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레시틴 오가노겔을 이용한 난용성 제니스테인의 용해도 향상

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Development of Lecithin Organogel to Improve Solubility of Genistein

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요 약: 오가노겔은 반고형상이며 3차원의 네트워크 구조로 이루어진 친유성 용매로 이루어져 있다. 본 연구에서는 유상과 수상에서 모두 난용성 특징을 가진 제니스테인을 포함하는 레시틴 오가노겔을 개발하였다. 이 시스템은 안정할 뿐만 아니라 경피 흡수 실험에서도 높은 흡수율을 보였다. 본 오가노겔 제형에 적합한 원료들을 선별한 결과, 수화된 레시틴, 해바라기유, dipropylene glycol (DPG), polyethylene glycol (PEG)이 이 시스템에서 주로 사용되었다. 레시틴 오가노겔의 제조에 적합한 원료의 함량은 phase ternary diagram 작성을 통하여 결정하였다. 제조된 레시틴 오가노겔을 organoleptic characteristics, stability, pH, rheology, phase transition temperatures, microscopic analysis, skin penetration 실험을 통해 평가하였다. 본 연구 결과를 통해 본 논문에서 제시하는 레시틴 오가노겔 제형은 안정한 상태에서 난용성 물질을 높은 농도로 피부에 효과적으로 전달할 수 있는 제형으로 활용될 수 있을 것이라 생각된다.

Abstract: Organogels are semi-solid systems that consist of an apolar solvent as the liquid phase within a three-dimensional networked structure. In this study, we developed a stable and skin penetration-enhanced Lecithin Organogel (LO) containing genistein, which is one of the poorly soluble active ingredients in both polar and apolar phase. After screening of various components (type of gelators, organic and aqueous phase), hydrogenated lecithin (HL), sunflower oil (SO), dipropylene glycol (DPG), and polyethylene glycol (PEG) were mainly used in this formulation. Phase ternary diagram was employed for optimization of the composition in the LO. The formulated LO were evaluated for its organoleptic characteristics, stability, pH, rheology, phase transition temperatures, microscopic analysis and skin penetration. The optimized stable LO system can be utilized as an effective and stable cosmetic formulation that can carry poorly soluble active ingredients at high concentration for topical dermal delivery.

Keywords: *cosmetic, organogel, lecithin, skin penetration, genistein*

1. Introduction

Genistein, a major component in soybeans[1], has known that it has antioxidant properties and anti-proliferative effects[2], with no toxicity in rats[3]. Thus, genistein can be an anti-aging agent in cosmetic formulation[4]. However, because of the low aqueous solubility of genistein, there is a difficulty of dissolution in the cosmetic formulation[5]. Also, several studies have reported that genistein barely penetrated through the skin of hairless mice when administered in conventional creams[6].

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Organogels are semi-solid systems that consist of an apolar solvent as the liquid phase within a three-dimensional networked structure[7]. Organogels have been studied as a matrix for topical drug delivery[8]. There are also several studies proving that water can be substituted for such polar organic substances as glycerol, ethylene glycol and formamide[9,10]. Because of this reason, this system could be a great potential drug delivery system for poorly soluble active agents[8].

Organogels can be further classified based on the organogelator[11]. Among the organogels, lecithin organogels (LO), in which lecithin is used as an organogelator, are the most investigated organogels for topical delivery of active agents[7,12-21]. It has some advantages such as its biocompatibility, its amphiphilic nature, facilitating dissolution of various drug classes, as well as their permeation enhancement properties[22].

Thus, in the present work, we selected genistein as a poorly soluble active agent, and attempted to stabilize it in the LO with the hydrogenated lecithin (HL) for the organogelator. HL was much proper organogelator than unsaturated lecithin in this study. For oil phase solvent, sunflower oil (SO) was used, and dipropylene glycol (DPG) and polyethylene glycol (PEG) were used as polar phase solvent. It is also determined that genistein penetrated through a pig skin effectively in the LO.

2. Materials and Methods

2.1. Materials

Hydrogenated lecithin (HL) (Emulmetik 950) was purchased from LucasMeyer (France). Sunflower oil (SO) (high oleic sunflower oil deodorized), polyethylene (Perfomalene 400), PEG-8 (PEG-400), and dipropylene glycol (DPG) (DPG-FC) were purchased at Aldivia (France), New Phase Technologies (USA), Surfachem (England), and SKC Inc. (USA), respectively. Genistein was purchased at Xi'an natural field bio-technique (China). A porcine skin (thickness is 0.7 mm) was purchased at Medi Kinetics (Korea). Triple distilled water (TDW) was used throughout the study.

