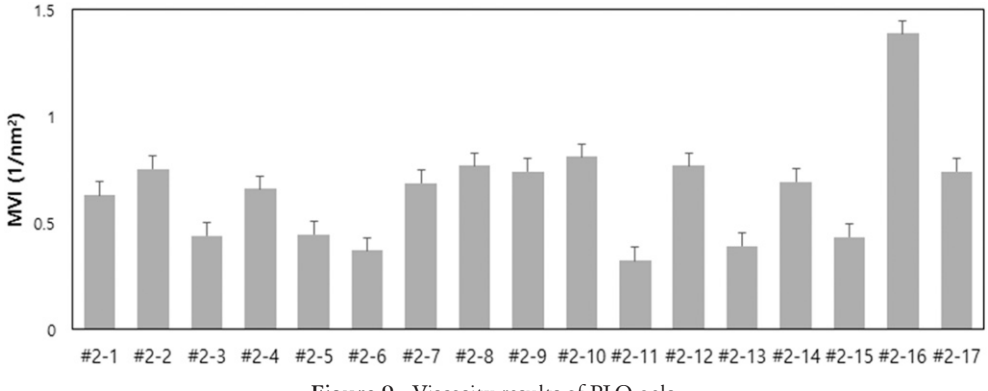


Figure 9. Viscosity results of PLO gels.

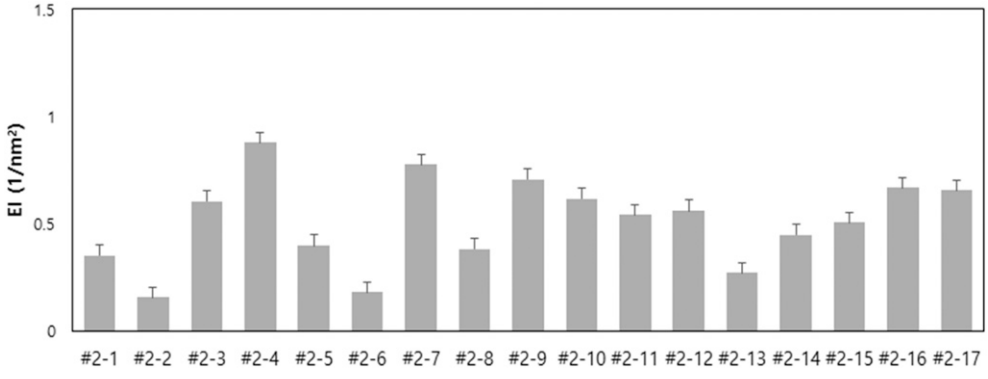


Figure 10. Elasticity results of PLO gels.

significant impact of independent variables on response variables in the response surface model (33).

FACTORS AFFECTING ELASTICITY (Y_1)

The results of the ANOVA on elasticity indicated that a linear model was appropriate. The p value was 0.0040 (<0.05); F value was 7.3545, having a significance; and the coefficient of determination (R^2) was 0.6292. The response surface model for elasticity is as follows:

