รายงานวิจัยฉบับสมบูรณ์
โครงการบทบาทของเทอร์ปีนต่อการตั้งตำรับเหมาะสมที่สุดของลิโพโซมสำหรับการนำส่งทางผิวหนังของละลายน้ำน้อยโดยใช้วิธีพื้นผิวตอบสนอง

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สัญญาเลขที่ MRG5980260

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สนับสนุนโดยสำนักงานคณะกรรมการการอุดมศึกษาและสำนักงานกองทุนสนับสนุนการวิจัย

บทคัดย่อ

รหัสโครงการ: MRG5980260

ชื่อโครงการ: บทบาทของเทอร์ปีนต่อการตั้งตำรับเหมาะสมที่สุดของลิโพโซมสำหรับการนำส่งทางผิวหนังของละลายน้ำน้อยโดยใช้วิธีพื้นผิวตอบสนอง

ชื่อนักวิจัย และสถาบัน: ผู้ช่วยศาสตราจารย์ ดร. สุรีวัลย์ ดวงจิตต์ คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี

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บทคัดย่อ:

วัตถุประสงค์ของการศึกษานี้เพื่อออกแบบและพัฒนาแบบสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด (transinvasome; OTV) แบบพร้อมกันเพื่อเพิ่มการนำส่งแคปไซซินทางผิวหนัง โดยใช้การออกแบบประสมกลาง (composite experimental design) แบบซ้ำที่จุดศูนย์กลาง (duplicate centroids) เตรียมต้นแบบสูตรตำรับทรานสอินเวโซม (transinvasomes; TVs) 10 สูตร โดยศึกษาส่วนประกอบไขมันของสูตรตำรับเป็นปัจจัยสูตรตำรับ (X_n) และปัจจัยตอบสนอง (Y_n) สูตรตำรับทรานสอินเวโซมประกอบด้วยฟอสฟาทิดิล คอเลสเตอรอลความเข้มข้นคงที่ และแคปไซซินร้อยละ 0.15 และศึกษาปัจจัยร้อยละของลิโมนีน (X_1) และโคคามายด์ไดเอทาโนลามีน (X_2) คุณลักษณะทางเคมีกายภาพ ได้แก่ ขนาดอนุภาค การกระจายขนาด ศักย์ไฟฟ้าซีตา ความสามารถในการกักเก็บยา และการซึมผ่านผิวหนังของสูตรตำรับทรานสอินเวโซมศึกษาโดยวิธีการทดลอง ความสัมพันธ์ระหว่างปัจจัยสูตรตำรับ ปัจจัยตอบสนอง และความคงตัวของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุดทำนายโดยใช้ Design Expert® software ความถูกต้องและความน่าเชื่อถือของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด (OTV) ที่ทำนายโดยโปรแกรมคอมพิวเตอร์ยืนยันผลโดยวิธีการทดลองเป็นสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุดโดยการทดลอง (experimental transinvasome formulation; ETV) ผลการวิจัยพบว่าการซึมผ่านผิวหนังของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุดโดยการทดลอง (ETV) ใกล้เคียงกับสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด (OTV) และมากกว่าอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับลิโพโซมมาตรฐานและผลิตภัณฑ์ที่มีจำหน่ายในท้องตลาด พื้นผิวตอบสนองที่ทำนายโดยโปรแกรมคอมพิวเตอร์ช่วยให้เข้าใจความสัมพันธ์ที่ซับซ้อนระหว่างปัจจัยสูตรตำรับและปัจจัยตอบสนองและความคงตัวของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด

คำหลัก : ลิโพโซม เทอร์ปีน ระบบนำส่งยาทางผิวหนัง การหาสูตรที่เหมาะสมที่สุด วิธีพื้นผิวตอบสนอง

Abstract

Project Code : MRG5980260

Project Title : Role of terpenes on formulation optimization of liposomes for transdermal delivery of lipophilic drug using response surface method

Investigator : Asst.Prof.Dr.Sureewan Duangjit, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University

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Project Period : 2 Years

Abstract:

The aim of this study was to design and develop simultaneous optimal transinvasome formulations (OTV) to enhance the transdermal delivery of capsaicin. Using a central composite experimental design with duplicate centroids, ten model formulations of transinvasomes (TVs) were demonstrated. The lipid compositions of the TV formulations were determined as formulation factors (X_n) and response variables (Y_n). TV formulations containing a constant concentration of phosphatidylcholine, cholesterol and 0.15% capsaicin and various percentages of *d*-limonene (X_1) and cocamide diethanolamine (X_2) were prepared. The physicochemical characteristics, e.g., the vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability, of the TV formulations were experimentally investigated. The relationship among the formulation factor, the response variables and the stability of the OTV was predicted using Design Expert® software. The accuracy and reliability of the OTV predicted using computer software were experimentally confirmed and investigated as an experimental transinvasome formulation (ETV). The results indicated that the skin permeability of the ETV was close to the OTV and was significantly higher than that of conventional liposomes and commercial products. The response surfaces estimated by the computer software were helpful in understanding the complicated relationship among the formulation factor, the response variables, and the stability of the TV formulation.

Keywords : Liposomes; Terpenes; Transdermal drug delivery; Optimization; Response surface method

สารบัญ

กิตติกรรมประกาศ

โครงการวิจัยนี้ได้รับทุนอุดหนุนการวิจัยประเภททุนพัฒนาศักยภาพในการทำงานวิจัยของอาจารย์รุ่นใหม่ (MRG) จากสำนักงานกองทุนสนับสนุนการวิจัยและสำนักงานคณะกรรมการการอุดมศึกษา ปีงบประมาณ 2559 ผู้วิจัยขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย สำนักงานคณะกรรมการการอุดมศึกษา และมหาวิทยาลัยอุบลราชธานี ที่สนับสนุนด้านทุนวิจัย สถานที่และเครื่องมือในการทำวิจัย ขอขอบคุณนักวิจัยที่ปรึกษารองศาสตราจารย์ ดร.ธนะเศรษฐ์ ง้าวหิรัญพัฒน์ คณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร ผู้วิจัยขอขอบคุณผู้มีส่วนในงานวิจัยทุกท่านที่มีส่วนช่วยให้งานวิจัยสำเร็จลุล่วง และหากงานวิจัยนี้มีข้อผิดพลาดประการใด ผู้วิจัยขออภัยมา ณ โอกาสนี้

คณะผู้จัดทำ

สิงหาคม 2561

บทคัดย่อ

วัตถุประสงค์ของการศึกษานี้เพื่อออกแบบและพัฒนาแบบสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด (transinvasome; OTV) แบบพร้อมกันเพื่อเพิ่มการนำส่งแคปไซซินทางผิวหนัง โดยใช้การออกแบบประสมกลาง (composite experimental design) แบบซ้ำที่จุดศูนย์กลาง (duplicate centroids) เตรียมต้นแบบสูตรตำรับทรานสอินเวโซม (transinvasomes; TVs) 10 สูตร โดยศึกษาส่วนประกอบไขมันของสูตรตำรับเป็นปัจจัยสูตรตำรับ (X_n) และปัจจัยตอบสนอง (Y_n) สูตรตำรับทรานสอินเวโซมประกอบด้วยฟอสฟาทิดิล คอเลสเตอรอลความเข้มข้นคงที่ และแคปไซซินร้อยละ 0.15 และศึกษาปัจจัยร้อยละของลิโมนีน (X_1) และโคคามายด์ไดเอทาโนลามีน (X_2) คุณลักษณะทางเคมีกายภาพ ได้แก่ ขนาดอนุภาค การกระจายขนาด ศักย์ไฟฟ้าซีตา ความสามารถในการกักเก็บยา และการซึมผ่านผิวหนังของสูตรตำรับทรานสอินเวโซมศึกษาโดยวิธีการทดลอง ความสัมพันธ์ระหว่างปัจจัยสูตรตำรับ ปัจจัยตอบสนอง และความคงตัวของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุดทำนายโดยใช้ Design Expert® software ความถูกต้องและความน่าเชื่อถือของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด (OTV) ที่ทำนายโดยโปรแกรมคอมพิวเตอร์ยืนยันผลโดยวิธีการทดลองเป็นสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุดโดยการทดลอง (experimental transinvasome formulation; ETV) ผลการวิจัยพบว่าการซึมผ่านผิวหนังของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุดโดยการทดลอง (ETV) ใกล้เคียงกับสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด (OTV) และมากกว่าอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับลิโพโซมมาตรฐานและผลิตภัณฑ์ที่มีจำหน่ายในท้องตลาด พื้นผิวตอบสนองที่ทำนายโดยโปรแกรมคอมพิวเตอร์ช่วยให้เข้าใจความสัมพันธ์ที่ซับซ้อนระหว่างปัจจัยสูตรตำรับและปัจจัยตอบสนองและความคงตัวของสูตรตำรับทรานสอินเวโซมที่เหมาะสมที่สุด

Abstract

The aim of this study was to design and develop simultaneous optimal transinvasome formulations (OTV) to enhance the transdermal delivery of capsaicin. Using a central composite experimental design with duplicate centroids, ten model formulations of transinvasomes (TVs) were demonstrated. The lipid compositions of the TV formulations were determined as formulation factors (X_n) and response variables (Y_n). TV formulations containing a constant concentration of phosphatidylcholine, cholesterol and 0.15% capsaicin and various percentages of *d*-limonene (X_1) and cocamide diethanolamine (X_2) were prepared. The physicochemical characteristics, e.g., the vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability, of the TV formulations were experimentally investigated. The relationship among the formulation factor, the response variables and the stability of the OTV was predicted using Design Expert® software. The accuracy and reliability of the OTV predicted using computer software were experimentally confirmed and investigated as an experimental transinvasome formulation (ETV). The results indicated that the skin permeability of the ETV was close to the OTV and was significantly higher than that of conventional liposomes and commercial products. The response surfaces estimated by the computer software were helpful in understanding the complicated relationship among the formulation factor, the response variables, and the stability of the TV formulation.

บทที่ 1 ที่มาและความสำคัญของปัญหา

(Introduction to the research problem and its significance)

1.1 Introduction

Topical and transdermal drug delivery (TDD) has numerous advantages over conventional drug delivery (oral and injectable drug delivery), such as first pass metabolism avoidance, non-invasiveness and improved patient compliance. As a primary limitation of TDD, the stratum corneum (SC) is the outermost layer of skin and an excellent skin permeability barrier. Several theoretical strategies have been introduced to overcome intact SC, such as (1) drug and vehicle interactions, including the use of drug or prodrugs, chemical potential, ion pairs, and eutectic systems; (2) vesicles and particles, e.g., liposomes and high-velocity particles; (3) SC-modified materials, e.g., skin hydration and chemical enhancers; (4) SC bypassed or removed materials, e.g., microneedles, ablation and follicular delivery; and (5) electrically-assisted methods, e.g., ultrasound, iontophoresis, electroporation, magnetophoresis, radio waves and photomechanical waves.(Barry, 2006)

The combination of two different strategies to enhance TDD has been shown to substantially improve the skin permeation of various drugs.(Trommer and Neubert, 2006) The combination of liposomes (vesicles and particles) and chemical or penetration enhancers (SC modified) has frequently been demonstrated in TDD research.(El Maghraby *et al.*, 2008) Penetration enhancers that improve TDD via several mechanisms have been identified, and their potential mechanisms of action differ: (1) modify SC conformation, (2) modify the desmosomes between corneocytes and (3) modify intercellular lipids.(Williams and Barry, 2004) The integration of various types of penetration enhancers improves the approach to enhancing TDD because of differences in the enhancers' mechanisms of action.(Chattaraj and Walker, 1995) The synergistic effect of solutions of terpenes and ethanol systems or the combination of various types of surfactant systems markedly enhances TDD compared with the application of individual components alone.(Cazares-Delgadillo *et al.*, 2005) Moreover, the formulation exhibits increased safety compared with single penetration enhancers by reducing skin irritation.(Karande *et al.*, 2004)

Several intensive studies have suggested that the skin efficacy, skin safety and formulation stability of vesicle formulations are primarily influenced by lipid composition or formulation factors. Hence, only specially designed vesicles have been shown to facilitate transdermal drug delivery, e.g., transfersomes, ethosomes, fexosomes, menthosomes and invasomes. Previous studies have introduced novel elastic vesicles to enhance transdermal

drug delivery, the so called transethosomes (TELs). TELs are novel, modified vesicles of elastic liposomes between transfersomes and ethosomes, comprising phospholipids, ethanol, water and permeation enhancers or surfactants (oleic acid). TELs can deliver drugs into deep skin regions and enhance both *in vitro* and *in vivo* skin deposition over that of conventional elastic liposomes (CELs) and conventional liposomes (CLPs).(Song *et al.*, 2012) The precise ratios of various penetration enhancers in TELs directly affect physicochemical characteristics, skin permeability, skin safety and formulation stability.

The aim of this study was to design and develop simultaneous optimal transinvasome formulations (OTV) to enhance the transdermal delivery of capsaicin using Design Expert® software. Transinvasomes (TVs) are novel elastic liposomes that integrate the excellent characteristics of transfersomes and invasomes for TDD. TV formulations containing a fixed concentration of phosphatidylcholine, cholesterol, 0.15% capsaicin and various percentages of formulation factor (X_n), e.g., *d*-limonene (main penetration enhancer of invasomes) and cocamide diethanolamine (main penetration enhancer of transfersomes), were prepared. The physicochemical characteristics (Y_n) (e.g., vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability) of the TV formulations were measured. The response surface method (RSM) was applied to understand the relationship between formulation factors and response variables, and the OTV was subsequently predicted using Design Expert® software. The accuracy and reliability of the predicted OTV were experimentally confirmed and evaluated as experimental transinvasome formulations (ETVs). Furthermore, the estimated response surfaces were utilized to clarify the relationship between formulation factor and formulation stability.