2.2. Methods

2.2.1. Preparation of the Formulated LO

The optimization of LO was carried out by altering the proportion of materials forming LO. The gel forming area was plotted on the ternary phase diagram. The LO was prepared by the method described by Nirod Baran et al. with slight modification[23]. In brief, the weighed HL was dissolved in SO, being incubated over 90 ℃ (apolar phase). Then, polar phase (in this study, a mixture of PEG-8 and DPG or TDW), also heated to over 90 ℃, was added dropwise to the apolar phase. This solution was cooled to 25 ℃. Genistein (0.5% w/v in the total formulation) was dissolved in polar phase over 90 ℃ before adding to the apolar phase.

2.2.2. Organoleptic Evaluation

Freshly prepared samples were observed for their color, appearance, texture, and surface.

2.2.3. Stability Studies

The samples were stored at four different storage conditions, i.e., in refrigerated condition (5 ℃), room temperature (25 ℃), high temperature (50 ℃) and cycled temperature (CT; -10 ℃ ~ 45 ℃) for 3 months. The samples were evaluated on every 20^{th} day for phase separation and surface change for 3 months[24].

2.2.4. pH Measurement

The pH of the solution containing 2 g of the optimized LO in 30 mL of TDW was detected with a pH meter (827 pH lab, Metrohm, Swiss).

2.2.5. Rheology Analysis

The rheological test was utilized by RS-50 rheometer (HAAKE, Germany) with cone & plate sensor system. All samples were prepared freshly. A cone with an angle of 4° and a diameter of 35 mm was used. The gap between the cone and plate was 0.05 mm. The whole procedure was carried out at room temperature[22]. G', the storage modulus was measured by oscillation test[25]. The conditions of oscillation test were as following;

the frequency was 1 Hz, and shear stress rage was 0 ~ 150 Pa.

2.2.6. Differential Scanning Calorimetry

The samples were subjected to differential scanning calorimetry (DSC) to analyze thermal properties with Hart DSC-Ⅱ (DSC 4100, Calorimetry Sciences Co., USA) over a temperature range of 25 - 90 ℃ at a heating rate of 1 ℃/min[22,26].

2.2.7. Microscopic Analysis

A sample was placed on a microscopic slide, covered with a cover glass and observed with an optical microscope (Leica DM1000, Leica Microsystems, Germany) equipped with a camera (Leica DFC 295, Leica Microsystems, Germany).

2.2.8. Skin Permeation Studies

Skin permeation was evaluated using a Franz-type diffusion cell supplied by Kwang Jin Science (Korea). The sample-applied skin was mounted in the diffusion cell with the dermal side facing downward into the receiving media 30% (v/v) PEG-8[26]. The receiving media, which was maintained at 35 ± 0.5 ℃, was stirred at 100 rpm on a magnetic stirrer[22]. The study was conducted for 24 h. The experiments were carried out in duplicate. The amount of genistein in the receiving media and skin was determined by high-performance liquid chromatography (HPLC). The HPLC system consisted of 1260 series pump, and UV/visible detector all from Agilent (USA). UV/visible detector was set at 260 nm. Genistein was analyzed using a Phenomenes C 18 column (2.6 μm, 4.6 × 50 mm inner diameter). The mobile phase consisted of water, with 10% acetonitrile from the beginning to 5 min, and with 90% acetonitrile from 5 min to 7 min, finally with 10% acetonitrile from 8 min to 11 min in the flow rate of 1 mL/min. The injection volume was 2 μL.

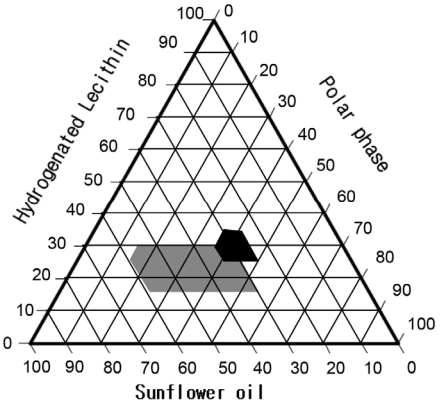
2.2.8.1. Skin Penetration

After permeation studies, the skin was removed from the diffusion cell, and was wiped out 3 - 4 times with a cotton swab immersed in Dulbecco's phosphate buffered saline (Gibco by life technologies, Korea). Stratum corneum was removed using a tape stripping technique[27]. The skin was tape-stripped 4 times with adhesive tapes, D100 - D-Squame Standard Sampling Discs (Cudurm, USA). The remaining skin was broken into small pieces after freezing at -20 ℃. The remaining skin was analyzed by HPLC.

