Development of Deformable Liposomes for Transdermal Delivery of Extraction from Chili Pepper

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Abstract

Introduction: Capsaicin is a main pungent ingredient in chili peppers. It can stimulate the release of vasoactive neuropeptides (substance P) and calcitonin gene-related peptide (CGRP) from C-fiber nerve ending. Thus capsaicin is used as a medication for the temporary relief of neuralgia, rheumatism, lumbago and sciatica. However, it has been reported that the strong pungency, the short half-life and the poor water solubility of capsaicin lead to its limitation in the development of capsaicin formulations as pharmaceutical products. To achieve this aim, deformable liposome formulation with novel non-ionic surfactant was developed. **Methods:** The liposomes containing a constant amount of 0.15% capsaicin, phosphatidylcholine, cholesterol and non-ionic surfactant (e.g., Comperlan® KD and Tween® 20) were prepared. Conventional liposomes (CLP) and three deformable liposomes (DLP) were evaluated for physical properties (e.g. size, size distribution, zeta potential), drug content and skin permeability. **Results:** The results revealed that the liposome formulations containing 0.15% capsaicin were smaller than 100 nm in size, narrow size distribution (0.01-0.20) and had negative zeta potential value (less than -10 mV). The skin permeability of the deformable liposomes composed of Comperlan® KD and Tween® 20 was significantly higher than CLP and commercial product (control). Moreover, the application of deformable liposomes significantly disrupted the microstructure of the stratum corneum. **Conclusion:** The novel deformable liposome composed of the combination of non-ionic surfactants was successfully developed as a transdermal delivery carrier for chili pepper extract.

Keywords: Liposomes, Chili pepper extract, Capsaicin, Comperlan® KD, Skin delivery

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1. Introduction

Chili pepper (CP) is a popularly consumed by many people around the world. CP is not only admix hot and spicy taste to food but also own potential usefulness in medication (Luo, 2011). Capsaicin is the major active compound of CP (Figure 1). CP is a primary source of natural capsaicin. Capsaicin (8-methyl N-vanillyl-6-non-enamide) is a main pungent ingredient that has been extensively studied in several medical and pharmaceutical fields. It has been shown that capsaicin was used orally or topically for pain relief by neuralgia, rheumatism, lumbago or sciatica (Fraenkel, 2004, Huang, 2008). Capsaicin was added to several pharmaceutical product i.e., solutions or creams at a concentration of 0.15% or lower (Knotkova, 2008). Generally, solutions or creams containing capsaicin have poor efficacy for chronic pain (Luo, 2011). Moreover, the strong pungency, the significant first pass metabolism, the short half-life and the poor water solubility of capsaicin lead to its limitation in the development of capsaicin formulations as novel pharmaceutical products.

Nowadays, several novel delivery systems have been intensively investigated for transdermal delivery carries. Last a few decades, the first generation of deformable liposomes (DLP) have been introduced by Cevc and Blume so called transfersomes® (Cevc and Blume, 1992). Therefore, DLP seems to be an alternative carrier for chili pepper extract, since it has been reported that DLP could deliver the drug into deep skin region. DLP composed of phospholipid and a single chain surfactant called edge activator. An edge activator is having a high radius of curvature which could destabilize the lipid bilayer of liposomes and increase the bilayer deformability. The incorporation of edge activators resulted in different physicochemical properties of DLP. The specially designed vesicle was shown to be able to allow transdermal drug delivery, such as transfersomes, ethosomes, niosomes, flexosomes, invasomes and menthosomes. Numerous surfactants were utilized as edge activators i.e., sodium cholate, strearylamine, Tween® 60, or Span® 60 (Elsayed, 2006). However, non-ionic surfactants are the most common type of surfactant used in preparing liposomes due to their ideal benefits such as high compatibility, high stability and less toxicity compared to other (i.e., anionic, cationic or zwitterionic surfactants) (Kumar and Rajeshwarrao, 2011).

The aim of this study was to develop and characterize CP-loaded DLP and investigate the penetration-enhancing ability of non-ionic surfactants. DLP containinga constant amount of phosphatidylcholine (PC), cholesterol (Chol) and CP, and various type of non-ionic surfactant (Comperlan® KD and Tween® 20) were prepared. The physical properties (i.e., size, size distribution, and charge), drug content and skin permeability of CP-loaded DLP were evaluated. Furthermore, the possibility mechanism for enhancing skin permeation was investigated by Fourier transform infrared (FT-IR) spectroscopy and Differential scanning calorimetry (DSC).
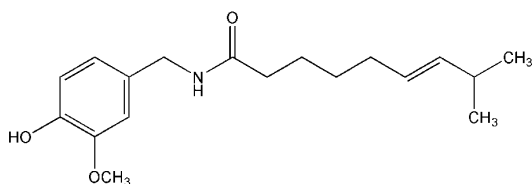
Figure 1 The Chemical structure of capsaicin