1.2 Objectives

1.2.1 To design and develop optimal liposome formulation as a transdermal delivery carrier for enhancing skin permeation of lipophilic using the computer programs.

1.2.2 To investigate the influence of the formulation factor (terpenes and co-solvents) such as types and concentrations on physicochemical characteristics of liposome (e.g., vesicle size, size distribution, zeta potential, drug content, entrapment efficiency), morphology, thermal properties, stability of the formulation and *in vitro* skin permeation enhancement by response surface method.

1.3 Expected benefits

1.3.1 The novel liposomal systems with high potential for enhancing transdermal delivery of lipophilic drugs are optimized and prepared using the computer-based design; the reliability and its reproducibility are confirmed by the experiment.

1.3.2 The response surface in of each response variable could be utilized as the fundamental knowledge to easily understand the complicated relationships between formulation factor and physicochemical characteristics.

1.4 Scope of research

1.4.1 The model liposome formulations are experimentally prepared and investigated. The ratio of each composition is obtained from the design of the experiment.

1.4.2 The physicochemical characteristics e.g., vesicle size, size distribution, zeta potential, elasticity, drug content, entrapment efficiency, stability, morphology, thermal properties and the skin permeability of the model formulations are investigated.

1.4.3 The response surfaces and the optimal liposome formulation are calculated using the computer program.

1.4.4 The response surfaces can be used for understanding the relationship between the fromulation foactors and the physicochemical characteristics. The predicted optimal liposome formulation is confirmed the accuracy and reliability by the experimental optimal liposome formulation. The predicted optimal liposome formulation is experimentally prepared and evaluated.

บาที่ 2 ทบทวนวรรณกรรม

(Review literatures)

The pharmaceutical formulations are consisted of several formulation factors or composition and method of preparation variables. Several responses relating to the physicochemical characteristics, the stability, the effectiveness, as well as safety must be optimized simultaneous. The difficulties in the quantitative approach to develop the formulations are approximating the relationship between causal factors and pharmaceutical responses. Furthermore, another difficulty is an optimal formulation for one property is not always desirable for the other characteristics. This is called a multi-objective optimization problem. Consequently, expertise and experience are required to design and develop an acceptable pharmaceutical formulation (Takayama *et al.*, 2003). The response surface method (RSM) has widely been used for choosing the acceptable pharmaceutical formulations. The conventional pharmaceutical formulation was successfully applied using the RSM-S for obtaining the optimal formulation such as griseofulvin solid dispersions (Takai *et al.*, 1984), indomethacin gel ointment (Takayama *et al.*, 1990), ketoprofen hydrogels (Takayama and Nagai, 1991), sustained-release tablet of chlorpheniramine maleate (Hirata *et al.*, 1992), controlled-release theophylline tablet (Matsumura *et al.*, 1994) and ondansetron hydrogels (Obata *et al.*, 2010). In addition, predicted value of diltiazem hydrochloride release profile estimated by RSM-S also coincided well with the release profile of optimal formulation measured by the experiment (Kikuchi and Takayama, 2010). Thus, the novel pharmaceutical formulation performed under the design of experiments and RSM-S may have an approach for achieving research and development for the best pharmaceutical formulation. The RSM includes (a) statistical factorial experimental designs, (b) modeling between causal factors and response variables, and (c) multi-objective optimization for seeking the best formulation under a data set of pragmatic constraints. Composite experimental design can be applied for selecting rationale model formulations, which are consisted of many formulation factors and method of preparation variables. Compared to a normal analysis based on a one-factor-at-a-time experiments, we can greatly reduce the number of the experiments for the preparation of model formulations. Response variables of these model formulations are predicted quantitatively by the combination of causal factors. Generally, multiple regression analysis has been applied on the basis of a quadratic polynomial equation, since theoretical relationships between causal factors and response variables are not clear. Finally, multi-objective optimization algorithms are applied for predicting the best formulation (Takayama and Nagai, 1991).

2.1 Liposomes and analogs

Liposomes are lipid vesicles that fully enclose an aqueous volume (Williams, 2003). Lipid molecules are usually phospholipids with or without some additives. The lipid may be arranged in one or more bilayers. The lipid component affects the characteristics and properties of the resulting liposomes so, for example, the addition of relatively small amounts of cholesterol tends to stabilize the membrane and hence the liposomes would be somewhat more rigid than non-cholesterol adding vesicle. Liposomes can entrap lipophilic molecules within the membrane or they can entrap hydrophilic molecules within their aqueous core. Liposomes can be classified in many ways, depending for example on their method of preparation, on their size, or by their lamellarity as summarized in Table 1. The novel designing liposomes have also been reported upon, and tend to be named after the lipid source from which they derive, such as niosomes using non-ionic surfactant, ethosomes using ethanol or menthosomes using menthol as their vesicle composition. However, the major vesicle component still was phospholipid and most commonly; the film hydration method is used. The discussion below concentrates on the most common liposome types of current interest for drug delivery to and through the skin, namely conventional (traditional) liposomes and the more specialized variants e.g., transfersomes, niosomes, ethosomes and other highly deformable (or elastic) liposomes.

Table 1 Classification of liposomes

Classification of liposomes	Composition and/or characteristics
By composition	
- Conventional (traditional) liposomes	Phospholipid (with and without cholesterol)
- Transfersomes	Phospholipid + Edge activator (surfactant)
- Niosomes	Non-ionic surfactant + Cholesterol
- Ethosomes	Phospholipid + Ethanol
- Invasomes	Phospholipid + Ethanol + Terpenes
- Flexosomes	Phospholipid + Non-ionic surfactant + Edge activator
- Menthosomes	Phospholipid + Edge activator + Menthol
By size and lamellarity	
- Small unilamellar vesicles (SUV)	25-100 nm
- Large unilamellar vesicles (LUV)	100-400 nm
- Multilamellar vesicles (MLV)	0.2-10 μm

2.1.1 Conventional (traditional) liposomes

Conventional liposomes or traditional liposomes are the first generation of phospholipid vesicles that fully enclose an aqueous volume. Conventional liposomes

molecules are usually phospholipids with or without Cholesterol addition. Cholesterol may be included to improve bilayers characteristics of liposomes (Vemuri and Rhodes, 1995); increasing the microviscosity of the bilayers, reducing permeability of the membrane to water soluble molecules, stabilizing the membrane and increasing rigidity of the vesicles.

2.1.2 Novel deformable liposomes

Intensive research of highly deformable (ultraflexible or elastic) liposomes led to the introduction and development, over the past 15 years, of a new class of highly deformable (ultraflexible or elastic) liposomes that have been named Transfersomes®. While conventional liposomes were reported to have mainly localizing or rarely transdermal effects, deformable liposomes were reported to penetrate intact skin, carrying therapeutic concentrations of drugs, but only when applied under non-occluded conditions (Cevc and Blume., 1992).

2.1.2.1 Deformable liposomes (Transfersomes®) are the first generation of elastic vesicles introduced by Cevc and Blume (Cevc and Blume., 1992). They consist of phospholipids and an edge activator. An edge activator is often a single chain surfactant, having a high radius of curvature that destabilizes lipid bilayers of the vesicles and increases deformability of the bilayers (Cevc, 1996; Cevc *et al.*, 1996; Honeywell-Nguyen and Bouwstra, 2005). Sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80 and dipotassium glycyrrhizinate were employed as edge activators (El Maghraby *et al.*, 1999; El Maghraby *et al.*, 2000, 2001a-b; Trotta *et al.*, 2002). Preparation of deformable liposomes involves methods similar to those used in preparation of traditional liposomes. Most commonly, the film hydration method is used. The effects of incorporation of different edge activators on physicochemical characteristics (i.e., vesicle size, zeta potential, entrapment efficiency, among others) of deformable liposomes were extensively investigated in several studies (Elsayed *et al.*, 2007; Jain *et al.*, 2003; Oh *et al.*, 2006). The interaction between edge activators and liposomes was also investigated (El Maghraby *et al.*, 2001a-a, 2004).

2.1.2.2 Niosomes are a novel and efficient approach to drug delivery. They are mainly composed of nonionic surfactants and cholesterol and the enclosed interior usually contains a buffer solution at appropriate pH. Niosomes are usually prepared by various methods such as nitrogen bubble method, film hydration method, from proniosomes, etc. which affect their formations along with the properties of the drug, cholesterol content and amount, structure and type of surfactant. As a drug delivery

system, niosomes are more stable as compared to other vesicular systems and are cheap. They are found to improve the stability of the entrapped drug. They can also be prepared with different structural characteristics (composition, fluidity and size), and for particular routes of administration. Niosomes are useful in the delivery of various therapeutically active moieties such as gene delivery, COPD drug delivery, anti-cancer agents, anti-inflammatory agents, and anti-infective agents. Various types of drug deliveries can be possible using niosomes like targeting, ophthalmic, topical, parenteral, transdermal, cosmetics, etc. While selecting a suitable drug through niosomal drug delivery, it should be kept in mind that niosomes encapsulating hydrophobic drugs and macromolecules are more stable than niosomes encapsulating low molecular weight drugs. These factors also affect niosome stability in vivo (Mahale *et al.*, 2012).

2.1.2.3 Ethosomes are another novel lipid carrier, recently developed by Touitou et al. (Touitou *et al.*, 2000; Touitou *et al.*, 2000b), showing enhanced skin delivery. The ethosomal system is composed of phospholipid, ethanol and water (Touitou *et al.*, 2000). Although, liposomal formulations containing up to 10% ethanol and up to 15% propylene glycol were described by Foldvari et al. (Foldvari *et al.*, 1993), the use of high ethanol content was first described by Touitou et al. (Touitou *et al.*, 1997) for ethosomes. Due to the interdigitation effect of ethanol on lipid bilayers, it was believed that high concentrations of ethanol are detrimental to liposomal formulations. However, ethosomes which are novel permeation-enhancing lipid vesicles embodying high concentration (20–45%) of ethanol were developed and investigated. Ethosomes are most commonly prepared as described by Touitou et al. (Touitou *et al.*, 2000). Briefly, the lipids and the drug are dissolved in ethanol. The aqueous component is added slowly in a fine stream at constant rate in a well-sealed container with constant mixing. Mixing is then continued for additional few minutes.

Several studies investigated the effect of ethanol on physicochemical characteristics of the ethosomal vesicles (Dayan and Touitou, 2000; Elsayed *et al.*, 2007; Lopez-Pinto *et al.*, 2005; Touitou *et al.*, 2000). One reported characteristic of ethosomes is their small size relative to liposomes, when both are obtained by preparation methods not involving any size reduction steps (Dayan and Touitou, 2000). This reduction in vesicle size could be explained as a result of incorporation of high ethanol concentration. Ethanol confers a surface negative net charge to the liposome which causes the size of vesicles to decrease (Lopez-Pinto *et al.*, 2005; Touitou *et al.*, 2000). The size of ethosomal vesicles was reported to increase with decreasing ethanol

concentration in the ethanol concentration range of 20–45% (Touitou *et al.*, 2000). The effect of phospholipid concentration on the size of ethosomal vesicles was also investigated (Elsayed *et al.*, 2007; Touitou *et al.*, 2000). Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles (Touitou *et al.*, 2000) as well as by the presence of ethanol in ethosomes, which allows for better solubility of many drugs.

2.1.2.4 Invasomes was developed and introduced by Verma (Verma, 2002). Several studies of Dragicevic-Curic et al. (Dragicevic-Curic *et al.*, 2008a, 2009b, c) revealed that the invasome dispersion containing 3.3% (w/v) ethanol and 1% (w/v) of the terpene mixture (cineole: citral: d-limonene; 45:45:10, v/v) could significantly enhance skin penetration of the highly hydrophobic photosensitizer temoporfin (mTHPC). Invasomes enhanced mTHPC-deposition in SC compared to liposomes without terpenes and conventional liposomes, and they were efficient in delivering mTHPC to deeper skin layers (Dragicevic-Curic *et al.*, 2008a). Invasomes were of a small particle size (<150 nm), high homogeneity (<0.3), mostly unilamellar and spherical, but also deformed vesicles were detected. Invasomes containing 1% (w/v) cineole provided the highest skin penetration enhancement of mTHPC, i.e. they provided high amounts of mTHPC in the SC and deeper skin layers, indicating that also incorporation of a single terpene into invasomes could provide efficient nano-carriers of mTHPC. These invasomes could be considered as a promising tool for delivering the photosensitizer mTHPC to the skin. However, in contrast to most invasomes, being effective nano-carriers of mTHPC, there were also formulations less effective than liposomes containing 3.3% (w/v) ethanol and one formulation were less efficient than conventional liposomes.

