Effect of Terpene on Physicochemical Properties and Skin Permeability of Capsaicin Loaded Solid Lipid Nanoparticles

Sureewan Duangjit[1,3], Chanida Pucharean[2], Pajeemon Yoddee[2], Kulda Kanlayawuttipong[2], Tanasait Ngawhirunpat[3,4*]

Abstract

Introduction: Capsaicin (CAP) is the major active compound of chili piper. Previous study has been reported that CAP was used orally or topically for pain relief by rheumatism, neuralgia, lumbago or sciatica. However, the significant first pass metabolism, the short half-life and the poor water solubility of CAP lead to its limitation in the development of CAP formulations as novel pharmaceutical products. The objective of this study was to investigate the effect of terpene on physicochemical properties and skin permeability of CAP loaded solid lipid nanoparticles (SLN). **Method:** The SLN containing a constant amount of 0.15% CAP, cetyl palmitate, transcutol P, Tween 20, Tween 80, deionized water and various percentage of terpene as a potential penetration enhancer from 0 to 15% were prepared. SLN formulations were evaluated for physicochemical properties (e.g. size, size distribution, zeta potential and entrapment efficiency), and skin permeability. The terpene used in SLN formulations was defined as formulation factors (X_n), while the physicochemical properties and skin permeability were defined as response variables (Y_n). **Results:** The results indicated that the percentage of terpene significantly affected the entrapment efficiency and skin permeability of SLN formulation. The skin permeability of all SLNs was significantly higher than commercial product. The 10% terpene incorporated in SLN showed the maximum entrapment efficiency and skin permeability. **Conclusion:** The incorporation of terpene into the formulation was beneficial for the development of SLNs for enhanced transdermal delivery of CAP. The optimal percentage of terpene used in SLN was up to 10%. We are success in showing the effect of terpene on physicochemical properties and skin permeability of capsaicin loaded SLN. Further study is required to confirm the influence of terpene on the stability of SLN.

Keywords: Terpene, Lipid nanoparticles, Chili pepper extract, Capsaicin, Transdermal delivery

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ผลของเทอร์ปีนต่อคุณลักษณะทางเคมีกายภาพและความสามารถในการซึมผ่านผิวหนังของแคปไซซินที่ถูกกักเก็บในอนุภาคไขมันของแข็ง

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บทคัดย่อ

บทนำ: แคปไซซินเป็นสารออกฤทธิ์สำคัญที่พบในพริก การศึกษาก่อนหน้าพบว่าแคปไซซินรูปแบบยารับประทานและยาใช้ภายนอกช่วยบรรเทาอาการปวดได้ เช่น ปวดข้อรูมาตอยด์ ปวดเส้นประสาท ปวดเอวหรือสะโพก อย่างไรก็ตาม การเกิดเมตาบอลิซึมครั้งแรกที่ตับ มีค่าครึ่งชีวิตสั้น และมีค่าการละลายน้ำน้อยของแคปไซซิน ทำให้เป็นข้อจำกัดในการพัฒนาสูตรตำรับแคปไซซินสำหรับเป็นเภสัชภัณฑ์รูปแบบใหม่ วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาผลของเทอร์ปีนต่อคุณลักษณะทางเคมีกายภาพและความสามารถในการซึมผ่านผิวหนังของแคปไซซินที่ถูกกักเก็บในอนุภาคไขมันของแข็ง **วิธีการดำเนินการวิจัย:** เตรียมอนุภาคไขมันของแข็งที่ประกอบด้วย 0.15% capsaicin, cetyl palmitate, transcutol P, Tween 20, Tween 80, deionized water ในสัดส่วนคงที่ และเปรียบเทียบร้อยละของเทอร์ปีนที่ใช้เป็นสารเร่งการซึมผ่านผิวหนังจากร้อยละ 0 ถึง 15 ศึกษาคุณลักษณะทางเคมีกายภาพ (ได้แก่ ขนาดอนุภาค การกระจายขนาด ประจุ และการกักเก็บตัวยา) และศึกษาความสามารถในการซึมผ่านผิวหนังของอนุภาคไขมันของแข็ง กำหนดให้เทอร์ปีนที่ใช้ในอนุภาคไขมันของแข็งเป็นปัจจัยสูตรตำรับ (X_n) และคุณลักษณะทางเคมีกายภาพและความสามารถในการซึมผ่านผิวหนังเป็นปัจจัยตอบสนอง (Y_n) **ผลการศึกษาวิจัย:** ผลการศึกษาบ่งชี้ว่าร้อยละของเทอร์ปีนมีผลต่อการกักเก็บตัวยาและความสามารถในการซึมผ่านผิวหนังของอนุภาคไขมันของแข็ง อนุภาคไขมันของแข็งทุกสูตรตำรับมีความสามารถในการซึมผ่านผิวหนังมากกว่าผลิตภัณฑ์ที่มีในท้องตลาดอย่างมีนัยสำคัญ พบว่าอนุภาคไขมันของแข็งที่มีเทอร์ปีนร้อยละ 10 มีการกักเก็บตัวยาและความสามารถในการซึมผ่านผิวหนังมีค่าสูงที่สุด **สรุปผลการวิจัย:** การเติมเทอร์ปีนลงในสูตรตำรับเป็นประโยชน์ในการพัฒนาอนุภาคไขมันของแข็งสำหรับนำส่งแคปไซซินทางผิวหนัง อนุภาคไขมันของแข็งที่เหมาะสมสามารถเติมเทอร์ปีนได้ถึงร้อยละ 10 ผู้วิจัยประสบความสำเร็จในการศึกษาผลของเทอร์ปีนต่อคุณลักษณะทางเคมีกายภาพและความสามารถในการซึมผ่านผิวหนังของอนุภาคไขมันของแข็งกักเก็บแคปไซซิน การศึกษาต่อไปต้องการยืนยันผลของเทอร์ปีนต่อความคงตัวของอนุภาคไขมันของแข็ง