2. Materials and methods

Materials

Synthetic chili pepper extract (98% capsaicin) (CP) was purchased from Hunan Huacheng Biotech, Inc. (Changsha, China). Phosphatidylcholine (PC) was purchased from LIPOID GmbH (Cologne, Germany). Cholesterol (Chol) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Poly-sorbate-20 (Tween® 20) was purchased from the NOF Corporation (Osaka, Japan). Cocamide diethanolamine (DEA) (Comperlan® KD) were obtained from BASF (Thai) Co. Ltd. (Bangkok, Thailand). All other chemicals were commercially available and of analytical and high-performance liquid chromatography (HPLC) grade.

Preparation of deformable liposomes

Deformable liposomes containing a controlled amount of PC, Chol and chili pepper extract, and various type of non-ionic surfactant (Comperlan® KD and Tween® 20) were prepared (Duangjit, 2013, Duangjit, 2010). The deformable liposomes incorporated CP was prepared by thin-film hydration method. Briefly, all components (e.g. PC, Chol, CP and non-ionic surfactants) were dissolved in chloroform/methanol (2:1 v/v ratio). The liposome components were mixed and evaporated under nitrogen gas stream. The lipid film was placed in a desiccator for 6 h to remove the organic solvent. The dried film was hydrated with the phosphate buffer solution (pH 7.4). Following hydration, the liposome suspensions were sonicated in a bath for 30 min using bath type sonicator and then sonicated for 2 cycles of 30min using probe-sonicator. Deformable liposome were freshly prepared or stored in airtight container at 4ºC prior to use.

Evaluation of size, size distribution, zeta potential

The vesicle size,size distribution and zeta potential of deformable liposomes were measured by photon correlation spectroscopy (Zetasizer Nano series, Malvern Instrument, UK). Twenty µl of the liposome formulations were diluted with the appropriate amount of deionized water. All liposome samples were performed. At least three independent samples were taken, and the vesicle size, size distribution and zeta potentialwere 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 deformable liposomes with 0.1% Triton® X-100 at a 1:1 v/v ratio and appropriate dilution with phosphate buffer pH 7.4. The vesicle/Triton® X-100 solution was centrifuged at 15,000 rpm at 25 ºC for 15 min. The supernatant was filtered with a 0.45 µm nylon syringe filter.

In vitro skin permeation study

A Franz diffusion cell with an available diffusion area of 2.01 cm^2 was employed. 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). The receiving chamber was filled with 6.5 ml of phosphate buffer solution (pH 7.4, 321 ºC) and the donor chamber was filled with 1 ml CP-loaded deformable liposomes (DLP), CP-loaded conventional liposomes (CLP) or CP-solutions (Sol). At appropriate times, 0.5 ml ofthe receiver medium was withdrawn and the same volume of fresh buffer solution was replaced to the receiver chamber. The concentration CP in the aliquot was analyzed using a HPLC.

HPLC analysis

CP concentration was determined 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–0.01% phosphoric acid with the volume ratio of 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 CP was in the range of 1-100 μg/ml with a correlation coefficient of 0.999.

Characterization of snake skin after skin permeation

Following the skin permeation study, the shed snake skin was washed 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 spectrum of the treated skin with the MX suspension was also recorded and used as a control. Then, the skin sample (2 mg) was weighed into an aluminum crucible pan and was heated from 25 to 300ºC at a heating rate of 10 ºC/min using a Metter Toledo STARe System (DSC822e Module, Switzerland). All DSC measurements were collected under a nitrogen atmosphere with a flow rate of 100 mL/min. The DSC thermogram of the treated skin with CP solution was also recorded and used as a control.

Data analysis

The data were reported as mean S.E. (n=3) and statistical analysis of the data was carried out using one way ANOVA followed by a LSD *post hoc* test. A p-value of less than 0.05 was considered to be significant.

Results and discussion

Physical properties of deformable liposomes: The physical properties (i.e., size, size distribution, zeta potential) and capsaicin content of the deformable liposome formulations were shown in Figure 2 (A-D). The increasing of non-ionic surfactant (i.e., Comperlan® KD, Tween® 20) resulted in significant difference in physical