2.1.2.5 Flexosomes are introduced by Song and Kim (Song and Kim, 2006a). To increase topical delivery of low-molecular-weight heparin (LMWH), cationic, neutral, and anionic flexible liposomes or cFlexosome, nFlexosome, and aFlexosome, respectively were prepared. The effects of surface charge of Flexosome on physicochemical properties and skin penetration of LMWH were also investigated. Among the different formulations of Flexosome, cFlexosome demonstrated three-time higher entrapment efficiency of LMWH, and better physicochemical stability than nFlexosome and aFlexosome. In vitro skin penetration and in vivo localization into the deeper skin layer

of LMWH were significantly greater from cFlexosome compared to other formulations. Changes of skin surface charge after LMWH-cFlexosome application were investigated as a function of time. In the process of skin penetration, the Flexosomes act as drug carrier with the associated LMWH. Overall, macromolecular LMWH could be delivered deeply into the skin by topical application of cFlexosome for the treatment of superficial thrombosis, subcutaneous wounds, bruise, and burns.

Moreover, flexosomes provided significantly enhanced mTHPC-amount in the skin compared to conventional liposomes. Cationic flexosomes delivered the highest mTHPC-amount to SC and deeper skin layers. In addition, mTHPC was not found in the acceptor compartment regardless of the applied vesicle formulation, indicating no risk of systemic side-effects (i.e. photosensitivity) (Dragicevic-Curic *et al.*, 2010).

2.1.2.6 Menthosomes, the latest deformable liposomes, was developed and introduced by Duangjit et al (Duangjit *et al.*, 2012a). Menthosomes, novel ultradeformable carriers consist of phospholipids, menthol and edge activator. Menthosomes was optimized using a nonlinear response-surface method incorporating thin-plate spline interpolation (RSM-S), which accuracy and reliability of the optimal formulation was also confirmed in several intensive studies (Kikuchi and Takayama, 2010; Obata *et al.*, 2010). The result showed that the estimated values by the computer programs were very close to experimental values of skin permeability at 2-12 and steady-state flux value of meloxicam (Duangjit *et al.*, 2012a).

2.2 Terpenes

Terpenes, a class of skin penetration enhancers obtained from natural sources, offer advantages over many other penetration enhancers, in part because they are categorized as generally regarded as safe substances by the U.S. Food and Drug Administration (Thakur *et al.*, 2006). Structurally, terpenes consist of isoprene units (C_5H_8) and can be classified as hydrocarbons, alcohols, ketones, and oxides. Monoterpenes and some sesquiterpenes are the major components of essential oils and have been widely studied as skin-penetration enhancers for various drugs, such as propranolol (Kunta *et al.*, 1997), tamoxifen (Zhao and Singh, 1998), clonazepam (Puglia *et al.*, 2001), lorazepam (Puglia *et al.*, 2001), haloperidol (Vaddi *et al.*, 2002), nicardipine (Krishnaiah *et al.*, 2002), zidovudine (Narishetty and Panchagnula, 2005), diclofenac sodium (Nokhodchi *et al.*, 2007). Liposomes containing terpenes have been reported to enhance the skin pen-etration of a number of drugs. However, its mechanism of action on the skin has not yet been clearly elucidated (Subongkot *et al.*, 2012). The potent terpenes

derivatives including limonene, menthol, cineole, geraniol, citral, camphor, seliene have been used in drug delivery systems (Dragicevic-Curic *et al.*, 2009a) (Figure 1).

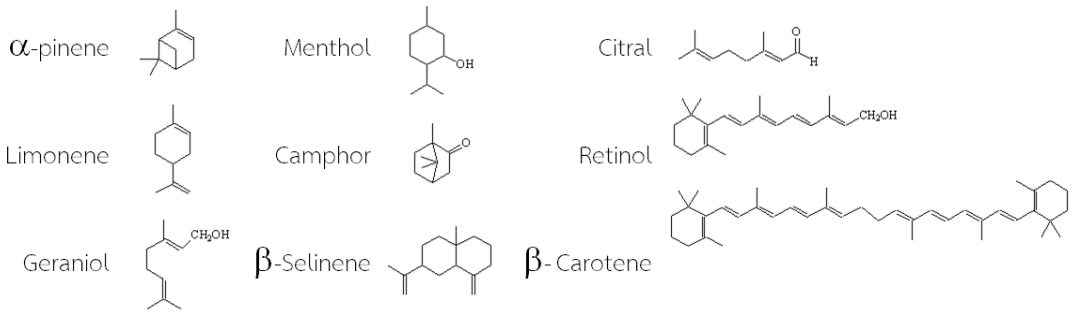

Figure 1 The different structure s of terpenes

2.3 Capsaicin

Capsaicin (8-methyl N-vanillyl-6-nonenamide) is a natural alkaloid (capsaicinoid) and is the major active pungent ingredient extracted from chili pepper. Capsaicin is a fat-soluble, odorless, pungent-tasting, off-white solid with a melting point between 62-65°C and a molecular weight of 305.4 kDa. Capsaicin is reliable because of its hot pungent taste and ability to cause a burning sensation to mammalian tissues. Because capsaicin is water-insoluble, alcohol and other organic solvents are utilized to solubilize capsaicin in conventional topical preparations and sprays. Capsaicin is applied to topical pharmaceutical treatments for a diversity of diseases, including musculoskeletal inflammation, rheumatism, post hepatic neuralgia, lumbago and sciatica.(Huang *et al.*, 2008) The mechanisms of action of capsaicin have been extensively studied over the past few decades. Capsaicin can release substance P from the afferent nociceptive neurons, and the resulting depletion of substance P causes the desensitization of small afferent sensory neurons.(Hayman and Kam, 2008) However, a significant hepatic first-pass metabolism has been detected upon the oral administration of capsaicin in rats and mice(Donnerer *et al.*, 1990), and the strong pungency of capsaicin limits its clinical applications. Currently, several studies reported the topical and transdermal delivery of capsaicin using novel carriers e.g., niosomes or ME, however the high concentration used (0.75% w/w capsaicin)(Tavano *et al.*, 2011) and, the use of ethanol or benzyl alcohol at high level are still their limitation.(Huang *et al.*, 2008; Zhang *et al.*, 2008) Therefore, ME with proper co-surfactant systems may hold the promise of being the proper formulation for the transdermal delivery of low dose capsaicin (0.15% w/w) with low concentration of surfactant system.

บทที่ 3 วิธีการทดลอง
(Methodology)

3.1 Materials

Phosphatidylcholine (PC) was supplied as a special gift from LIPOID GmbH (Cologne, Germany). Cholesterol (CHOL) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Capsaicin (CAP) and *d*-limonene (Lim) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cocamide diethanolamine (Com) (Comperlan® KD) was obtained from BASF (Thai) Co. Ltd. (Bangkok, Thailand). Polysorbate-20 (Tween® 20, T20) was purchased from the NOF Corporation (Osaka, Japan). All other chemicals were commercially available and of analytical and high-performance liquid chromatography (HPLC) grade.

3.2 Capsaicin-loaded transinvasomes preparation

Model formulations of transinvasomes (TV) comprising a controlled amount of 10 mM phosphatidylcholine (PC), 1 mM cholesterol (CHOL), 2% Tween 20 (T20), 0.15% capsaicin (CAP) and various concentrations of the penetration enhancers limonene (Lim) and cocamide diethanolamine (Com) at 0.5-1.5% and 10-30%mol, respectively, were experimentally prepared. Ten model formulations of TV were obtained from a central composite experimental design with duplicate centroids, as shown in Table 1. The percentages of Lim (X_1) and Com (X_2) were selected as formulation factors. TVs were prepared by the sonication method.(Subongkot *et al.*, 2012) Briefly, lipid mixtures of PC, CHOL, Com and CAP were dissolved in a mixture of chloroform/methanol (2:1 volume ratio). The solvent mixture was evaporated under a nitrogen gas stream. The lipid film was placed in a desiccator for at least 6 h to remove the remaining solvent. The dried lipid thin film was hydrated with 0.1 M phosphate buffer solution (PBS, pH 7.4), and a mixture of T20 and Lim was also incorporated during this process. The vesicles were subsequently sonicated for two cycles of 30 min using a bath-type sonicator (5510J-DTH Branson Ultrasonics, Danbury, USA). The TV formulations were freshly prepared or stored in airtight containers at 4°C prior to use.

3.3 Experimental design and statistical analysis

Design Expert® software (Design Expert® version 8, Stat-Ease, Inc., MN, U.S.A.) was used to evaluate the relationship between the formulation factors and the physicochemical characteristics (as response variables) of the TV formulation. The central composite experimental design, containing the axial points to define the quadratic term, was constructed.

The dual replicates of the center point were executed to resist the effect of noise and to ensure reliability. In pre-formulation study, the lipid composition ranges of limonene (Lim) and cocamide diethanolamine (Com) were screened to establish the minimum and maximum levels in the TV formulation. The results suggested that the concentration of 5-1.5% Lim and 10-30% Com was utilized to prepare TV formulations in this study. Table 1 shows the central composite experimental design with the formulation factors (X_1 and X_2) and response variables (Y_n), e.g., vesicle size (Y_1), size distribution (Y_2), zeta potential (Y_4), entrapment efficiency (Y_3) and skin permeation flux (Y_5). The regression analysis of the dataset was carried out with statistical software, presuming the quadratic mode with interactions among factors. The term of the quadratic polynomial model (O'Reilly Beringhs *et al.*, 2013; Woo *et al.*, 2015) of the coded factor can be determined as:

$$Y = \beta_0 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2 + \beta_1X_1 + \beta_2X_2 \quad (1)$$

where Y represents response variables associated with each formulation factor level combination, X_1^2 and X_2^2 are the quadratic factors, X_1X_2 represents the binary interactions among factors, and X_1 and X_2 are the formulation factors *d*-limonene (Lim) and cocamide diethanolamine (Com), respectively. β_0 is the mean value, and $\beta_1, \beta_2, \beta_{12}, \beta_{11}$ and β_{22} are regression coefficients. Analysis of variance (ANOVA) was executed to identify the significance of the quadratic factors (X_n^2), binary interactions (X_nX_m) and single factors (X_n) in relation to the influence on the response analysis. The responses were considered significant at a coefficient p value < 0.05.

3.4 Vesicle size, size distribution and zeta potential measurement

The average vesicle sizes, size distributions and zeta potentials of the TV were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano series, Malvern Instruments, UK). Twenty microliters of the TV formulation were diluted with 1480 µL of deionized water. All measurements were performed in triplicate at 25°C.

3.5 Entrapment efficiency of the CAP-loaded TV formulation measurement

The concentration of CAP in the TV formulation was determined by HPLC analysis after disruption of the TV with Triton® X-100 (0.1% v/v) at a 1:1 volume ratio and was then appropriately diluted with PBS, pH 7.4. The Triton® X-100/TV vesicle mixture was centrifuged at 10,000 rpm at 4°C for 10 min. The supernatant was filtered with a 0.45 µm nylon syringe filter.

The entrapment efficiencies of the CAP-loaded TV formulation were calculated according to the following equation:

$$\% \text{ entrapment efficiency} = (C_L/C_i) \times 100,$$

where C_L is the concentration of the CAP-loaded TV formulation, as described in the above methods, and C_i is the initial concentration of CAP added to the formulation.

3.6 *In vitro* skin-permeation

The SC is well known as an excellent barrier of human skin. Therefore, the shed snakeskin of *Naja kaouthia* was used as a model for skin permeation studies because of its similarity to human SC. (Wonglertnirant *et al.*, 2012) The shed snakeskin was donated by the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand. The entire shed snakeskin was obtained from different snakes immediately after shedding and stored at -10°C prior to use. The estimated thickness of the skin was 0.02-0.03 mm. The shed snakeskin was divided into 10-12 pieces, and cut into round sections of 2.5 cm × 2.5 cm. After thawing, the shed snakeskin was immediately placed on a Franz diffusion cell. A Franz diffusion cell with an available permeation area of 2.01 cm^2 and a water jacket at 32±1°C was employed. The SC side of the shed snakeskin faced the donor chamber and was filled with 1 mL of CAP formulations. The receiving chamber was filled with 6.5 mL of receiver medium (PBS pH 7.4: ethanol; 1:1 v/v) under sink conditions and stirred by a magnetic stirrer at 500 rpm. At appropriate intervals, 0.5 mL aliquots of the receiver medium were withdrawn and immediately replaced with an equal volume of fresh medium. The CAP in the receiver medium was analyzed by HPLC. The cumulative amount of CAP that permeated through the shed snakeskin was plotted as a function of time. The steady-state flux (J) was defined after calculating the slope of the linear portion of the plot.

Following the *in vitro* skin permeation study, the shed snakeskin was washed with water and blotted dry. The spectrum of the shed snakeskin was recorded in the range of 500-4000 cm^{-1} using a FT-IR spectrophotometer (Nicolet 4700, Thermo Scientific, USA). An X-ray diffractometer (XRD) (MiniFlex II, Rigaku Co., Tokyo, Japan) was used to investigate and confirm the possible mechanism underlying the effect of the different formulations on the skin permeation of capsaicin. The same shed snakeskin was cut into small pieces, approximately 2 cm × 2 cm, and attached to an aluminum well sample holder. XRD was used with Cu Kα, scanning from 2θ = 5 – 45°. The voltage and operating current were 30 kV and 15 mA, respectively.

3.7 Simultaneous optimization

The optimization of the TV formulation based on the RSM was performed using the dataset obtained from model formulations. The formulation factors Lim (X_1) and Com (X_2) and the response variables of the model formulation, e.g., vesicle size (Y_1), the size distribution (Y_2), the zeta potential (Y_3), the entrapment efficiency (Y_4) and the skin permeability (Y_5), were defined. The simultaneous OTV formulation was estimated using the appropriate characteristics prescribed in a previous study. (Duangjit *et al.*, 2014a) Briefly, an appropriate TV formulation was designed to minimize the vesicle size, size distribution and zeta potential and to maximize the entrapment efficiency and skin permeation flux. Once the RSM-estimated OTV formulation was obtained, the reliability and desirability were evaluated by linear correlation, and the corresponding residual plot, as previously described. (O'Reilly Beringhs *et al.*, 2013) Moreover, experiments were carried out to confirm the reliability of the predicted values under optimal conditions.