3. Results and Discussion

3.1. Preparation of the Formulated LO

To formulate the LO, investigation of proper ingredients was performed, first. To make a normal LO, unhydrogenated lecithin has been used for an organogelator[28,29], and it has been known that when synthetic lecithins containing residues of saturated fatty acids are examined, the organogel is not formed[29]. However, in this case, the polar phase forming LO is almost comprised of water[30]. As mentioned earlier, if the water used in the LO, it is difficult to stabilize the active agent which is insoluble in both water and oil. In this study, to stabilize the active agent, we used only polyol as the polar phase. The LO with unhydrogenated lecithin appeared very unstable, while the LO with hydrogenated lecithin showed a firm structure, when polyol was used. Therefore, to stabilize the poorly soluble active agent, only polyols has to be used as polar phase, and hydrogenated lecithin has to be used as an organogelator at the same time. Several kinds of oils, lecithins and polyols were examined and HL (> 94% phosphatidylcholine), SO, DPG and PEG-8 were selected. Poorly purified lecithin does not form organogels, and it has been demonstrated that lecithin should contain at least 95% phosphatidylcholine in the case of unsaturated lecithin[27,31]. In this study, we used the hydrogenated lecithin containing > 94% phosphatidylcholine. It was found that as the amount of phosphatidylcholine decreased in the hydrogenated lecithin, more unstable oganogels were formed, and sometimes did not form, at all (data not shown). This suggests that the purity of hydrogenated lecithin is important to form the LO, as well. Also, the proportion



* LO: Lecithin organogel * HL: Hydrogenated lecithin * SO: Sunflower oil

Figure 1. Phase diagram of the LO with HL, polar phase and SO.

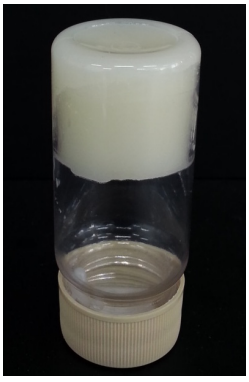
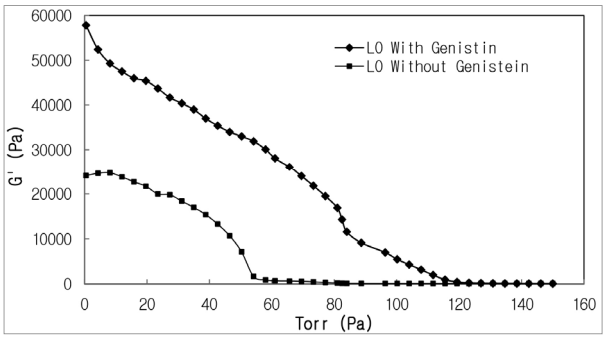


Figure 2. Appearance of the lecithin organogel.

of DPG and PEG-8 was studied to dissolute genistein effectively and form LO stably at the same time. Phase diagram for HL, SO and polar phase was constructed. A gel was formed in the grey colored region, which was composed of HL, SO, DPG and PEG-8 (DPG : PEG-8 = 1 : 2), and the black colored region, which was composed of HL, SO and TDW (Figure 1). We found that organogels consisting of DPG and PEG-8 (polyols) instead of TDW had broader range of possible organogel-formation (HL: 15 ~ 30%, SO: 30 ~ 60%, polyols: 14 ~ 54%), while the range of possible organogel-formation containing TDW was that HL was from 25% to 35%, TDW was from 35% to 50%, and SO was from 25% to 35% (Figure 1). A sol was formed or a separated liquid phase was shown in the rest of the phase diagram.



* LO: Lecithin organogel

Figure 3. Plot of the elasticity versus shear of the LO with genistein and without genistein.

3.2. Organoleptic Characteristics

The LO was yellowish in color and had smooth in texture (Figure 2). The surface of the gel was even and any lumps and grittiness were not shown in the gel.

3.3. Stability Studies

The LO was stored at four different conditions (5, 25, 50 ℃ and CT) for 60 days. It was found that the LO was generally stable at all temperatures in the case of phase separation and surface change, whereas at 50 ℃, there was a slight surface change on the 60th day (i.e., syneresis phenomenon).