Table V
Box-Behnken Design Experiments for PLO Gel Optimization

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
		A: Hydrogenated lecithin	B: PEG-400	C: Poloxamer 407	Elasticity	Viscosity	DSC
Run	Space type	wt%	wt%	wt%	EI (1/nm^2)	MVI (1/nm^2)	mW/mg
#2–1	Center	3.0	20.0	20.0	0.3511	0.6300	0.53319
#2–2	IB factor	1.0	25.0	20.0	0.1555	0.7539	0.47859
#2–3	IB factor	5.0	20.0	15.0	0.6021	0.4382	0.44563
#2–4	IB factor	5.0	15.0	20.0	0.8769	0.6585	0.37812
#2–5	IB factor	3.0	25.0	15.0	0.3936	0.4468	0.36887
#2–6	IB factor	1.0	20.0	15.0	0.1801	0.3688	0.24754
#2–7	Center	3.0	20.0	20.0	0.7743	0.6874	0.53816
#2–8	Center	3.0	20.0	20.0	0.3804	0.7676	0.54413
#2–9	IB factor	3.0	25.0	25.0	0.7060	0.7417	0.63741
#2–10	IB factor	1.0	25.0	25.0	0.6160	0.8087	0.50225
#2–11	IB factor	3.0	15.0	15.0	0.5406	0.3230	0.38335
#2–12	IB factor	3.0	25.0	25.0	0.5591	0.7662	0.47410
#2–13	IB factor	5.0	20.0	20.0	0.2696	0.3910	0.59556
#2–14	Center	3.0	20.0	20.0	0.4471	0.6925	0.55291
#2–15	IB factor	5.0	25.0	25.0	0.5036	0.4329	0.88435
#2–16	IB factor	1.0	20.0	20.0	0.6652	1.3879	0.55577
#2–17	Center	3.0	20.0	20.0	0.6547	0.7400	0.59108

Table VI
ANOVA for RSM Model

	Y_1, elasticity	Y_2, viscosity	Y_3, DSC
Run	EI ($1/nm^2$)	MVI ($1/nm^2$)	mW/mg
p-value	0.0040	0.0215	0.0107
F-value	7.3545	5.0995	5.6237
R^2	0.6292	0.8677	0.5648
Model	Linear	Quadratic	Linear

$$Y_1 = 0.5103 + 0.0527A - 0.2077B + 0.0789C, \quad (1)$$

where Y_1 represents the elasticity, A is the hydrogenated lecithin content, B is the PEG-400 content, and C is the poloxamer 407 content. Using equation 1, coefficient was high in the order of PEG-400 B (0.2077), poloxamer 407 C (0.0789), and hydrogenated lecithin A (0.0527). Main effect was significant in the order of B, C, and A.

Figure 11 depicts the 3D response surface graph using a regression equation. Figure 11A is the graph that presents the effect of the amounts of hydrogenated lecithin and PEG-400 on elasticity when a fixed amount of poloxamer 407 was mixed in the formulated PLO gel. This graph reveals that elasticity increased with an increase in the content of hydrogenated lecithin and a decrease in the content of PEG-400. Figure 11B represents the effect of the amounts of hydrogenated lecithin and poloxamer 407 on elasticity when a fixed amount of PEG-400 was mixed. This graph reveals that the content of hydrogenated lecithin and poloxamer 407 had almost no effect on elasticity. Figure 11C shows the effect of the amounts of poloxamer 407 and PEG-400 when a fixed amount of hydrogenated lecithin was mixed. This graph demonstrates that elasticity increased with an increase in the content of poloxamer 407 and a decrease in the content of PEG-400. The aforementioned findings suggest that PEG-400 concentration appeared to have the most significant impact on elasticity, whereas the content of hydrogenated lecithin and poloxamer 407 had insignificant influence on elasticity.

FACTORS AFFECTING VISCOSITY (Y_2)

The results of the ANOVA on viscosity indicated that a quadratic model was appropriate. The p value was 0.0215 (<0.05); F value was 5.0995, having a significance; and the coefficient of determination (R^2) was 0.8677. The response surface model for viscosity is as follows:

$$\begin{aligned} Y_2 &= 0.7035 - 0.1748A - 0.2050B + 0.0358C + 0.0916AB \\ &\quad - 0.1113AC - 0.0248BC + 0.0186A^2 + 0.0758B^2 - 0.2099C^2, \end{aligned} \quad (2)$$

where Y_2 represents the viscosity. In the aforementioned equation, hydrogenated lecithin and PEG-400 were shown to have a reciprocity relation with viscosity, a response variable. The squared value of poloxamer 407 content was found to have a reciprocity relation with viscosity. As shown in Table VI, the p value of each coefficient revealed that the