3.8 Stability evaluation

The physicochemical stabilities of the TV formulations were evaluated by monitoring the formulations for at least 90 days after their initial preparation. Various TV formulations were stored in glass bottles with plastic plugs at 4±1°C for 90 days to determine the stability of the formulations. The physicochemical stabilities of TV formulations were evaluated by visual inspection for sedimentation. The vesicle size, size distribution and zeta potential were determined by PCS. The CAP remaining in the TV formulation was analyzed by HPLC after 90 days. The dataset obtained from the evaluation of the physicochemical stabilities was analyzed using Design Expert® software to predict the relationship between the formulation factors and the formulation stability.

3.9 HPLC analysis

The concentration of CAP in the samples was analyzed by HPLC. All measurements were stored at 4°C until analysis. The HPLC 1100 system (Agilent 1100 Series HPLC System, Agilent Technologies, U.S.A.) was used. The analytical column was an Eclipse XDB-C18 column (particle size = 5 µm; column dimension = 4.6 mm × 150 mm), and a mobile phase comprising diluted phosphoric acid in reversed osmosis water (1 in 1000):acetonitrile (50:50), a flow rate of 1 mL/ min, and a UV detector set at 280 nm were used for all determinations. (Duangjit *et al.*, 2015a) The calibration curve for CAP was within the range of 1–100 µg/mL, with a correlation coefficient of 0.999.

บทที่ 4 ผลการทดลองและวิจารณ์ผลการทดลอง
(Results and discussion)

4.1 Experimental design

Among the various formulation factors that define the feasibility of TV for the transdermal delivery of CAP, knowledge of the behavior of the factors associated with efficacy, skin safety and formulation stability is crucial in high efficiency formulations. Used to design and develop a simultaneous OTV to enhance the transdermal delivery of CAP, penetration enhancers are compounds known for their ability to improve the skin permeability of the formulation (efficacy) and also affect skin irritation (skin safety) and formulation stability. Therefore, formulation factors (Lim and Com) were considered because the skin permeation flux (as efficacy parameter) should be relatively high, whereas the skin irritation (as skin safety parameter) and the formulation stability (as stability parameter) should remain relatively low. In this respect, Lim and Com play important roles as potential penetration enhancers because adequate percentages of these components may improve the skin permeation flux of the TV formulation. Nevertheless, the Lim extracted from plants is a good candidate for a permeation enhancer because of its relatively low skin irritation potential. In addition, Com has been extensively used as a nonionic surfactant in various cosmetics and topical personal care products, reflecting its rare allergic contact dermatitis potential. (Shaughnessy *et al.*, 2014) Therefore, the formulation factors used in this study were safe with respect to skin irritation. Other response variables considered included the vesicle size (Y_1), size distribution (Y_2), zeta potential (Y_3), entrapment efficiency (Y_4) and skin permeability (Y_5) of each TV formulation because the principle of vesicle formulation is based on these physicochemical characteristics. (Duangjit *et al.*, 2012b) The values of the response variables for different experimental conditions are shown in Table 2.

4.2 Factors influencing TV characteristics

The statistical analysis data, e.g., the significant model, regression coefficient value, analysis of variance (p value), for the response variables are shown in Table 3. The quadratic model for all responses was strongly significant (except zeta potential), and the model maximized the R^2 coefficients. Adequate precisions of 11.8920 (vesicle size), 9.2350 (size distribution), 2.6580 (zeta potential), 11.1670 (entrapment efficiency) and 9.9290 (skin permeation flux) indicated an adequate signal/noise ratio (an adequate precision ratio greater

than 4 is desirable). Moreover, the lack of fit values for all responses were over 0.05, implying that the lack of fit was not significant relative to the pure error. Non-significant lack of fit values represented a good model in this study. The response surface, the linear correlation and the corresponding residual plot of the response variables of the model TV formulation are shown in Fig. 2. The ANOVA of the formulation factors for each response is detailed separately below.

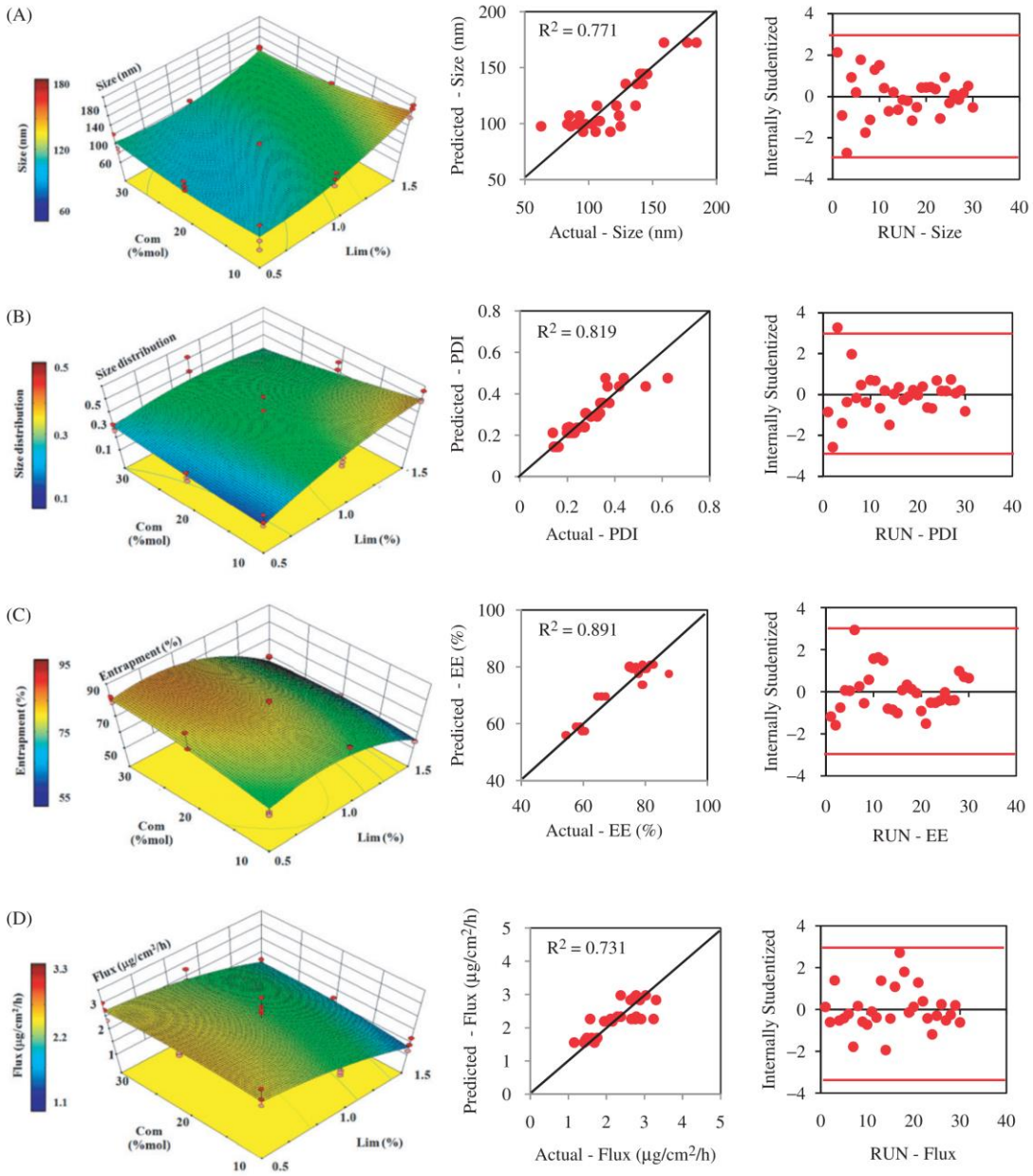

Fig. 2 The response surface (left), the linear correlation (middle) and the corresponding residual plot (right) of the (A) size, (B) size distribution, (C) entrapment efficiency and (D) skin permeation flux of the model transinvasome formulation.

Table 2 The compositions (formulation factors; X_n) and physicochemical characteristics (response variables; Y_n) of vesicle formulations

Form.	Formulation factors						Response variables				
	Fixed factors		Variable factor				Physicochemical characteristics (mean±SD)				
	PC (mM)	CHOL (mM)	X_1	Lim (%)	X_2	Com (%mol)	Size (nm) (Y_1)	Size distribution (Y_2)	Zeta potential (-mV) (Y_3)	Entrapment (%) (Y_4)	Flux ($\mu g/cm^2/h$) (Y_5)
1	10	1	-1	0.5	-1	10	91.18±27.51	0.47±0.12	17.20±3.55	65.83±1.34	2.94±0.34
2	10	1	-1	0.5	0	20	104.58±9.29	0.44±0.09	18.38±3.45	81.02±5.66	2.77±0.06
3	10	1	-1	0.5	1	30	100.43±18.00	0.35±0.04	19.00±2.85	81.12±1.85	2.73±0.33
4	10	1	0	1.0	-1	10	121.54±14.20	0.25±0.05	19.88±3.51	79.04±0.25	2.05±0.11
5	10	1	0	1.0	0	20	96.86±5.53	0.19±0.04	16.87±1.69	76.12±0.39	2.15±0.59
6	10	1	0	1.0	0	20	90.78±6.09	0.21±0.02	16.96±2.31	79.87±0.48	2.93±0.29
7	10	1	0	1.0	1	30	108.43±1.12	0.15±0.02	17.81±1.76	77.24±2.48	2.48±0.26
8	10	1	1	1.5	-1	10	173.38±11.50	0.30±0.04	16.24±1.80	54.38±0.19	1.42±0.27
9	10	1	1	1.5	0	20	142.73±3.83	0.31±0.03	17.48±1.43	58.07±0.74	1.56±0.14
10	10	1	1	1.5	1	30	136.23±6.18	0.23±0.02	15.89±0.81	59.89±0.57	1.62±0.14
ETV	10	1	-	0.5	-	20	101.39±2.63	0.19±0.02	18.80±1.05	80.84±1.24	3.12±0.20
CIV	10	1	-	0.5	-	-	96.75±1.64	0.41±0.06	-5.81±0.27	82.14±2.70	2.21±0.19
CTS	10	1	-	-	-	20	97.55±2.64	0.21±0.01	-2.79±1.51	64.92±0.80	1.24±0.16
CLP	10	1	-	-	-	-	109.23±0.49	0.24±0.01	-3.82±0.71	62.79±1.80	1.22±0.20

PC; phosphatidylcholine, Chol; cholesterol, Lim; limonene, Com; cocamide diethanolamine

Table 3 Terms of the significant model, regression coefficient value, and analysis of variance (p value) for the response variables

Polynomial term	Vesicle size		Size distribution		Zeta potential		Entrapment efficiency		Skin permeation flux	
	coefficient	p value	coefficient	p value	coefficient	p value	coefficient	p p value	coefficient	p p value
Model	-	<0.0001*	-	0.0002*	-	0.6280	-	<0.0001*	-	<0.0001*
Intercept	99.59	-	0.24	-	-17.49	-	136.91	-	2.46	-
X_1: Lim	25.80	<0.0001	0.038	0.0001	0.83	0.1398	-19.98	<0.0001	-0.64	<0.0001
X_2: Com	-6.84	0.0645	-0.013	0.1344	0.10	0.8499	2.30	0.3678	0.07	0.4099
X_1X_2	-11.60	0.0130	-0.036	0.0020	0.54	0.4249	-6.52	0.0437	0.10	0.3229
X_1^2	18.99	0.0026	-0.026	0.0704	0.44	0.6157	-19.85	<0.0001	-0.21	0.1218
X_2^2	9.61	0.1023	0.0046	0.7356	-0.47	0.5901	-6.76	0.1048	-0.11	0.4142
R^2	0.7711		0.8192		0.1275		0.8066		0.7306	
Adjusted R^2	0.7234		0.5353		-0.0543		0.7663		0.6744	
Predicted R^2	0.6091		0.4363		-0.3593		0.6908		0.6093	
Adequate precision	11.8920		9.2350		2.658		11.1670		9.9290	
Lack of Fit	1.99	0.1457	2.47	0.0904	1.74	0.1889	1.40	0.2703	0.88	0.4663

* significant

4.2.1 Vesicle size response

The formulation factors with a significant influence on vesicle size were the Lim and Com percentages in the TV formulation. The Lim percentage supported a positive influence (coefficient = 25.80), as an increase in Lim percentage leads to an increase in vesicle size (Table 3). This effect corresponded to the results of a previous study(Dragicevic-Curic *et al.*, 2008b) showing that the vesicle size of temoporfin-loaded invasomes increased with increasing concentrations of terpenes from 0, 0.5 and 1%, respectively. Furthermore, the quadratic term of Lim (X_1^2) was considered to be a strong influence on vesicle size (p value < 0.05), revealing a nonlinear relationship and reaching a plateau at 1.5% (Fig. 2A). The response surface of the vesicle size suggested that with the incorporation of Lim up to 1.5%, the vesicle size of the TV may increase more than 180 nm. To formulate the TV formulation using a vesicle size smaller than 100 nm, the Lim percentage should not be more than 1.0%.