3.4. pH Measurement

The pH of LO was found to be 6.58 ± 0.25, which is proper for skin topical application.

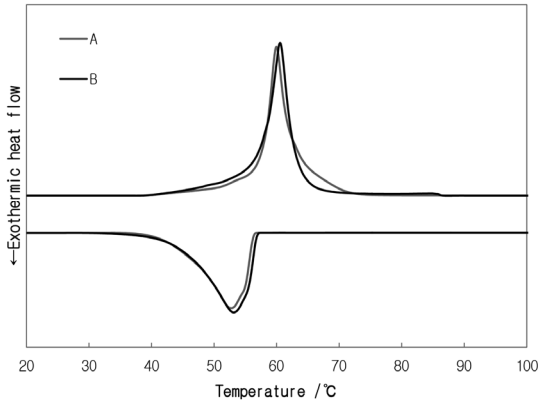
3.5. Rheology Analysis

Figure 3 shows the shear-thinning behavior of the LOs. As the shear stress increased, a decrease of G' was observed. Also, there was a gap in G' of the LO with genistein and the LO without genistein. Andrade et al. reported that drug incorporation resulted in an increase in the melting temperature of nanoparticles[32] and Satapathy et al. suggested that there was an increase in

Table 1. Stability of the LO

Time (days)	Temperature (℃)	Phase separation	Surface change
0	25	-	-
20	25	-	-
40	25	-	-
60	25	-	-
20	5	-	-
40	5	-	-
60	5	-	-
20	50	-	-
40	50	-	-
60	50	-	+
20	CT	-	-
40	CT	-	-
60	CT	-	-

* CT : -10 ℃ ~ 45 ℃, - : no separation and no change, + : slight separation and slight change, ++ : considerable separation and considerable change.
* LO: Lecithin organogel



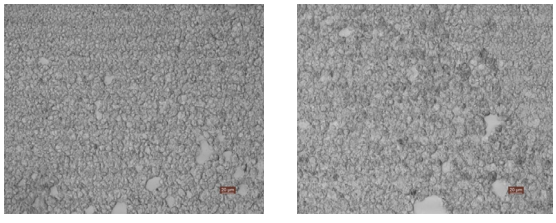
* LO: Lecithin organogel

Figure 4. The DSC thermograms of LO with genistein (A) and LO without genistein (B).

the stability of the organogel as a drug was incorporated within its structure[22]. Therefore, it seems that genistein induced the matrix to be rigid. And there are similar reports that drug could increase matrix organization.

3.6. Differential Scanning Calorimetry

Figure 4 shows the DSC curves of the LO with genistein and the LO without genistein. The endothermic peak of the LO with genistein was 60.0 ℃ and the LO without genistein showed the endothermic peak at 60.5 ℃. There was no significant difference depending on the drug incorporation in the endothermic peak temperature and exothermic peak temperature.



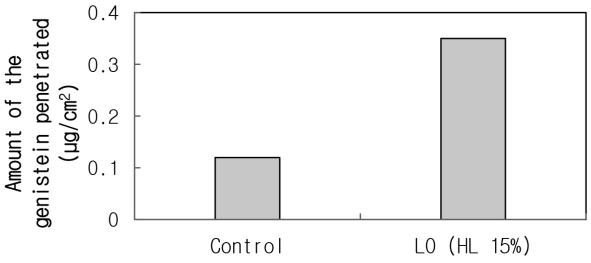
(a) (b)

* LO: Lecithin organogel

Figure 5. Microscopic images of the LO without genistein (a) and with genistein (b). The scale bar is 20 μm.

3.7. Microscopic Analysis

The optical photomicrograph is portrayed in Figure 5. Figure 5(a) is the LO without genistein, and Figure 5(b) is the LO without genistein. With genistein incorporation,



* HL: Hydrogenated lecithin * LO: Lecithin organogel

Figure 6. Skin penetration of genistein in the remaining skin and the receiving media after 24 h of the experiences in Franz diffusion cells (Control: HL free organogel, LO: LO with 15 % HL).

there was a slight increase in size of micelles. And, both micrographs show a homogeneous organization in the LO.