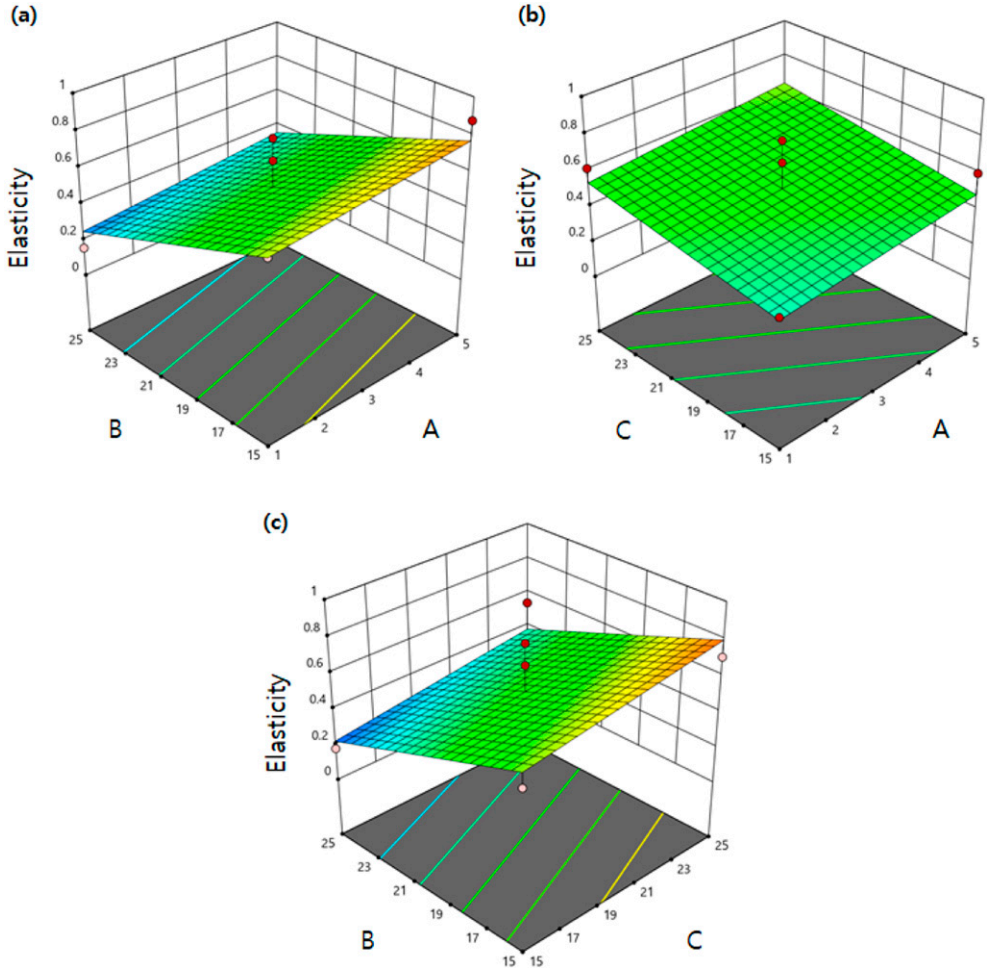


Figure 11. Response surface for elasticity results of PLO gels as a (A) hydrogenated lecithin and PEG-400, (B) hydrogenated lecithin and poloxamer 407, and (C) poloxamer 407 and PEG-400. (A) hydrogenated lecithin, (B) PEG-400, and (C) poloxamer 407.

content of hydrogenated lecithin and PEG-400 was found to have a significant effect on viscosity (<0.05), and the squared value of poloxamer 407 content was shown to have a significant effect on viscosity (<0.05). The 3D response surface graph demonstrates that viscosity decreased with an increase in the content of hydrogenated lecithin and PEG-400, and decreased in a curved-shape form with an increase in the poloxamer 407 content (Figure 12).