The Com percentage provided a negative influence (coefficient = -6.84), suggesting that an increase in the Com percentage leads to a decrease in the vesicle size. This effect can be attributed to the fact that the insertion of Com (as single chain surfactant) into the TV increases the curvature, resulting decreased vesicle size. (Park *et al.*, 2011) Moreover, the vesicle size decreases as the percentage of the surfactant increases, thereby leading to crucially stabilized vesicles because the small vesicles have more chances to collide with each other.(van Zyl *et al.*, 2004) However, at high cocamide diethanolamine (Com) monomer to phosphatidylcholine (PC) (Com:PC) weight ratios, vesicles smaller than 50 nm are nearly translucent. Therefore, an increase in Com percentage will not necessarily lead to a dramatic vesicle size reduction but may lead to the presence of mixed-micelles in the formulation. Furthermore, vesicle size can be controlled by the method of preparation, e.g., the ultrasonication process or the drug loading in the formulation, as well as the percentage of surfactant used.(Landfester *et al.*, 1999; Song and Kim, 2006b) However, the quadratic term of Com (X_2^2) indicated that the percentage of Com did not evidently influence the vesicle size (p value = 0.1023) (Fig. 2A). The response surface of the vesicle size suggested that the incorporation of Com at various percentages between 10 and 30% also resulted in nano-size range of all formulations (no more than 200 nm).

4.2.2 Size distribution response

The only factor that significantly influenced the reduction in the size distribution was the Com percentage in the TV formulation. The Com percentage exerted a negative influence (coefficient = -0.013), suggesting that an increase in the Com percentage leads to a decrease

in the size distribution (Table 3). However, an increase in the Lim percentage leads to an increase in the size distribution (Fig. 2B). Among the quadratic factors (X_1^2 and X_2^2), binary interactions among factors (X_1X_2) and single formulation factors ($X_1$and X_2) that determine the feasibility of significantly affecting the size distribution, Lim (X_1) was the most significant, as the p value = 0.0001. Dragicevic-Curic et al. reported that an increase in the Lim percentage (from 0.5-1.0%) leads to an increase in the size distribution, with acceptable homogeneity (PDI < 0.3).

4.2.3 Zeta potential response

The quadratic term of the Lim and Com factors (X_1^2 and X_2^2; p value > 0.05) was not significant because both formulation factors act as penetration enhancers with no intrinsic charge (non-ionic compounds). The zeta potential was one of the most important response variables influencing the skin permeability of the vesicle formulation. (Duangjit *et al.*, 2012b) Therefore, in case of the ionic compound the result may show inversely. This study suggested that the response surface of the zeta potential should be considered case by case. Nevertheless, the TV formulation in this study showed a negative surface charge because of the influence of PC as a zwitterionic compound with an isoelectric point (pI) of approximately 6-7.(Chain and Kemp, 1934) The experimental condition included a pH of 7.4, which was higher than the pI of the PC carrying a net negative charge.(Petelska and Figaszewski, 2002)

4.2.4 Entrapment efficiency response

The Lim percentage was a strongly significant factor influencing the entrapment efficiency of the CAP-loaded TV formulations (p value < 0.0001). The Lim percentage exerted a negative influence (coefficient = -19.98). In addition, the quadratic term of the Lim was also verified as being strongly significant (X_1^2; p value < 0.0001). An increase in the Lim percentage led to a decrease in the entrapment efficiency of CAP in the formulation. Moreover, the response surface of the entrapment efficiency showed an entrapment efficiency of CAP-loaded TV formulations of more than 80% when the incorporation of Lim was not more than 1% (Fig. 2C). The increase in the Lim percentage (log P 4.23)(El-Kattan *et al.*, 2001) increased the lipophilicity in the lipid bilayer of the TV vesicles, which may compete with CAP (as lipophilic drug, log P 3.8)(Hanson *et al.*) when arranged in the lipid bilayer(Rangsimawong *et al.*, 2014; Varman and Singh, 2012) and hence the CAP was excluded as it assembled into the bilayer of the TV vesicles.

4.2.5 Skin permeation flux response

The only factor that had a significant influence on skin permeation flux was the Lim percentage in the TV formulation. The Lim percentage exerted a negative influence (coefficient = -0.64), demonstrating that an increase in the Lim percentage leads to a decrease in the skin permeation flux (Table 3). This result might be attributed to the intrinsic property of Lim. As the partition coefficient of Lim (log P) is 4.23(El-Kattan *et al.*, 2001), Lim strongly exhibited lipophilic property. Therefore, CAP would rather in remain in the TV formulation more than permeate through the release medium. The response surface indicated that for a satisfactory skin permeation flux greater than 2.5 $\mu g/cm^2/h$, the Lim should not be more than 1.0%, whereas the percentage of Com should be between 10-25% (Fig. 2D). However, several studies have previously suggested that a ratio of 1% for terpenes is appropriate for enhancing the transdermal delivery of various drugs, e.g., carboxyfluorescein,(Chen *et al.*, 2011) sodium fluorescein,(Rangsimawong *et al.*, 2014) and temoporfin.(Chen *et al.*, 2011; Dragicevic-Curic *et al.*, 2008b; Subongkot *et al.*, 2012) This result can be attributed to the fact that the addition of Com may simultaneously affect the skin permeability of TV formulations to enhance the transdermal delivery of CAP. Therefore, TV formulations with a 0.5% Lim percentage presented an adequate skin permeation flux in this study. Moreover, Com was not only used as a potential penetration enhancer in the TV formulation but was also utilized to increase the solubility of Lim in TV vesicles instead of ethanol. Ethanol (as solubilizing agent for Lim), which was used in vesicle formulations with Lim or other terpene derivatives (e.g., cineol, methanol, carveol) in previous studies,(Dragicevic-Curic *et al.*, 2008b) may limit the formulation stability of the vesicle formulation because of the evaporation of ethanol after storage. Thus, Com was used as an alternative solubilizing agent and non-ionic surfactant for the vesicle formulation incorporating Lim or terpene derivatives instead of volatile substances, such as ethanol.

The reliability of all response surfaces was confirmed by linear correlation, and the corresponding residual plots between the experimental run and the internally studentized residuals are shown in Fig. 2 in the middle and right columns, respectively. These results indicated that the linear correlation of almost all response surfaces was between 0.7-0.9, indicating good correlation. Furthermore, under the completely randomized run, the vertical distribution of the internally studentized residuals was within the line from top-to-bottom. This result suggested that all points fell within the limits of a 95% confidence interval (CI).

4.3 Optimal TV formulation

The conformity between the adjusted and the predicted R^2 values, as a difference lower than 0.2 of the mathematical model obtained by the response surface method (RSM), permits the safe estimation of the optimal TV formulation. The numerical optimization identifies an appropriate point that maximizes the desirability index based on pre-established values. The optimization options of a goal can be altered after adjusting the limits, weights and importance. For all formulation factors and response variables, the goals were intergraded into one desirability index. The desirability of the optimal formulation includes a target function scoring from zero to one (outside of the limits to the target). The desirability index was absolutely dependent on how far the lower and upper limits were set in relation to the genuine optimal formulation.

The criteria focused on the minimization of the vesicle size, size distribution and zeta potential and the maximization of the entrapment efficiency and skin permeation flux. According to the dataset obtained from the statistical analysis shown in Table 4, the target of the optimization was to optimize the proportion of Lim (X_1) and Com (X_2) because the vesicle formulation that offered relatively high-skin permeability also contained high concentrations of penetration enhancers. The skin irritation indicates that the safety of the penetration enhancers used may restrict the consumption of this formulation. (Duangjit *et al.*, 2015a) However, the selected penetration enhancers in this study were based on the safety of their intrinsic properties, as described above.

Table 4 The response variables of the optimal formulation

Response	Actual value	Predicted value	95% Confidence Intervals (lower - upper)	Bias* (%)
Size (nm)	101.39±2.63	93.90	81.03- 106.76	7.98
Size distribution	0.19±0.02	0.19	0.16- 0.21	0.24
Charge (-mV)	18.80±1.05	18.32	16.95- 19.68	2.62
EE (%)	80.84±1.24	78.47	75.26- 81.68	3.03
Flux (μg/cm^2/h)	3.12±0.20	2.92	2.69- 3.14	6.95

* Bias was calculated as {(predicted value - experimental value)/experimental value}×100%.

Under the numerical optimization conditions, the integrated desirability index of the optimal TV formulation (OTV) containing 0.5% Lim (X_1) and 20% mol Com (X_2) was 0.771. A desirability index higher than 0.7 indicated that the model was capable of optimizing all of the

factors (formulation factors and response variables) simultaneously until an adequate level was reached.(Roustaei *et al.*, 2015) The individual desirability index of both Lim and Com predicted by this software was 1.000, indicating that this model was highly competent for the optimization requirements for each of the factors.

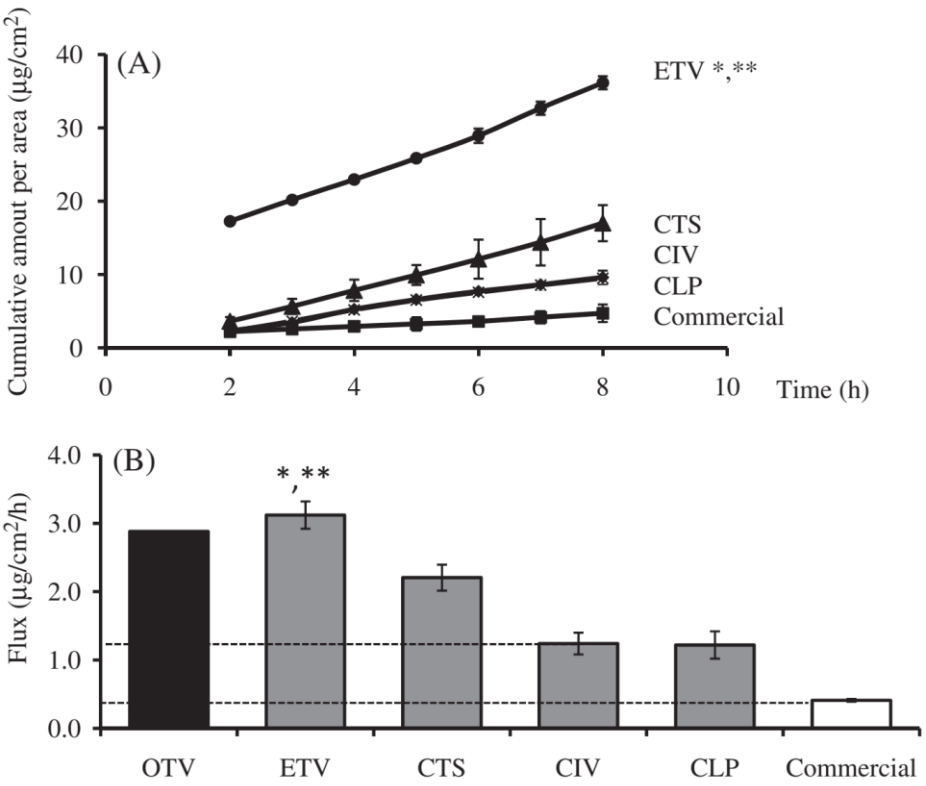

Fig. 3 (A) The skin permeation profile and (B) flux of different CAP formulations. * and ** indicate $p < 0.05$ compared with the commercial product and the conventional transinvasomes, respectively.

To confirm the accuracy and reliability of the OTV formulation predicted using Design Expert®, the experimental TV formulation (ETV), conventional transfersomes (CTS), conventional invasomes (CIV) and conventional liposomes (CLP) prepared and investigated experimentally. The cumulative skin permeation per area and the skin permeation flux of different vesicle formulations were compared with the commercial product of CAP (GoodSense® Capsaicin Arthritis Pain Relief, a product manufactured in the USA), as shown in Fig. 3. The concentration of CAP in all formulations was 0.15%. The cumulative skin permeation per area of the different formulations increased linearly after 2 h. The skin permeation flux of CAP through the skin model was defined as the slope of the linear portion of the plot. According to the skin permeation profile, the results showed that the skin permeation profile of ETV was

higher than that of the CTS, CIV, CLP and commercial products (Fig. 3A). The skin permeation flux of the ETV was significantly higher that of the CLP (p value = 0.004) and the commercial products (p value = 0.002), as shown in Fig. 3B. The skin permeation data indicated that the dramatic synergistic permeability-enhancing effect of two different types of surfactants, as in ETV, was greater than that of Com and Lim, as in CTS and CIV, respectively. This result strongly suggested that Lim and Com act synergistically (Davidsen *et al.*, 2002) to improve the transdermal delivery of CAP. The synergistic effect of Com and Lim may be due to the combination of different mechanisms of each intrinsic property. The ETV formulation may promote skin permeability by various mechanisms. The possible mechanisms by which this ETV formulation (as elastic liposomes) improved the skin delivery of CAP encompassed the penetration-enhancing mechanism and the vesicle adsorption to and/or fusion with the stratum corneum.(Duangjit *et al.*, 2014b; El Maghraby *et al.*, 2008)

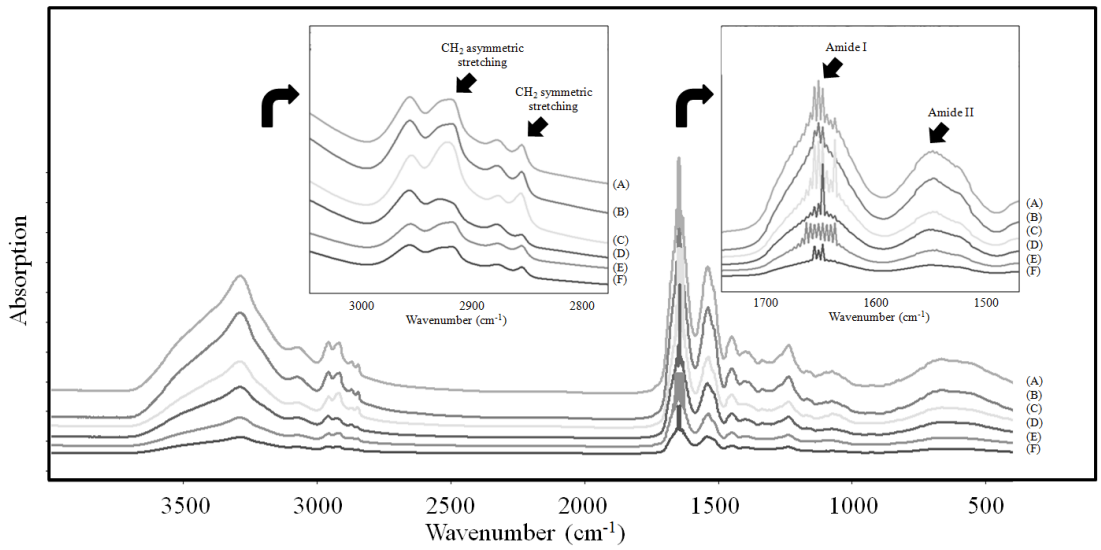

Fig. 4 The FT-IR spectra of the shed snakeskin after treatment with different formulations; (A) ETV, (B) commercial product, (C) CTS, (D) CIV and (E) CLP compared with the skin treated with (F) PBS.