คำสำคัญ: เทอร์ปีน อนุภาคไขมันขนาดนาโนเมตร สารสกัดพริก แคปไซซิน การนำส่งทางผิวหนัง
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Introduction

Capsaicin (CAP) is the major active compound derived from hot chili pepper (Figure 1). CAP not only admix hot and spicy taste to food but also own potential usefulness in medication. CAP was used orally or topically for pain relief by rheumatism, neuralgia, lumbago or sciatica (Fraenkel, 2004; Huang, 2008). CAP is a main pungent ingredient that has been widely studied in both medical and pharmaceutical applications (Baranidharan, 2013; Luo, 2011). Recently, the new product of high-concentration CAP 8% patch, also known as NGX-4010, which provides rapid, long-lasting pain relief with a single application, was introduced. However, the strong pungency of high concentration of CAP, the significant first pass metabolism, the short half-life and the poor water solubility of CAP lead to its limitation in the development of CAP formulations as novel pharmaceutical products.

In the development of novel drug delivery carriers for topical and transdermal drug delivery systems, the effectiveness of drug delivery systems depend on the capability of model drug penetrate the skin barrier in proper amounts to reach therapeutic levels (Pathan and CM, 2009). Nowadays, several novel drug delivery carriers have been introduced including solid lipid nanoparticles (SLNs). SLNs are the first generation of lipid nanoparticles. Generally, SLNs composed of solid lipids that have a solid state at room and body temperature. SLNs have been reported that can significantly increase safety, provide an enhanced efficiency and improve degradation of drugs (Muller, 2002). The degradation of drug loaded in SLN was lower than that of nanoemulsions or other lipid carriers (Charoenputtakun, 2014a). Moreover, SLNs can reduce the immunogenic responses or irritant effects and particularly intrinsic instability of vitamin A derivative (Muller, 2002). Therefore, SLNs are attractive as novel colloidal drug carriers for the topical and transdermal application. However, the limitation of SLNs have trended to slow down the drug release for several days to weeks, which inconvenience for acute condition of diseases (Bhushan, 2013). Therefore, the development of SLNs for overcoming this limitation was still required. The combination of several penetration enhancers, like Transcutol P, Tween 20, Tween 80 and other excipients may have an enhancement of the efficacy for target drug delivery (Martins, 2013). Thus, the addition of limonene as terpene penetration enhancer (Figure 1) in SLNs may improve their permeation efficacy and drug release across the skin membrane.