Using equation 2, coefficient was high in the order of PEG-400 *B* (0.2050), hydrogenated lecithin *A* (0.1748), and poloxamer 407 *C* (0.0358), indicating that the main effect was significant in the order of *B*, *A*, and *C*.

Figure 12 depicts the 3D response surface graph using a regression equation. Figure 12A is the graph that shows the effect of the amounts of hydrogenated lecithin and PEG-400 on viscosity when a fixed amount of poloxamer 407 was used. This graph reveals that viscosity gradually decreased as the amounts of hydrogenated lecithin and PEG-400 increased, but

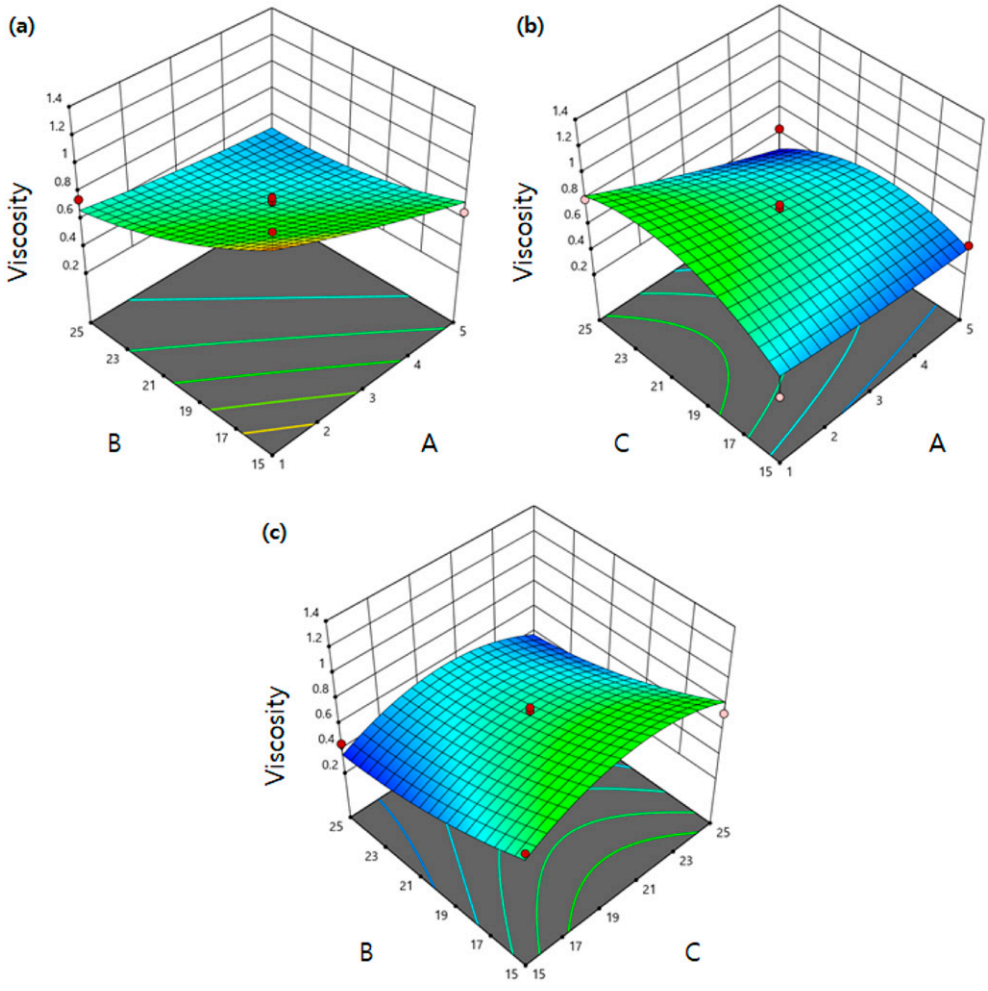


Figure 12. Response surface for viscosity results of PLO gels as (A) hydrogenated lecithin and PEG-400, (B) hydrogenated lecithin and poloxamer 407, and (C) poloxamer 407 and PEG-400. (A) hydrogenated lecithin, (B) PEG-400, and (C) poloxamer 407.