The possible mechanism of action of the different formulations was confirmed by the FT-IR spectra and X-ray diffractograms, as shown in Fig. 4 and Fig. 5, respectively. The spectrums between 1500 and 1700 cm^{-1}, and 2800 and 3000 cm^{-1} of the FT-IR spectra are due to the amide (I and II) and the CH_2 stretching patterns, respectively. The amide I patterns were split into a doublet (1600 to 1700 cm^{-1}), whereas the amide II pattern was broad peak (1500 to 1580 cm^{-1}). The alteration of the peak of the amide I and amide II patterns were used to

understand the organization and interaction of hydrogen bonds at the polar interface. The modified shapes of the CH_2 symmetric stretching (~ 2850 cm^{-1}) and asymmetric stretching (~ 2920 cm^{-1}) frequencies were attributed to changes in the conformational order or hydrocarbon chain fluidity and alkyl chain packing. (Yu *et al.*, 2012) The result indicated that the CH_2 stretching patterns of skin treated with ETV, CLP and commercial product did not markedly differ from those of intact skin treated with PBS. In contrast, the CH_2 asymmetric stretching peaks of the skin treated with the CTS and CIV shifted from 2920 to 2924 and 2931 cm^{-1}, respectively.

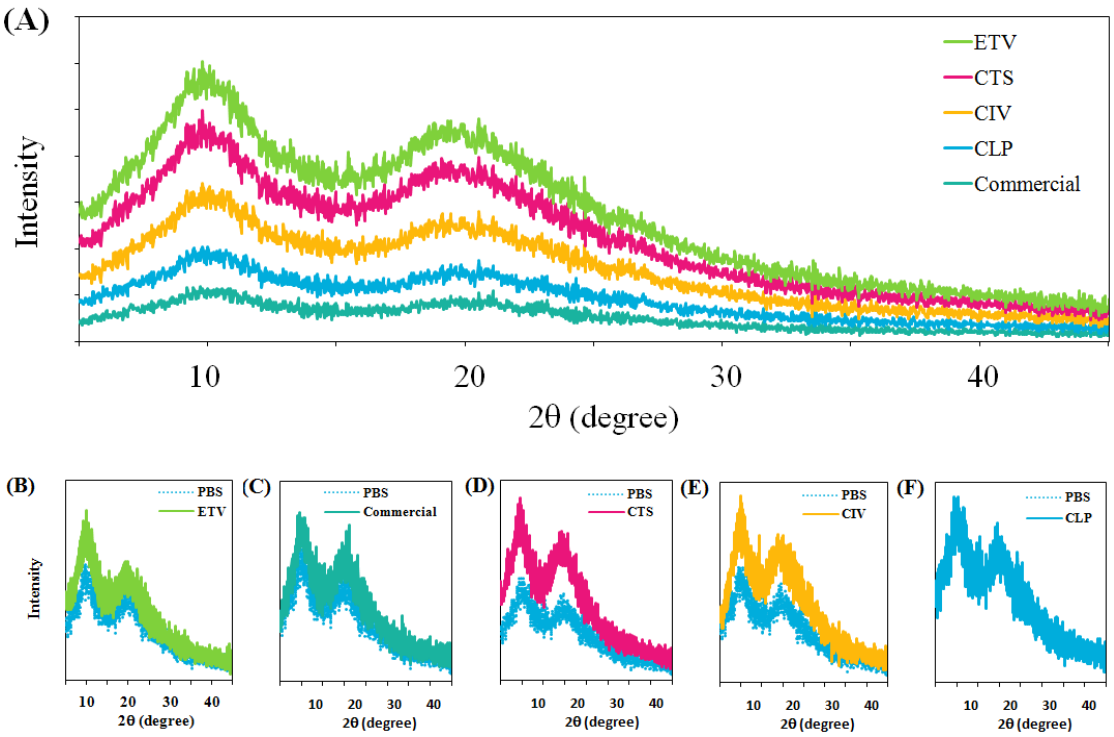

Fig. 5 The X-ray diffractogram of the shed snakeskin after treatment with different formulations (A) all diffractograms, (B) ETV, (C) commercial product, (D) CTS, (E) CIV and (F) CLP compared with the skin treated with PBS.

Furthermore, the amide I bands of the skin treated with the CTS and CIV changed from the intact skin treated with PBS, as shown in Fig. 4. The X-ray diffractograms at 2θ = 10 and 20° demonstrated the hexagonal packing of the alkyl chains of lipids in the skin. (Mizushima *et al.*, 1996) The X-ray diffractograms of the skin treated with ETV, CLP and commercial product did not significantly differ from those of the intact skin (as treated with PBS) as shown in Fig. 5B, 5C and 5F, respectively, whereas the skin treated with the CTS and CIV exhibited notable

differences, as shown in Fig. 5D and 5F, respectively. These results revealed that the combination of various types of surfactants may increase safety compared with single penetration enhancers by reducing skin irritation.

The experimental values of the confirmation experiments were close to the predicted values and fell within the 95% CI, as presented in Table 4. These results suggested the good estimation of the model for response analysis and confirmed the good reliability of the established model for the response observed in the RSM design. Furthermore, the reliability of these predicted values was also displayed as the percentage of bias (lower than 10%).(Singh *et al.*, 2011) These findings provide useful fundamental information for the development and design of novel vesicle formulations to enhance the transdermal delivery of CAP.

4.4 Stability of the TV formulation

The stability evaluation of ten model formulations of TV was performed after measuring the vesicle size, size distribution, zeta potential and entrapment efficiency initially and at 90 days after preparation. The TV formulations remained transparent, and no signs of sedimentation at 4±1°C for 90 days were observed. The physicochemical stabilities of ten TV formulations incorporating CAP are displayed in Fig. 6. The contour plots represent the relationship formulation factors (Lim and Com) on the formulation stability as the initial preparation (left column), 90 days after preparation (middle) and the different values between days 0 and 90 (right column). The contour plots of the percent difference between days 0 and 90 were useful for the estimation of the relationship between the formulation factors and the formulation stability**.** The small difference in vesicle size, size distribution and zeta potential at days 0-90 reflected the addition of a high percentage of Lim and Com in the TV formulation, as shown in the blue areas in Fig. 6A-C (right column). The percent difference in the vesicle size, size distribution and zeta potential was smaller than 10% after 90 days. In addition, the remaining entrapment efficiency of the TV formulation was approximately 80% after storage, as shown in the green areas in Fig. 6D (right column). The changes in the fraction of the formulation factors of TVs demonstrated the feasibility of the TV formulations to acquire favorable physicochemical stability.(Juškaitė *et al.*, 2015) The results revealed the ability of computer software to estimate the formulation stability of the vesicle formulation, which can reduce the number of measurements required to simultaneously determine the effects of both Lim and Com on the physicochemical stabilities of TV formulations.

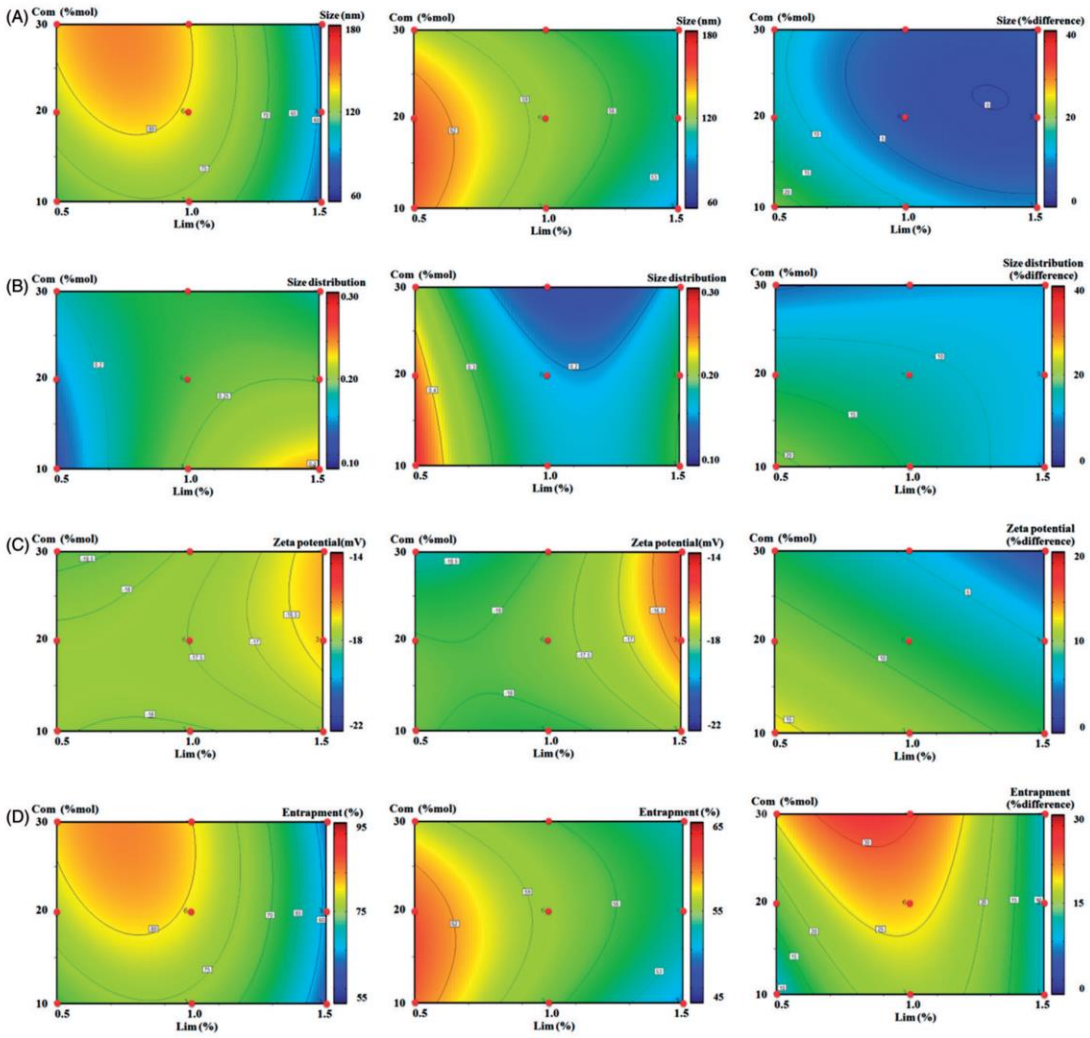

Fig. 6 The contour plots of the (A) size,(B) size distribution, (C) zeta potential and (D) entrapment efficiency of the model formulation of TV at initial preparation (left column), 90 days after preparation (middle column) and the percent difference between days 0 to 90 (right column).

บทที่ 5 สรุปผลการทดลองและข้อเสนอแนะสำหรับงานวิจัยในอนาคต
(Conclusion and suggestion)

The attempt to design and develop simultaneous OTV formulations to enhance the transdermal delivery of CAP using computer software presented an advancement challenge as the complicated relationship between various formulation factors and response variables must be considered. The design of experimental and computer software was applied to reduce the number of experimental formulations and to model the optimal TV formulation with proper characteristics. The accuracy and reliability of OTV formulations were experimentally verified by the formulation and evaluation of ETV. The results indicated that the experimental value agreed well with the predicted response variables. Considering the skin permeation data of the ETV formulation, we successfully showed the feasibility of the transdermal delivery of CAP using an ETV formulation. Moreover, the RSM was applied to construct a probabilistic graphical model of the formulation stability pattern and elucidated the complicated relationships between the formulation factors and the response variables.