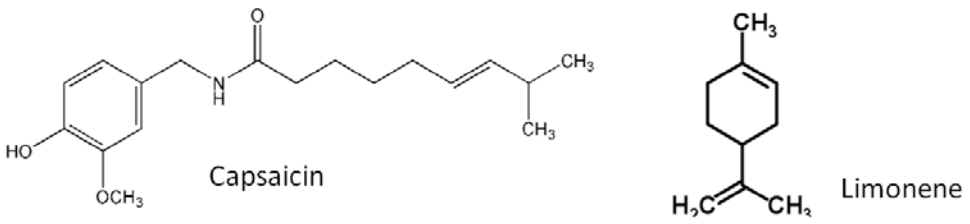

Figure 1 The chemical structure of capsaicin and limoene (as terpene penetration enhancer)

The objective of this study was to investigate the effect of terpene on physicochemical properties and skin permeability of CAP loaded solid lipid nanoparticles (SLN). The SLN containing a constant amount of 0.15% CAP, cetyl palmitate, transcutol P, Tween 20, Tween 80, deionized water and various percentage of terpene as a potential penetration enhancer from 0 to 15% were prepared. SLN formulations were evaluated for physicochemical properties (e.g. size, size distribution, zeta potential and entrapment efficiency), and skin permeability. The terpene used in SLN formulations was set as formulation factors (X_n), while the physicochemical properties and skin permeability were set as response variables (Y_n). Moreover, the possibility mechanism for enhancing transdermal delivery was investigated by fourier transform infrared (FT-IR) spectroscopy.

Materials and methods

Materials

Synthetic chili pepper extract (98% capsaicin) (CAP) was purchased from Hunan Huacheng Biotech, Inc. (Changsha, China). Terpene was purchased from Sigma-Aldrich (St. Louis, MO, USA). Cetyl palmitate (CP) was purchased from SABO SpA (Levate, Italy). Diethylene glycol monoethyl ether (transcutol P) was purchased from Ajax Finechem, (Auckland, New Zealand). Polysorbate-20 (Tween® 20, T20) and poltsorbate-80 (Tween® 80, T80) was purchased from the NOF Corporation (Osaka, Japan). All other chemicals were commercially available and of analytical and high-performance liquid chromatography (HPLC) grade.

Preparation of solid lipid nanoparticle (SLN)

The model SLN were prepared according to the SLN obtained from our previous study (Charoenputtakhun, 2014a; Charoenputtakun, 2014b). The SLN containing a constant amount of 0.15% CAP, cetyl palmitate, transcutol P, Tween 20, Tween 80, deionized water and various percentage of terpene as a potential penetration enhancer from 0 to 15% were prepared. SLN formulations were evaluated for physicochemical properties (e.g. size, size distribution, zeta potential and entrapment efficiency), and skin permeability. The terpene used in SLN formulations was defined as formulation factors (X_n), while the physicochemical properties and skin permeability were defined as response variables (Y_n). CAP-loaded SLN were prepared by the sonication method, as shown in Table 1.

Table 1 The composition of different SLN formulations

SLN	Oil phase (% w/w)				Water phase (% w/w)	
	CAP	CP	Trans P	Terpene	T20:T80	DI water
1	0.15	30	1.2	-	10	to 100
2	0.15	30	1.2	1	10	to 100
3	0.15	30	1.2	2	10	to 100
4	0.15	30	1.2	5	10	to 100
5	0.15	30	1.2	10	10	to 100
6	0.15	30	1.2	15	10	to 100

Evaluation of size, size distribution, zeta potential

The vesicle size, size distribution and zeta potential of SLN were measured by photon correlation spectroscopy (Zetasizer Nano series, Malvern Instrument, UK). Ten µl of the SLN formulations were diluted with the appropriate amount of deionized water. All SLN samples were performed. At least three independent samples were taken, and the vesicle size, size distribution and zeta potential were measured at least three times, at room temperature (25 ºC).

Determination of CP in the formulation

The concentration of CP in the formulation was determined by HPLC analysis after disruption of the SLN with methanol at a 1:1 v/v ratio and appropriate dilution with phosphate buffer pH 7.4. The SLN/methanol mixture was centrifuged at 15,000 rpm at 25 ºC for 15 min. The supernatant was filtered with a 0.45 μm nylon syringe filter and then analyze using HPLC.