properties of CP-loaded DLP formulation. The vesicle size of DLP-T was significantly smaller than CLP. The decreased vesicle size was due to the incorporation of non-ionic surfactants. The chemical structure of Tween® 20 was larger than comperlan® KD, thus the critical packing parameter of PC might be distinctly disrupted by Tween® 20. Tween® 20 may affect the ratio of the hydrophobic tail volume to the volume projected by the optimal hydrophilic head group area, namely the critical packing parameter (Bae, 2009), of PC. The size distribution of all liposome formulation was not significantly different. The zeta potential of all formulations was mild negative charge (less than -10 mV). Since the pH of the experimental condition (pH 7.4) was higher than the isoelectric point (PI) of PC (PI = 6), the PC carrier the net negative charge. Therefore, the total net charge of the deformable liposome may be mainly affected by PC (Duangjit, 2010). Furthermore, the CP content in the formulation of DLP was significantly higher than CLP. These results revealed that the incorporation of non-ionic surfactants can increase the solubility of CP in deformable liposome bilayer. This result was consistency with Fang et al. (Fang, 2008) that the drug content was increased when surfactant was incorporated in liposome formulation. These results suggested that the novel non-ionic surfactant (Comperlan® KD) was significantly improve the CP content in DLP, while the size, size distribution and zeta potential of the DLP was not considerably disrupted.
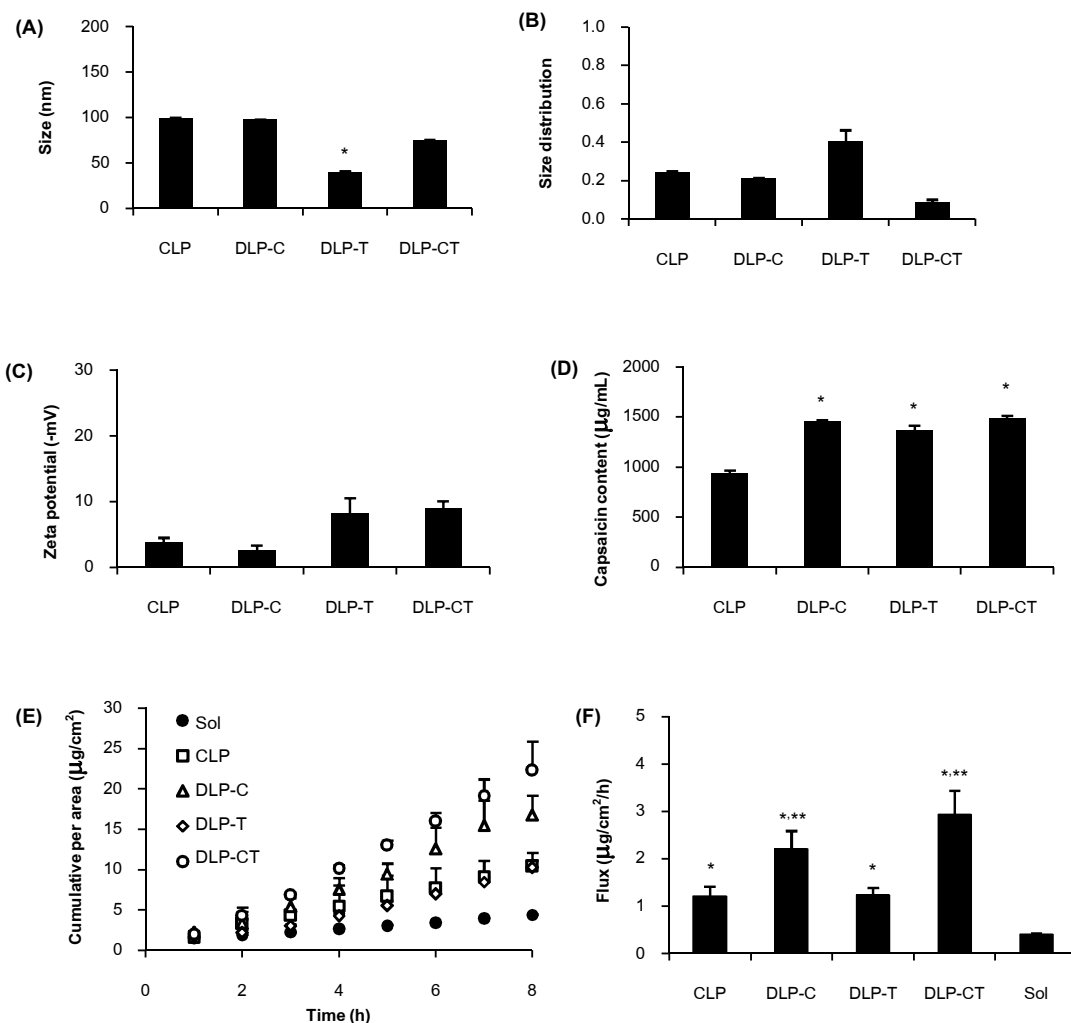
Figure 2 represents the physical properties, drug content and the skin permeability of conventional liposome, deformable liposomes and solution of extraction from chili pepper: (A) size, (B) size distribution, (C) zeta potential, (D) drug content, (E) skin permeation profiles and (F) skin permeation flux.