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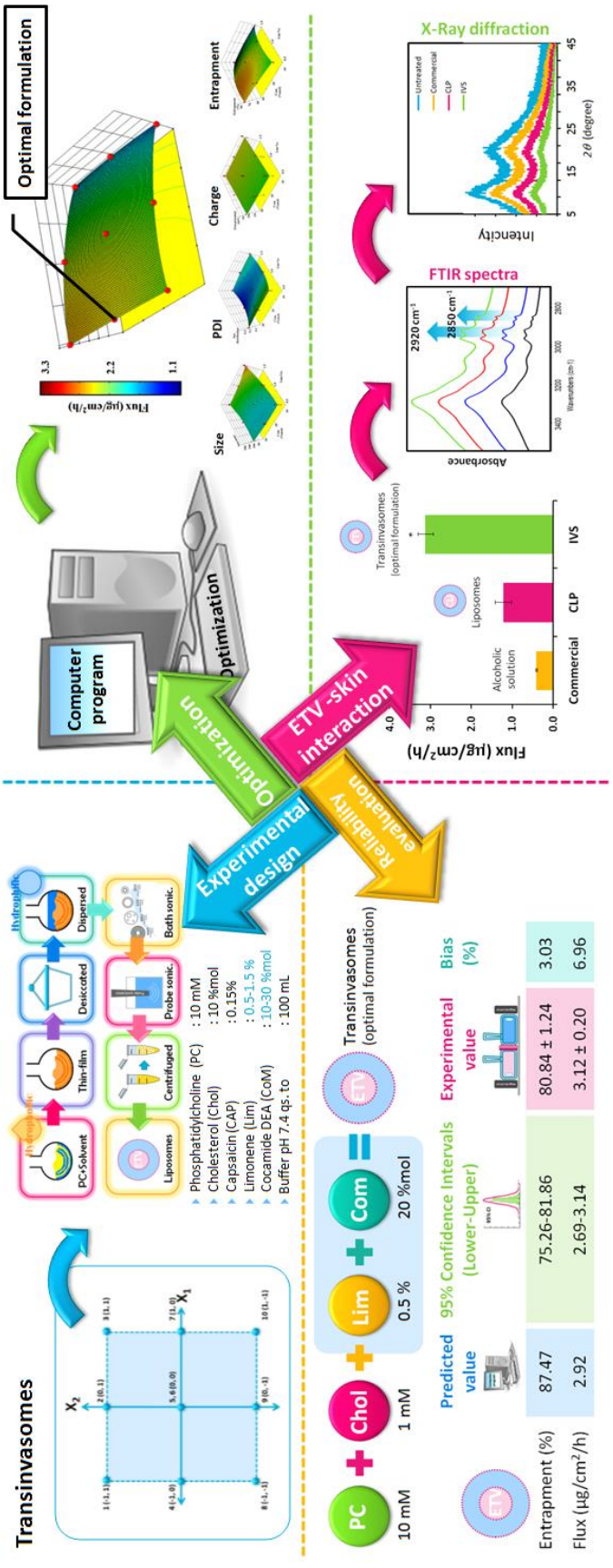
Optimal formulation
Flux (µg/cm²/h)
3.3
2.2
1.1
Entrapment
Charge
PDI
Size
Computer program
Optimization
Optimization
Experimental design
ETV-skin interaction
Reliability evaluation
X-Ray diffraction
Untreated
Commercial
CLP
IVS
2θ (degree)
Intencity
FTIR spectra
2920 cm⁻¹
2850 cm⁻¹
Absorbance
Transinvasomes (optimal formulation)
Liposomes
Alcoholic solution
Commercial
CLP
IVS
Flux (µg/cm²/h)
Transinvasomes
PC-Solvent
Thin-film
Desiccated
Dispersed
Liposomes
Centrifuged
Probe sonic.
Bath sonic.
Hydrophilic
Phosphatidylcholine (PC) : 10 mM
Cholesterol (Chol) : 10 %mol
Capsaicin (CAP) : 0.15%
Limonene (Lim) : 0.5-1.5 %
Cocamide DEA (CoM) : 10-30 %mol
Buffer pH 7.4 qs. to : 100 mL
Transinvasomes
PC
10 mM
Chol
1 mM
Lim
0.5 %
Com
20 %mol
Transinvasomes (optimal formulation)
Predicted value
95% Confidence Intervals (Lower-Upper)
Experimental value
Bias (%)
Entrapment (%) 87.47 75.26-81.86 80.84 ± 1.24 3.03
Flux (µg/cm²/h) 2.92 2.69-3.14 3.12 ± 0.20 6.96

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ประวัตินักวิจัย

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สถานที่ติดต่อ : กลุ่มเภสัชเคมีและเทคโนโลยีเภสัชกรรม

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16. **Duangjit S**, Sila-On W, Puapempoonsiri U, Ngawhirunpat T, Bumrungthai S, Chuchote T. Characterization of nano-vesicular containing tomato extract for dermal delivery: Liposomes, transfersomes and niosomes. The International Conference of Pharmaceutical Sciences and Medicines 2017 (ICPAM 2017). Burapha University, Bansean Chonburi, Thailand.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า) หรือผลงานตามที่คาดไว้ในสัญญาโครงการ	- Sureewan Duangjit, Tassanan Nimcharoenwan, Nutcha Chomya, Natthaporn Locharoenrat & Tanasait Ngawhirunpat. Computational design strategy: an approach to enhancing the transdermal delivery of optimal capsaicin-loaded transinvasomes. Drug Development and Industrial Pharmacy 2017; 43(1): 98-107 [IF=2.295]. **Published [เอกสารแนบ A]** - ชื่อเรื่องที่คาดว่าจะพิมพ์: Role of terpenes as permeation enhancement for transdermal drug delivery of novel liposomes: response surface method ชื่อวารสารที่คาดว่าจะตีพิมพ์: Int J of Pharm
2. การนำผลงานวิจัยไปใช้ประโยชน์	-
3. เชิงพาณิชย์ (มีการนำไปผลิต/ขาย/ก่อให้เกิดรายได้ หรือมีการนำไปประยุกต์ใช้โดยภาคธุรกิจ/บุคคลทั่วไป)	- ผลิตเป็นผลิตภัณฑ์ต้นแบบเพื่อเป็นสินค้าทดลองใช้ แต่ห้ามจำหน่าย ให้กับคณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี **[เอกสารแนบ B]**
4. เชิงนโยบาย (มีการกำหนดนโยบายอิงงานวิจัย/เกิดมาตรการใหม่/เปลี่ยนแปลงระเบียบข้อบังคับหรือวิธีทำงาน)	-
5. เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)	- การนำผลิตภัณฑ์ต้นแบบเพื่อเป็นสินค้าทดลองใช้ แต่ห้ามจำหน่ายในงานแสดงสินค้าจัดโดย อุทยานวิทยาศาสตร์ มหาวิทยาลัยอุบลราชธานี **[เอกสารแนบ C]**
6. เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)	- การนำองค์ความรู้ใหม่ที่ได้ไปใช้ในการเรียนการสอนรายวิชาเภสัชภัณฑ์และระบบนำส่งยารูปแบบใหม่ และรายวิชาการวิจัยและพัฒนาเครื่องสำอางและผลิตภัณฑ์สุขภาพขั้นสูง

7. อื่น ๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

การนำเสนอผลงานในที่ประชุมวิชาการ

- Duangjit S, Ngawhirunpat T. Optimization of solid lipid nanoparticles gels for transdermal delivery of capsaicin by response surface method. In: Seoul International Conference on Engineering and Applied Science (SICEAS 2017); 2017 Feb 7; Seoul, South Korea. 2017. p.407-414 **[Poster presentation; Proceeding]** **[เอกสารแนบ D]**

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การจดสิทธิบัตร [คำข้อรับสิทธิบัตร/อนุสิทธิบัตร]

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RESEARCH ARTICLE

Computational design strategy: an approach to enhancing the transdermal delivery of optimal capsaicin-loaded transinvasomes

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ABSTRACT

The aim of this study was to design and develop simultaneous optimal transinvasome formulations (OTV) to enhance the transdermal delivery of capsaicin. Using a central composite experimental design with duplicate centroids, 10 model formulations of transinvasomes (TVs) were demonstrated. The lipid compositions of the TV formulations were determined as formulation factors (X_n) and response variables (Y_n), respectively. TV formulations containing a constant concentration of phosphatidylcholine, cholesterol, 0.15% capsaicin, and various percentages of *d*-limonene (X_1) and cocamide diethanolamine (X_2) were prepared. The physicochemical characteristics, e.g. the vesicle size, size distribution, zeta potential, entrapment efficiency, and skin permeability, of the TV formulations were experimentally investigated. The relationship among the formulation factor, the response variables, and the OTV was predicted using Design Expert® software. The accuracy and reliability of the OTV predicted using computer software were experimentally confirmed and investigated as an experimental transinvasome formulation (ETV). The results indicated that the skin permeability of the ETV was close to the OTV and was significantly higher than that of conventional liposomes and commercial products. The response surfaces estimated by the computer software were helpful in understanding the complicated relationship among the formulation factor, the response variables, and the stability of the TV formulation.

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Introduction

Topical and transdermal drug delivery (TDD) has numerous advantages over conventional drug delivery (oral and injectable drug delivery), such as first-pass metabolism avoidance, non-invasiveness, and improved patient compliance. As a primary limitation of TDD, the stratum corneum (SC) is the outermost layer of skin and an excellent skin permeability barrier. Several theoretical strategies have been introduced to overcome intact SC, such as (1) drug and vehicle interactions, including the use of drug or prodrugs, chemical potential, ion pairs, and eutectic systems; (2) vesicles and particles, e.g. liposomes and high-velocity particles; (3) SC-modified materials, e.g. skin hydration and chemical enhancers; (4) SC bypassed or removed materials, e.g. microneedles, ablation, and follicular delivery; and (5) electrically assisted methods, e.g. ultrasound, iontophoresis, electroporation, magnetophoresis, radio waves, and photomechanical waves[1].

The combination of two different strategies to enhance TDD has been shown to substantially improve the skin permeation of various drugs[2]. The combination of liposomes (vesicles and particles) and chemical or penetration enhancers (SC modified) has frequently been demonstrated in TDD research[3]. Penetration enhancers that improve TDD via several mechanisms have been identified, and their potential mechanisms of action differ: (1) modify SC conformation, (2) modify the desmosomes between corneocytes, and (3) modify intercellular lipids[4]. The integration of various types of penetration enhancers improves the approach to enhancing TDD because of differences in the enhancers' mechanisms of action[5]. The synergistic effect of solutions of terpenes and ethanol systems or the combination of various types of surfactant systems markedly enhances TDD compared with the application of individual components alone[6]. Moreover, the formulation exhibits increased safety compared with single penetration enhancers by reducing skin irritation[7].

Several intensive studies have suggested that the skin efficacy, skin safety, and formulation stability of vesicle formulations are primarily influenced by lipid composition or formulation factors. Hence, only specially designed vesicles have been shown to facilitate TDD, e.g. transfersomes, ethosomes, fexosomes, menthosomes, and invasomes. Previous studies have introduced novel elastic vesicles to enhance TDD, the so-called transethosomes (TELs). TELs are novel, modified vesicles of elastic liposomes between transfersomes and ethosomes, comprising phospholipids, ethanol, water, and permeation enhancers or surfactants (oleic acid). TELs can deliver drugs into deep skin regions and enhance both *in vitro* and *in vivo* skin deposition over that of conventional elastic liposomes (CELs) and conventional liposomes (CLPs)[8]. The precise ratios of various penetration enhancers in TELs directly affect physicochemical characteristics, skin permeability, skin safety, and formulation stability.

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Table 1. The compositions (formulation factors: X_n) and physicochemical characteristics (response variables: Y_n) of vesicle formulations.

	Formulation factors						Response variables				
	Fixed factors		Variable factor				Physicochemical characteristics (mean ± SD)				
Form	PC (mM)	CHOL (mM)	X_1	Lim (%)	X_2	Com (%mol)	Size (nm) (Y_1)	Size distribution (Y_2)	Zeta potential (−mV) (Y_3)	Entrapment (%) (Y_4)	Flux (μg/cm^2/h) (Y_5)
1	10	1	−1	0.5	−1	10	91.18 ± 27.51	0.47 ± 0.12	17.20 ± 3.55	65.83 ± 1.34	2.94 ± 0.34
2	10	1	−1	0.5	0	20	104.58 ± 9.29	0.44 ± 0.09	18.38 ± 3.45	81.02 ± 5.66	2.77 ± 0.06
3	10	1	−1	0.5	1	30	100.43 ± 18.00	0.35 ± 0.04	19.00 ± 2.85	81.12 ± 1.85	2.73 ± 0.33
4	10	1	0	1.0	−1	10	121.54 ± 14.20	0.25 ± 0.05	19.88 ± 3.51	79.04 ± 0.25	2.05 ± 0.11
5	10	1	0	1.0	0	20	96.86 ± 5.53	0.19 ± 0.04	16.87 ± 1.69	76.12 ± 0.39	2.15 ± 0.59
6	10	1	0	1.0	0	20	90.78 ± 6.09	0.21 ± 0.02	16.96 ± 2.31	79.87 ± 0.48	2.93 ± 0.29
7	10	1	0	1.0	1	30	108.43 ± 1.12	0.15 ± 0.02	17.81 ± 1.76	77.24 ± 2.48	2.48 ± 0.26
8	10	1	1	1.5	−1	10	173.38 ± 11.50	0.30 ± 0.04	16.24 ± 1.80	54.38 ± 0.19	1.42 ± 0.27
9	10	1	1	1.5	0	20	142.73 ± 3.83	0.31 ± 0.03	17.48 ± 1.43	58.07 ± 0.74	1.56 ± 0.14
10	10	1	1	1.5	1	30	136.23 ± 6.18	0.23 ± 0.02	15.89 ± 0.81	59.89 ± 0.57	1.62 ± 0.14
ETV	10	1	–	0.5	–	20	101.39 ± 2.63	0.19 ± 0.02	18.80 ± 1.05	80.84 ± 1.24	3.12 ± 0.20
CIV	10	1	–	0.5	–	–	96.75 ± 1.64	0.41 ± 0.06	−5.81 ± 0.27	82.14 ± 2.70	2.21 ± 0.19
CTS	10	1	–	–	–	20	97.55 ± 2.64	0.21 ± 0.01	−2.79 ± 1.51	64.92 ± 0.80	1.24 ± 0.16
CLP	10	1	–	–	–	–	109.23 ± 0.49	0.24 ± 0.01	−3.82 ± 0.71	62.79 ± 1.80	1.22 ± 0.20

PC: phosphatidylcholine; Chol: cholesterol; Lim: *d*-limonene; Com: cocamide diethanolamine.