***In vitro* skin permeation study**

The shed snake skin from the Siamese cobra (*Naja kaouthia*) was used as a model membrane in our skin permeation study because the similarity of shed snake skin to human skin in lipid content and permeability (Duangjit, 2010). A Franz diffusion cell with an available diffusion area of 2.01 cm^2 was employed. The receiving chamber was filled with 6.5 ml of phosphate buffer solution (pH 7.4, 32±1 ºC) and the donor chamber was filled with 1.5 ml CP-loaded SLN and commercial product (ethanolic solution). At the time intervals, 0.5 ml of the receiver medium was withdrawn and the same volume of fresh phosphate buffer solution was replaced. The concentration CP in the aliquot was analyzed using a HPLC.

Skin Characterization study

Following the skin permeation study, the shed snake skin was cleaned with distilled water, blotted dry and kept in desiccators. The spectrum of the skin sample was recorded in the range of 500-4000 cm^{-1} using a FT-IR spectrophotometer (Nicolet 4700, Thermo Scientific, USA). The FT-IR spectra of the skin that treated with the commercial product of CAP was recorded and used as a control.

HPLC analysis

The concentration of CAP was analyzed using a HPLC (Agilent Technology, U.S.A.). A C18

reversed-phase column (Symmetry®, VertiSep™, Vertical, Thailand) with dimensions of 5 µm, 4.6×150 mm was used. The mobile phase was a mixture of acetonitrile and 0.01% phosphoric acid (50:50). A UV detector was set at 227 nm for capsaicin detection. The injection volume was 20 µL and the flow rate was 1.0 ml/min at ambient temperature. The calibration curve for CAP was in the range of 1-100 µg/ml with a correlation coefficient of 0.999.

Data analysis

The data were reported as mean ± S.E. (n=3) and statistical analysis of the data was carried out using student's unpaired t-test. A p-value of less than 0.05 was considered to be significant.

Results and discussion

Physicochemical properties of SLNS

The physical appearance and the physicochemical properties i.e., vesicle size, size distribution, zeta potential and entrapment efficiency of CAP loaded SLNs are shown in Table 2. The physical appearance of CAP loaded SLNs was white liquid emulsions as same as milky solution. The presence of terpene (limonene) resulted in significant difference in vesicle size, size distribution, entrapment efficiency and skin permeability compared to the SLN formulation without terpene. The increasing of terpene from 1-15% resulted in no significant difference in the physical appearance, size distribution and zeta potential of SLN formulations.

The vesicle size of SLNs with terpene varies from 190-230 nm depending on the percentage of terpene. The vesicle size of SLNs tend to decrease, when the percentage of terpene increase. However, the method of preparation and application of technique like sonication may affect the vesicle size of nanocarriers (Chourasia, 2011). The effect of percentage of terpene on the vesicle size of SLN was not yet fully understood. However, the difference in terpene concentration of SLN formulations may affect the energy barriers required for stabile dispersion systems (Charoenputtakun, 2014b). All SLN formulation in this study was within nanosize (190-230 nm) under sonication method.

Table 2 The physical appearance and physicochemical properties of different SLN formulations

SLN	1	2	3	4	5	6
Physical appearance						
Size (nm)	227.21±6.92	228.42±3.95	211.17±1.04	194.33±1.07	195.83±0.75	192.37±2.11
PDI	0.25±0.02	0.18±0.02	0.12±0.00	0.12±0.01	0.10±0.01	0.10±0.01
Charge (-mV)	29.62±0.47	34.49±0.62	33.20±1.54	28.72±1.27	29.33±1.05	31.89±1.79
EE (%)	41.88±8.29	69.09±16.24	72.45±12.83	79.34±9.14	99.47±7.03	98.23±4.08
Flux (µg/cm3/h)	20.49±4.73	21.96±1.76	23.36±3.47	33.25±10.10	38.77±8.85	31.44±9.96

Polydispersity index (PDI)

The size distribution of SLN formulations was between 0.10-0.25. The lowering value in size distribution of SLN formulation represented the narrow size distribution. The presence of terpene showed the smaller size distribution than SLN without terpene. However, the size distribution of nanocarriers can also be affected by the method of preparation (Yordanov, 2010).