Skin permeation study ofdeformable liposomes: Figure 2E and 2F represents the skin permeability of CLP, DLP and the solution (Sol) of extraction from CP. The correlation coefficient (R^2) of skin permeation profile was between 0.97 and 0.99, indicating that the cumulative skin permeation of CP followed the zero order kinetic models as shown in Figure 2E. The skin permeation flux of CLP, DLP and Sol is exhibited in Figure 3B. The skin permeation flux of CP permeated through the skin of all vesicle formulation (CLP and DLP) was significantly higher than

Sol. In addition, the skin permeation flux of DLP incorporated comperlan® KD (DLP-C and DLP-CT) was also significantly higher than CLP. The incorporation of comperlan® KD in DLP increased the skin permeation flux of CP, while the incorporation of Tween® 20 in DLP did not. The high elasticity and high CP content may be the primary factors affecting the skin permeation of CP-loaded DLP. However, the DLP incorporated non-ionic surfactant, Comperlan® KD obviously improved the skin permeability compared to Tween® 20. This phenomenon may be resulted from the intrinsic properties of Comperlan® KD that was a proper penetration enhancer for CP-loaded DLP. Furthermore, the skin permeability of the DLP incorporated double non-ionic surfactants (DLP-CT) was slightly greater than DLP incorporated single non-ionic surfactant (DLP-C and DLP-T). The structure of non-ionic surfactant might also be the factor governing the intrinsic properties of non-ionic surfactants. These results suggested that Comperlan® KD was the non-ionic surfactant that significantly improved the skin permeability of capsaicin-loaded deformable liposomes, while the non-ionic surfactant, Tween® 20 was not.

Characterization of skin after skin permeation: The effect of non-ionic surfactant on the stratum corneum of the skin was evaluated by FT-IR and DSC. The FT-IR spectra region between 1500-1700 cm^{-1} represents the amide I (C=O stretching) and amide II (N-H stretching). The FT-IR spectra at amide I and II of the skins treated with CLP and DLP were split into multiple peaks at 1620-1690 cm^{-1} and 1510-1560cm^{-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 stratum corneum (Figure 3A). The FT-IR spectra near 1620-1690 cm^{-1} of the skin treated with vesicle formulation was significant difference from the control. The FT-IR spectra of DLP-CT was used to confirm the efficacy of Comperlan® KD incorporation in DLP as its highest skin permeability. Moreover, the DSC thermogram at 200-280C suggested the transition temperature of the skin sample was around 228C. The DSC thermogram of the skin treated with DLP-CT was significantly different from the skin treated with Sol (control) as displayed in Figure 3B. These results suggested that DLP-CT could deliver the CP through the skin by disruption the microstructure of the skin.
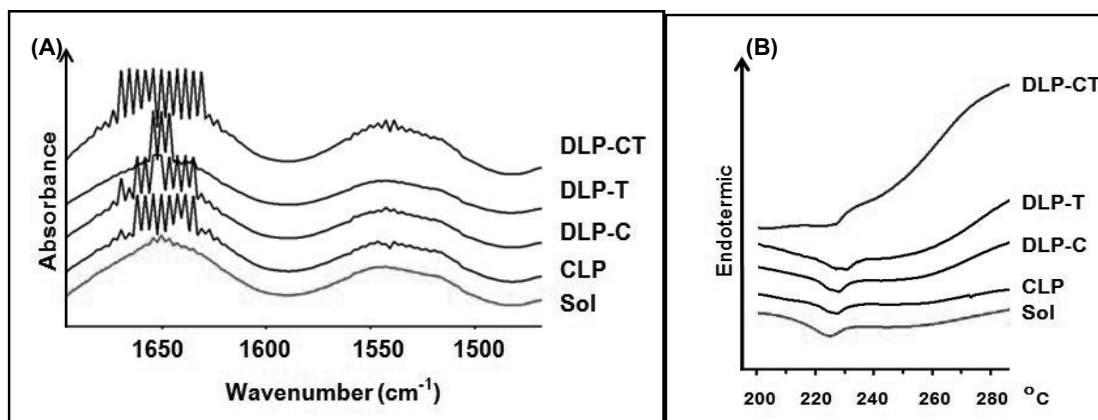
Figure 3 represents (A) the FT-IR spectra and (B) the DSC thermogram of the skin after the skin permeation study

4. Conclusion

In the current study, to improve the skin permeability of DLP for transdermal delivery, the incorporation of non-ionic surfactants (Comperlan® KD) was successfully developed as a transdermal delivery carrierfor CP extract. The physical properties, drug content and skin permeability of DLP formulations were depended on their compositions in the formulation. The combination of non-ionic surfactants in the DLP resulted in significantly improved the skin permeability of CP. Our study suggests the feasibility oftransdermal delivery carriers of DLP composed of non-ionic surfactants, Comperlan® KD as penetration en-hancer.

5. Acknowledgements

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