3.8. Skin Permeation Studies

To reveal the role of HL of LO in skin penetration, HL free organogel was formed. Polyethylene was used as organogelator, instead of HL to form HL free organogel. The HL free organogel and the LO, both were loaded with genistein 0.5% (w/v). The amount of the genistein penetrated is the total amount from the remaining skin and receiving media. Approximately, LO enhanced penetration of genistein 3 times compared to that of HL free organogel. It could be evidence that LO may enhance drug delivery. Bhatnagar and Vyas reported permeability of micellar-borne drug across the human skin was enhanced significantly over 10 times in unsaturated lecithin/iso-octane/water organogel system compared to that emulsified in the petroleum jelly[18]. From these, it could be inferred that LO enhances skin permeability of drug, in irrespective of whether the lecithin is hydrogenated or not.

4. Conclusions

Using our system, genistein was stabilized to be used at a concentration higher than any other cosmetics formula. From these results, we can conclude that the LO, which is composed of hydrogenated lecithin, sunflower oil, dipropylene glycol, and polyethylene glycol, can be used as an effective and stable cosmetic formulation that can carry poorly soluble active ingredients at high concentration for topical dermal delivery.

Reference

1. D. Locati, S. Morandi, A. Cupisti, L. Ghiadoni, and A. Arnoldi, Characterization and quantification of soy isoflavone metabolites in serum of renal transplanted patients by high-performance liquid chromatography/electrospray ionization mass spectrometry, *Rapid Commun. Mass Spectrom.*, **19**(23), 3473 (2005).
2. W. Huachen, R. Bowen, Q. Cai, S. Barnes, and Y. Wang, Antioxidant and antipromotional effects of the soybean isoflavone genistein, *Biol. Med.*, **208**(1), 124 (1995).
3. W. A. Fritz, L. Coward, J. Wang, and C. A. Lamartiniere, Dietary genistein : perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat, *Carcinogenesis*, **19**(12), 2151 (1998).
4. M. Jurzak and K. Adamczyk, Drug biochemistry influence of genistein on c-jun, c-fos and fos-b of ap-1 subunits expression in skin keratinocytes, fibroblasts and keloid fibroblasts cultured *in vitro*, *Acta Pol. Pharm.*, **70**(2), 205 (2013).
5. Z. R. Huang, C. F. Hung, Y. K. Lin, and J. Y. Fang, *In vitro* and *in vivo* evaluation of topical delivery and potential dermal use of soy isoflavones genistein and daidzein, *Int. J. Pharm.*, **364**(1), 36 (2008).
6. S. R. Georgetti, R. Casagrande, W. Verri, R. F. V. Lopez, and M. J. V. Fonseca, Evaluation of *in vivo* efficacy of topical formulations containing soybean extract, *Int. J. Pharm.*, **352**(1-2), 189 (2008).
7. A. Vintiloiu and J. Leroux, Organogels and their use in drug delivery—a review, *J. Control.*, **125**(3), 179 (2008).
8. S. Sahooa, N. Kumarb, C. Bhattacharyac, S. S.