The zeta potential of SLN formulations was comparable to each other. The SLN formulations had a negative zeta potential which was directly affect by the total net charge of all formulation component, i.e. cetyl palmitate or other anionic compounds. The result indicated that formulation component of SLNs may contribute to the negative charge of the formulations. Several penetration enhancers used in this formulation (i.e., Transcutol P, Tween 20, and Tween 80) were nonionic compounds. Thus the negative exhibited may be the effect of the total net charge of CAP, CP and terpene concentration. However, the nanocarriers with neutral or slight negative surface charge have been reported that increased ability for drug delivery to brain when compared to the positive charged nanocarriers (Gao and Jiang, 2006; Martins, 2013). Moreover, the advantage of using SLN formulation with negative surface charge was less toxic and have high stability than SLN formulation with positive surface charge (Kedmi, 2010). Therefore, the negative surface charge of SLN formulation in this study was an appropriate characteristic as optimal carriers for drug delivery systems.

The entrapment efficiency of SLNs with terpene was significantly higher than that of SLN without terpene. The entrapment efficiency was significantly increased in the order of 1-15% of terpene. This result revealed that the incorporation of terpene can increase the solubility of CAP in lipid matrix of SLNs. Several studies demonstrated that an increase in penetration enhancers resulted in an increase in the entrapment efficiency (Badran, 2012a; Badran, 2012b; Rangsimawong, 2014). The presence of terpene attributed to increase in entrapment efficiency, which frequently led to increase in lipophilicity of the vesicle, which consequently increased in the entrapment efficiency.

***In vitro* skin permeation study**

Figure 2 shows the graphic plot of the cumulative skin permeation per unit area and the steady-state flux of various CAP loaded SLN formulations over an incubation period of 2–8 hours. The skin permeability (skin permeation profile and skin permeation flux) of SLN formulation and commercial product were significantly different. The skin permeation profile of CAP loaded SLN formulations with different concentration of terpene (0, 1, 2, 5, 10 and 15%). The incorporation of terpene (limonene) to SLN formulations increase CAP skin permeation. The skin permeability of all CAP loaded SLNs was significantly higher than that of CAP loaded SLNs without terpene (0% terpene). This result was in accordance with the previous studies (Charoenputtakun, 2014b; T. Subongkot, 2012) the suggested that the skin permeation flux of sodium fluorescein was higher than that of cineole when encapsulated in liposomes containing limonene. The skin permeation enhancement by terpene may be due to increased CAP solubility within the skin or due

to increased drug partitioning into the stratum corneum (SC). Moreover, terpene has been used as permeation enhancer in the eutectic systems of ibuprofen formed with terpenes for enhancement transdermal drug delivery. Thus, the melting point reduction by eutectic system was correlated with a significant increase in transdermal permeation (Stott, 1998). The melting point depression by eutectic system may provide a method of enhancing transdermal delivery in this study. This phenomenon may be resulted from the intrinsic properties of terpene that was a proper penetration enhancer for CAP loaded SLN formulations.

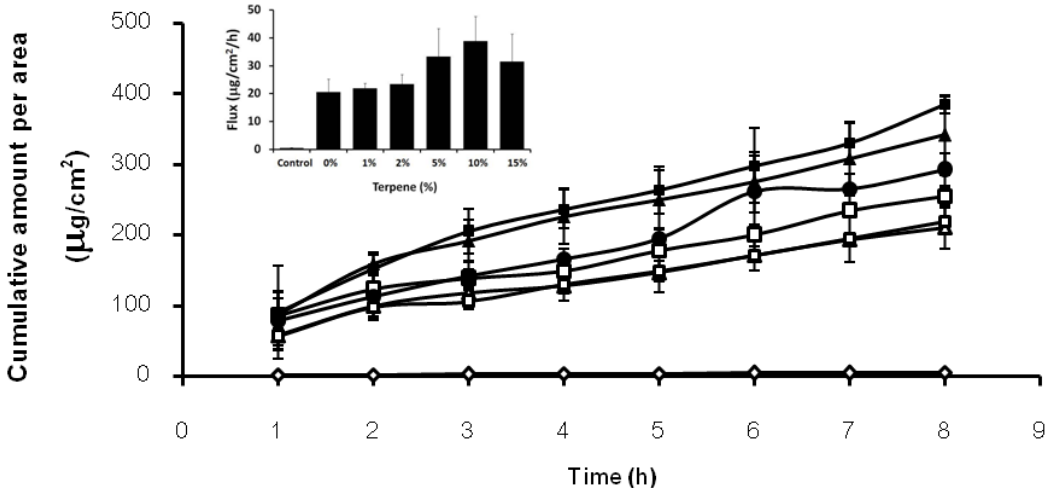

Figure 2 The skin permeation profile of different SLN formulations (◇; control solution, △; 0%, ○; 1%, □; 2%, ▲; 5%, ■; 10%, and ●; 15% terpene)