showed a drastic decrease with an interaction effect of hydrogenated lecithin and PEG-400. Figure 12B presents the effect of the content of hydrogenated lecithin and poloxamer 407 on viscosity when a fixed amount of PEG-400 was used. In this graph, viscosity exhibited a gradual decrease as the amount of hydrogenated lecithin increased, and then maintained constant after a gradual increase as poloxamer 407 content increased. Figure 12C is the graph that demonstrates the effect of the content of poloxamer 407 and PEG-400 on viscosity when a fixed amount of hydrogenated lecithin was used. In this graph, viscosity first showed a small increase and then it decreased with increasing poloxamer 407 concentration. Viscosity exhibited a drastic decrease as the concentration of PEG-400 showed an increase. To sum up the previous outcomes, the content of PEG-400 was found to have the most significant influence on viscosity, followed by hydrogenated lecithin. The content of poloxamer 407 alone seemed to have almost no impact, but had an influence to some extent in association with the other two compositions.

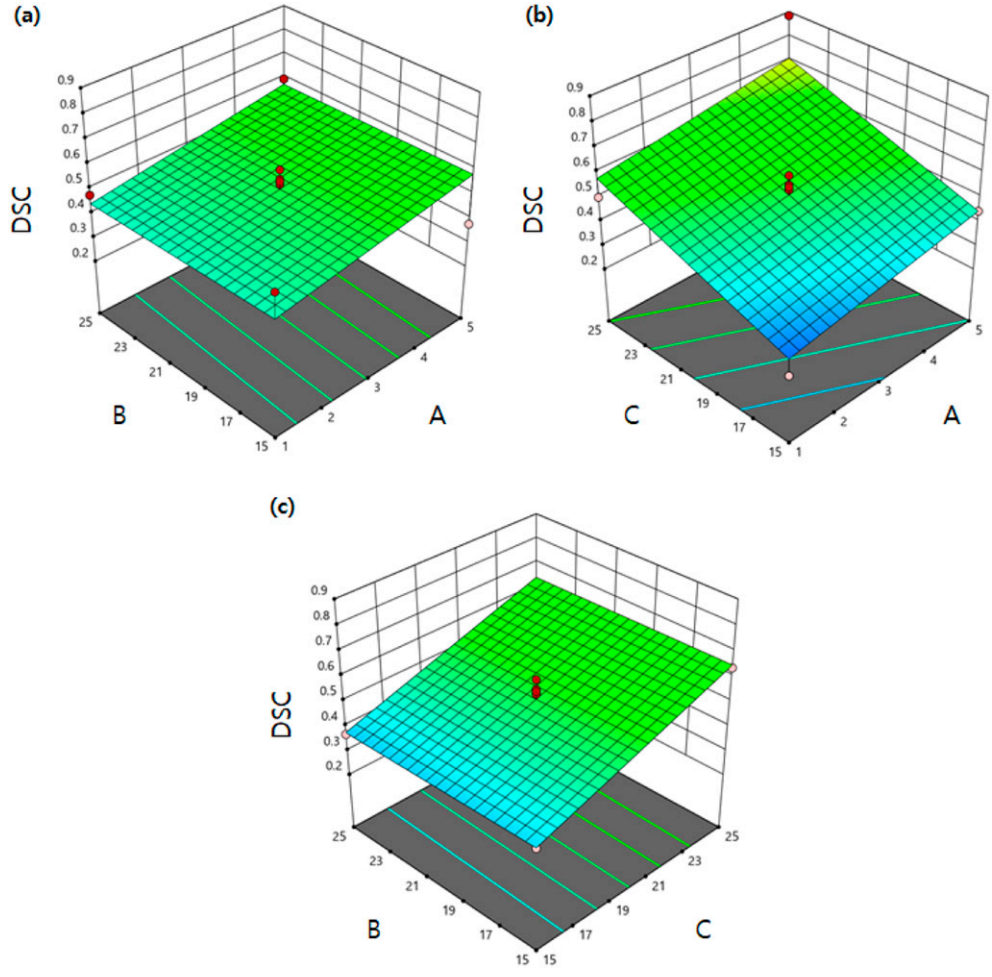


Figure 13. Response surface for DSC results of PLO gels as (A) hydrogenated lecithin and PEG-400, (B) hydrogenated lecithin and poloxamer 407, and (C) poloxamer 407 and PEG-400. (A) hydrogenated lecithin, (B) PEG-400, and (C): poloxamer 407.

FACTORS AFFECTING DSC (Y_3)

The results of the ANOVA on the gelation point indicated that a linear model was appropriate. The p value was 0.0107 (<0.05); F value was 5.6237, having a significance; and the coefficient of determination (R^2) was 0.5648. The response surface model proposed for DSC is as follows:

$$Y_3 = 0.5124 + 0.0649A - 0.0047B + 0.1316C, \quad (3)$$

where Y_3 represents the gelation point. Equation 3 demonstrated that a correlation was present between the content of poloxamer 407 and DSC. As shown in Table VI, the p value of each coefficient revealed that the concentration of poloxamer 407 was found to have a significant impact on DSC.

Table VII
Results of Contents and Diffusion Rate of PLO Gel in Niacinamide

Site	Sampling time (h)	Detected quantity (μg)	Penetration rate (%)	Permeation rate (%)