Sagirid, K. Jaine, K. Palf, and B. Nayak, Organogels: properties and applications in drug delivery, *Des. Monomers Polym.*, **14**(2), 95 (2011).
9. Y. A. Shchipunov and E. Shumilina, Lecithin bridging by hydrogen bonds in the organogel, *Mater. Sci. Eng.*, **3**(1), 43 (1995).
10. Y. A. Shchipunov and E. Shumilina, Lecithin organogels: role of polar solvent and nature of intermolecular interactions, *Colloid J. Russ. Acad. Sci.*, **58**(1), 117 (1996).
11. R. Kumar and O. P. Katare, Lecithin organogels as a potential phospholipid-structured system for topical drug delivery: a review, *AAPS Pharm. Sci. Tech.*, **6**(2), E298 (2005).
12. H. Willimann, P. Walde, P. L. Luisi, A. Gazzaniga, and F. troppolo, Lecithin organogel as matrix for transdermal transport of drugs, *J. Pharm. Sci.*, **81**(9), 871 (1992).
13. D. Grace, J. Rogers, K. Skeith, and K. Anderson, Topical diclofenac versus placebo: a double blind, randomized clinical trial in patients with osteoarthritis of the knee, *J. Rheumatol.*, **26**(12), 2659 (1999).
14. P. Mahler, F. Mahler, H. Duruz, M. Ramazzina, V. Liguori, and G. Mautone, Double-blind, randomized, controlled study on the efficacy and safety of a novel diclofenac epolamine gel formulated with lecithin for the treatment of sprains, strains and contusions, *Drugs Exp. Clin.*, **29**(1), 45 (2003).
15. G. Spacca, A. Cacchio, A. Forgacs, P. Monteforte, and G. Rovetta, Analgesic efficacy of a lecithin-vehiculated diclofenac epolamine gel in shoulder periarthritis and lateral epicondylitis: a placebo-controlled, multicenter, randomized, double-blind clinical trial, *Drugs Exp. Clin.*, **31**(4), 147 (2005).
16. G. Agrawal, M. Juneja, S. Agrawal, S. K. Jain, and S. S. Pancholi, Preparation and characterization of reverse micelle based organogels of piroxicam. *Die Pharm. Int. J. Pharm. Sci.*, **59**(3), 191 (2004).
17. C. Nastruzzi and R. Gambari, Antitumor activity of (trans) dermally delivered aromatic tetra-amidines, *J. Control Release.*, **29**(1), 53 (1994).
18. S. Bhatnagar and S. P. Vyas, Organogel-based system for transdermal delivery of propranolol, *J. Microencapsul.*, **11**(4), 431 (1994).
19. R. Aboofazeli, H. Zia, and T. E. Needham, Transdermal delivery of nicardipine: an approach to *in vitro* permeation enhancement, *Drug Deliv.*, **9**(4), 239 (2002).
20. I. Shaikh, K. R. Jadhav, P. S. Gide, V. J. Kadam, and S. S. Pisal, Topical delivery of aceclofenac from lecithin organogels: preformulation study, *Curr. Drug Deliv.*, **3**(4), 417 (2006).
21. F. Dreher and E. Wehrli, Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport, *J. Control Release.*, **45**(2), 131 (1997).
22. D. Satapathy, D. Biswas, B. Behera, S. S. Sagiri, K. Pal, and K. Pramanik, Sunflower-oil-based lecithin organogels as matrices for controlled drug delivery, *J. Appl. Polym. Sci.*, **129**(2), 585 (2013).
23. N. Baran, V. K. Singh, K. Pal, A. Anis, D. K. Pradhan, and K. Pramanik, Development and characterization of soy lecithin and palm oil-based organogels, *Polym. Plast. Technol. Eng.*, **53**(9), 865 (2014).
24. V. Agrawal, V. Gupta, S. Ramteke, and P. Trivedi, Preparation and evaluation of tubular micelles of pluronic lecithin organogel for transdermal delivery of sumatriptan, *AAPS Pharm. Sci. Tech.*, **11**(4), 1718 (2010).
25. M. Lescanne, P. Grondin, A. d'Aleo, F. Fages, J.-L. Pozzo, O. Mondain Nonval, P. Reinheimer, and A. Colin, Thixotropic organogels based on a simple N-hydroxyalkyl amide: rheological and aging properties, *Langmuir*, **20**(8), 3032 (2004).
26. K. Choi, H. Son, and S. Lee, The effect of glossiness and lattice structure of wax matrixes on using n-parrafin and branched wax, *J. Soc. Cosmet. Scientists Korea*, **36**(2), 99 (2010).
27. S. F. Taveira, A. Nomizo, and R. F. Lopez, Effect of the iontophoresis of a chitosan gel on doxorubicin skin penetration and cytotoxicity, *J. Control Release*,

134(1), 35 (2009).
28. A. Bhatia, B. Singh, K. Raza, S. Wadhwa, and O. P. Katare, Tamoxifen-loaded lecithin organogel (LO) for topical application: development, optimization and characterization, *Int. J. Pharm.*, **444**(1-2), 47 (2013).
29. J. Varshosaz, S. Andalib, M. Tabbakhian, and N. Ebrahimzadeh, Development of lecithin nano-emulsion based organogels for permeation enhancement of metoprolol through rat skin, *J. Nanomater.*, **2013**, 1 (2013).
30. S. Pradhan, S. S. Sagiri, V. K. Singh, K. Pal, S. S. Ray, and D. K. Pradhan, Palm oil-based organogels and microemulsions for delivery of antimicrobial drugs, *J. Appl. Polym. Sci.*, **131**(6), 39979 (2014).
31. R. Scartazzini and P. L. Luisi, Organogels from lecithins, *J. Phys. Chem.*, **40**(2), 829 (1988).
32. L. M. Andrade, C. de Fátima Reis, L. Maione-Silva, J. Luiz V., A. A. Alonso, R. C. Serpa, R. N. Marreto, E. M. Lima, and S. F. Taveira, Impact of lipid dynamic behavior on physical stability, *in vitro* release and skin permeation of genistein-loaded lipid nanoparticles, *Eur. J. Pharm. Biopharm.*, **88**(1), 40 (2014).