Skin Characterization study

The effect of CAP loaded SLNs on the SC of the skin was evaluated by FT-IR. The FT-IR spectra region between 2800-3000 cm^{-1} represents the CH_2 and CH_3 groups of lipids and the spectra region between 1500-1700 cm^{-1} represents the amide I (C=O) and amide II (N-H). The FT-IR spectra at amide I and II of the skins treated with SLN and commercial product were split into multiple peaks at 1650 cm^{-1} and 1550 cm^{-1}, respectively. The alteration of the FT-IR spectra at amide I and II was used as a marker for investigating the organization of the hydrogen bond in the SC as shown in Figure 3. The FT-IR spectra near 2820 and 2950 cm^{-1} of the skin treated with vesicle formulation was slightly difference from the commercial product. The FT-IR spectra of the skin treated with receiver medium and untreated was also investigated in this study (data not shown). The FT-IR spectra of the skin treated with SLN was used to confirm the efficacy of novel drug delivery carriers as its highest skin permeability. This is also possible that terpene penetration enhancers can increase drug permeability by causing rearrangement of lipid domains or conformations (Williams and Barry, 2004). Moreover, the skin treated with SLN and commercial product should be demonstrated using

differential scanning calorimetry (DSC) or X-ray diffraction to confirm the disruption of the microstructure of the skin in further study.

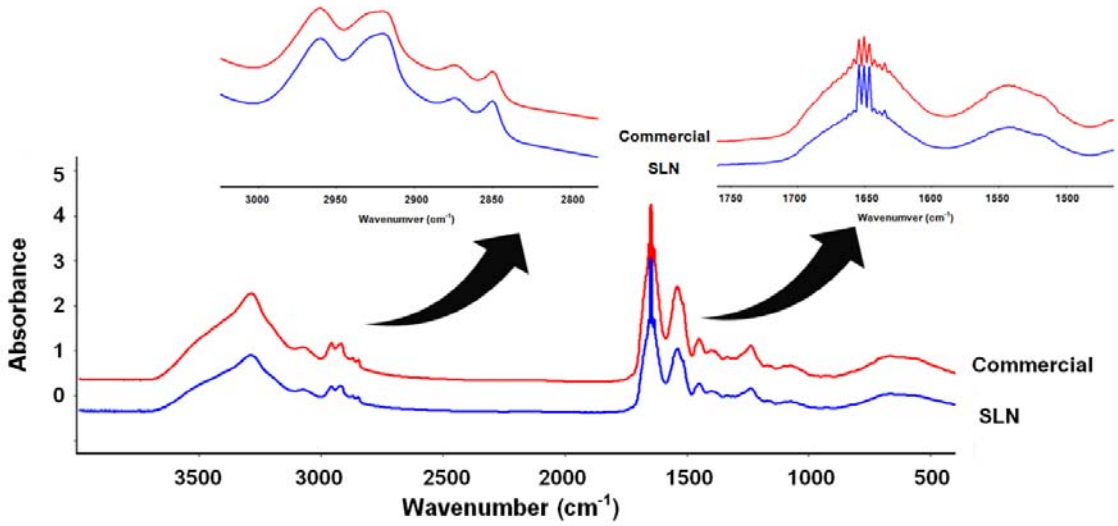

Figure 3 FT-IR spectra of the shed snake skin after the skin permeation study

Conclusion

In the current study, the investigation the effect of terpene on physicochemical properties and skin permeability of SLNs was successfully demonstrated as a transdermal delivery of CAP. The physicochemical properties (i.e., vesicle size, size distribution, entrapment efficiency) and skin permeability of SLN formulations were depended on terpene concentration. The incorporation of terpene in the SLNs resulted in significantly improved the skin permeability of CAP. Our study suggests the feasibility of transdermal delivery systems of SLNs composed of terpene at 10% limonene as the optimal ratio of terpene penetration enhancer.

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