Receptor	2	6.65 ± 0.72	0.30 ± 0.03	22.62 ± 3.59
	4	17.06 ± 0.51	0.47 ± 0.06	
	8	278.09 ± 97.26	12.52 ± 4.38	
Membrane	8	224.38 ± 70.74	10.10 ± 3.19	
PLO gel #2–1	-	2,221.08	-	-

The table shows the *p*-values of the coefficients and coefficients of each term of the skin permeation. The coefficient with a negative value indicates each relationship with 2 and 4 h, that *p*-value was under 0.05 ($p \leq 0.05$). But 4 and 8 h coefficient and 2 and 8 h coefficient p-value were over 0.05 ($*p > 0.05$).

Using equation 3, coefficient was high in the order of poloxamer 407 *C* (0.1316), hydrogenated lecithin *A* (0.0649), and PEG-400 *B* (0.0047), implying that the main effect was significant in the order of *C*, *A*, and *B*.

Figure 13 depicts the 3D response surface graph using a regression equation. Figure 13A is the graph that shows the effect of the amounts of hydrogenated lecithin and PEG-400 on DSC when a fixed amount of poloxamer 407 was mixed in the formulated gels. This graph demonstrates that the amounts of hydrogenated lecithin and PEG-400 had no impact on DSC. Figure 13B displays the effect of the content of hydrogenated lecithin and poloxamer 407 on DSC when a fixed amount of PEG-400 is mixed. This graph demonstrates that DSC increased with an increase in the content of hydrogenated lecithin and poloxamer 407. Figure 13C shows the effect of the amounts of poloxamer 407 and PEG-400 on DSC when a fixed amount of hydrogenated lecithin was mixed. This graph reveals that DSC showed an increase as the content of poloxamer 407 increased. The aforementioned findings suggest that the content of PEG-400 was found to have the most significant influence on DSC, followed by hydrogenated lecithin. PEG-400 seemed to have almost no impact.

IN VITRO SKIN PERMEATION TEST

The Franz diffusion cell method was applied to measure *in vitro* diffusion study. To obtain the transdermal absorption rate of PLO gel, niacinamide was used as an indicator component. A trend line was drawn with HPLC measurement according to niacinamide concentrations, and the R^2 value of the plotted points was one interpreted as greater reliability.

Based on HPLC measurements, the skin permeation efficiency of niacinamide in PLO gel was assessed using the following equation. In the evaluation of *in vitro* skin permeation efficiency of PLO gel, the formulated PLO gel (#2–1) was chosen, and the experiment was repeated four times. Sampling of the receptor was conducted over time (2, 4, and 8 h) using the Franz diffusion cell method. The amount of niacinamide was quantified *via* HPLC analysis by collecting the donor and the membrane after 8 h. Skin permeation efficiency (%) was calculated by substituting the quantified values of each niacinamide concentration to equation 4.

$$A = \frac{C_2 + C_3}{C_1} \times 100 \tag{4}$$

C_1 = Amount of niacinamide in original PLO gel (μg),

C_2 = Amount of niacinamide in receptor (μg),

C_3 = Amount of niacinamide in membrane (μg).

The skin permeation efficiency of the formulated PLO gel was calculated by substituting C_1, C_2, and C_3 values obtained by HPLC analysis into the aforementioned equation. As a result, skin permeation efficiency was 22.62±3.59% in the formulation #2–1, and this outcome reconfirmed the applicability of the formulated PLO gel as a TDDS.

CONCLUSION

This study was performed to investigate the applicability of (PLO gel) in cosmetics as a topical drug delivery system. To achieve this objective, the formulation and assessment of PLO gel were conducted using RSM by mixing three major compositions, including lecithin, PEG-400, and poloxamer 407 prepared at different ratios.

To evaluate the stability of PLO gel formulations, the elapsed time change was observed by storing these formulations at low temperature (4°C), room temperature (25°C), and constant temperature (45°C). The all-formulated organogels were stable at room and constant temperatures, but formulations #2–6 and #2–11 were unstable by exhibiting phase separation.

To examine the properties of the formulated PLO gel, the relatively smooth and spreadable formulation #2–3 was measured using SEM, and this PLO gel exhibited a bicontinuous microemulsion structure. To determine gelation temperature exhibiting phase separation at low temperatures, DSC measurement was performed. To evaluate the characteristics of the gel formulations, elasticity and viscosity were also measured.

RSM was used in analyzing the measurements of elasticity, viscosity, and DSC varying depending on the content ratios of hydrogenated lecithin, PEG-400, and poloxamer 407 that have a significant impact on the properties of the formulated PLO gel, and assessing the degree of influence of each composition. In the degree of influence on elasticity, the content of PEG-400 was most influential, followed by hydrogenated lecithin and poloxamer 407. In the degree of impact on viscosity, the amount of PEG-400 was most influential, followed by hydrogenated lecithin. The content of poloxamer 407 alone in the formulated gels appeared to have almost no effect, but had an influence to some extent in association with the other two compositions. In DSC analysis, the concentration of poloxamer 407 was most influential, followed by hydrogenated lecithin. The content of PEG-400 seemed to have almost no effect.

As previously introduced, PLO gel has shown problems by displaying flowability at low temperatures; issues have been raised concerning the safety of PLO gel in cosmetic formulations. This research has verified the possibility of PLO gel as a safe formulation without flowability at cold temperatures. In addition, skin permeation efficiency was measured using the transdermal Franz diffusion cell system, and the measured value was 22.62±3.59%. This outcome demonstrated that PLO gel is suitable as a TDDS.

In conclusion, the stability of PLO gel has to be maintained without phase separation at low temperature, room temperature, and constant temperature to evaluate the suitability of PLO gel as a cosmetic ingredient. In particular, stability has to be maintained without flowability at cold temperatures. Moreover, because excessively high viscoelasticity may influence the texture and application of the formulation when spreading it on the skin, the formulation with adequate viscoelasticity seems to be most appropriate. Based on the previous findings, the formulation #2–3 is suggested as the most suitable PLO gel in cosmetic formulations.

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