The aim of this study was to design and develop simultaneous optimal transinvasome formulations (OTV) to enhance the transdermal delivery of capsaicin (CAP) using Design Expert® software. Transinvasomes (TVs) are novel elastic liposomes that integrate the excellent characteristics of transfersomes and invasomes for TDD. TV formulations containing a fixed concentration of phosphatidylcholine (PC), cholesterol (CHOL), 0.15% CAP and various percentages of formulation factor (X_n), e.g. *d*-limonene (Lim, main penetration enhancer of invasomes) and cocamide diethanolamine (Com, main penetration enhancer of transfersomes), were prepared. The physicochemical characteristics (Y_n) (e.g. vesicle size, size distribution, zeta potential, entrapment efficiency, and skin permeability) of the TV formulations were measured. The response surface method (RSM) was applied to understand the relationship between formulation factors and response variables, and the OTV was subsequently predicted using Design Expert® software. The accuracy and reliability of the predicted OTV were experimentally confirmed and evaluated as experimental transinvasome formulations (ETVs). Furthermore, the estimated response surfaces were utilized to clarify the relationship between formulation factor and formulation stability.

Materials and methods

Materials

PC was supplied as a special gift from LIPOID GmbH (Cologne, Germany). CHOL was purchased from Wako Pure Chemical Industries (Osaka, Japan). CAP and Lim were purchased from Sigma-Aldrich (St. Louis, MO). Com (Comperlan® KD) was obtained from BASF (Thai) Co. Ltd. (Bangkok, Thailand). Polysorbate-20 (Tween 20®, T20) was purchased from the NOF Corporation (Osaka, Japan). All other chemicals were commercially available and of analytical and high-performance liquid chromatography (HPLC) grade.

Capsaicin-loaded transinvasomes preparation

Model formulations of transinvasomes (TV) comprising a controlled amount of 10 mM PC, 1 mM CHOL, 2% T20, 0.15% CAP, and various concentrations of the penetration enhancers Lim and Com at 0.5–1.5% and 10–30% mol, respectively, were experimentally prepared. Ten model formulations of TV were obtained from a central composite experimental design with duplicate centroids, as shown in Table 1. The percentages of Lim (X_1) and Com (X_2) were selected as formulation factors. TVs were prepared by the sonication method[9]. Briefly, lipid mixtures of PC, CHOL, Com, and CAP were dissolved in a mixture of chloroform/methanol (2:1 volume ratio). The solvent mixture was evaporated under a nitrogen gas stream. The lipid film was placed in a desiccator for at least 6 h to remove the remaining solvent. The dried lipid thin film was hydrated with 0.1 M phosphate buffer solution (PBS, pH 7.4), and a mixture of T20 and Lim was also incorporated during this process. The vesicles were subsequently sonicated for two cycles of 30 min using a bath-type sonicator (5510J-DTH Branson Ultrasonics, Danbury, CT). The TV formulations were freshly prepared or stored in airtight containers at 4 °C prior to use.

Experimental design and statistical analysis

Design Expert® software (Design Expert® version 8, Stat-Ease Inc., Minneapolis, MN) was used to evaluate the relationship between the formulation factors and the physicochemical characteristics (as response variables) of the TV formulation. The central composite experimental design, containing the axial points to define the quadratic term, was constructed. The dual replicates of the center point were executed to resist the effect of noise and to ensure reliability. In pre-formulation study, the lipid composition ranges of Lim and Com were screened to establish the minimum and maximum levels in the TV formulation. The results suggested that the concentration of 5–1.5% Lim and 10–30% Com was utilized to prepare TV formulations in this study. Table 1 shows the central composite experimental design with the formulation factors (X_1 and X_2) and response variables (Y_n), e.g. vesicle size (Y_1), size distribution (Y_2), zeta potential (Y_4), entrapment efficiency (Y_3), and skin permeation flux (Y_5). The regression analysis of the dataset was carried out with statistical software, presuming the quadratic mode with interactions among factors. The term of the quadratic polynomial model[10,11] of the coded factor can be determined as:

$$Y = \beta_0 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2 + \beta_1X_1 + \beta_2X_2 \quad (1)$$

where Y represents response variables associated with each formulation factor level combination, ${X_1}^2$ and ${X_2}^2$ are the quadratic factors, X_1X_2 represents the binary interactions among factors, and X_1 and X_2 are the formulation factors Lim and Com, respectively. β_0 is

the mean value, and β_1, β_2, β_{12}, β_{11}, and β_{22} are regression coefficients. Analysis of variance (ANOVA) was executed to identify the significance of the quadratic factors (X_n^2), binary interactions (X_nX_m) and single factors (X_n) in relation to the influence on the response analysis. The responses were considered significant at a coefficient p values $<.05$.

Vesicle size, size distribution, and zeta potential measurement

The average vesicle sizes, size distributions, and zeta potentials of the TV were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano series, Malvern Instruments, Malvern, UK). Twenty microliters of the TV formulation were diluted with 1480 μL of deionized water. All measurements were performed in triplicate at 25 °C.

Entrapment efficiency of the CAP-loaded TV formulation measurement

The concentration of CAP in the TV formulation was determined by HPLC analysis after disruption of the TV with Triton® X-100 (0.1% v/v) at a 1:1 volume ratio and was then appropriately diluted with PBS, pH 7.4. The Triton® X-100/TV vesicle mixture was centrifuged at 10,000 rpm at 4 °C for 10 min. The supernatant was filtered with a 0.45 μm nylon syringe filter. The entrapment efficiencies of the CAP-loaded TV formulation were calculated according to the following equation:

$$\% \text{ entrapment efficiency} = (C_L/C_i) \times 100,$$

where C_L is the concentration of the CAP-loaded TV formulation, as described in the above methods, and C_i is the initial concentration of CAP added to the formulation.

In vitro *skin-permeation*

The SC is well known as an excellent barrier of human skin. Therefore, the shed snakeskin of *Naja kaouthia* was used as a model for skin permeation studies because of its similarity to human SC[12]. The shed snakeskin was donated by the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand. The entire shed snakeskin was obtained from different snakes immediately after shedding and stored at −10 °C prior to use. The estimated thickness of the skin was 0.02–0.03 mm. The shed snakeskin was divided into 10–12 pieces and cut into round sections of 2.5 cm × 2.5 cm. After thawing, the shed snakeskin was immediately placed on a Franz diffusion cell. A Franz diffusion cell with an available permeation area of 2.01 cm^2 and a water jacket at 32 ± 1 °C was employed. The SC side of the shed snakeskin faced the donor chamber and was filled with 1 mL of CAP formulations. The receiving chamber was filled with 6.5 mL of receiver medium (PBS pH 7.4:ethanol; 1:1 v/v) under sink conditions and stirred by a magnetic stirrer at 500 rpm. At appropriate intervals, 0.5 mL aliquots of the receiver medium were withdrawn and immediately replaced with an equal volume of fresh medium. The CAP in the receiver medium was analyzed by HPLC. The cumulative amount of CAP that permeated through the shed snakeskin was plotted as a function of time. The steady-state flux (J) was defined after calculating the slope of the linear portion of the plot.

Following the *in vitro* skin permeation study, the shed snakeskin was washed with water and blotted dry. The spectrum of the shed snakeskin was recorded in the range of 500–4000 cm^{-1} using a FT-IR spectrophotometer (Nicolet 4700, Thermo Scientific, Madison, WI). An X-ray diffractometer (XRD) (MiniFlex II, Rigaku Co., Tokyo, Japan) was used to investigate and confirm the possible mechanism underlying the effect of the different formulations on the skin permeation of CAP. The same shed snakeskin was cut into small pieces, approximately 2 cm × 2 cm, and attached to an aluminum well sample holder. XRD was used with Cu Kα, scanning from $2\theta = 5$–45°. The voltage and operating current were 30 kV and 15 mA, respectively.

Simultaneous optimization

The optimization of the TV formulation based on the RSM was performed using the dataset obtained from model formulations. The formulation factors Lim (X_1) and Com (X_2) and the response variables of the model formulation, e.g. vesicle size (Y_1), the size distribution (Y_2), the zeta potential (Y_3), the entrapment efficiency (Y_4), and the skin permeability (Y_5), were defined. The simultaneous OTV formulation was estimated using the appropriate characteristics prescribed in a previous study[13]. Briefly, an appropriate TV formulation was designed to minimize the vesicle size, size distribution, and zeta potential and to maximize the entrapment efficiency and skin permeation flux. Once the RSM-estimated OTV formulation was obtained, the reliability and desirability were evaluated by linear correlation, and the corresponding residual plot, as previously described[11]. Moreover, experiments were carried out to confirm the reliability of the predicted values under optimal conditions.

Stability evaluation

The physicochemical stabilities of the TV formulations were evaluated by monitoring the formulations for at least 90 days after their initial preparation. Various TV formulations were stored in glass bottles with plastic plugs at 4 ± 1 °C for 90 days to determine the stability of the formulations. The physicochemical stabilities of TV formulations were evaluated by visual inspection for sedimentation. The vesicle size, size distribution, and zeta potential were determined by PCS. The CAP remaining in the TV formulation was analyzed by HPLC after 90 days. The dataset obtained from the evaluation of the physicochemical stabilities was analyzed using Design Expert® software to predict the relationship between the formulation factors and the formulation stability.

HPLC analysis

The concentration of CAP in the samples was analyzed by HPLC. All measurements were stored at 4 °C until analysis. The HPLC 1100 system (Agilent 1100 Series HPLC System, Agilent Technologies, Wilmington, DE) was used. The analytical column was an Eclipse XDB-C18 column (particle size =5 μm; column dimension =4.6 mm ×150 mm), and a mobile phase comprising diluted phosphoric acid in reversed osmosis water (1 in 1000):acetonitrile (50:50), a flow rate of 1 mL/min, and a UV detector set at 280 nm were used for all determinations[14]. The calibration curve for CAP was within the range of 1–100 μg/mL, with a correlation coefficient of .999.

Results and discussion

Experimental design

Among the various formulation factors that define the feasibility of TV for the transdermal delivery of CAP, knowledge of the

behavior of the factors associated with efficacy, skin safety, and formulation stability is crucial in high efficiency formulations. Used to design and develop a simultaneous OTV to enhance the transdermal delivery of CAP, penetration enhancers are compounds known for their ability to improve the skin permeability of the formulation (efficacy) and also affect skin irritation (skin safety) and formulation stability. Therefore, formulation factors (Lim and Com) were considered because the skin permeation flux (as efficacy parameter) should be relatively high, whereas the skin irritation (as skin safety parameter) and the formulation stability (as stability parameter) should remain relatively low. In this respect, Lim and Com play important roles as potential penetration enhancers because adequate percentages of these components may improve the skin permeation flux of the TV formulation. Nevertheless, the Lim extracted from plants is a good candidate for a permeation enhancer because of its relatively low skin irritation potential. In addition, Com has been extensively used as a nonionic surfactant in various cosmetics and topical personal care products, reflecting its rare allergic contact dermatitis potential[15]. Therefore, the formulation factors used in this study were safe with respect to skin irritation. Other response variables considered included the vesicle size (Y_1), size distribution (Y_2), zeta potential (Y_3), entrapment efficiency (Y_4), and skin permeability (Y_5) of each TV formulation because the principle of vesicle formulation is based on these physicochemical characteristics[16]. The values of the response variables for different experimental conditions are shown in Table 1.

Factors influencing TV characteristics

The statistical analysis data, e.g. the significant model, regression coefficient value, analysis of variance (p value), for the response variables are shown in Table 2. The quadratic model for all responses was strongly significant (except zeta potential), and the model maximized the R^2 coefficients. Adequate precisions of 11.8920 (vesicle size), 9.2350 (size distribution), 2.6580 (zeta potential), 11.1670 (entrapment efficiency), and 9.9290 (skin permeation flux) indicated an adequate signal/noise ratio (an adequate precision ratio greater than 4 is desirable). Moreover, the lack of fit values for all responses was over 0.05, implying that the lack of fit was not significant relative to the pure error. Non-significant lack of fit values represented a good model in this study. The response surface, the linear correlation and the corresponding residual plot of the response variables of the model TV formulation are shown in Figure 1. The ANOVA of the formulation factors for each response is detailed separately below.

Vesicle size response

The formulation factors with a significant influence on vesicle size were the Lim and Com percentages in the TV formulation. The Lim percentage supported a positive influence (coefficient =25.80), as an increase in Lim percentage leads to an increase in vesicle size (Table 2). This effect corresponded to the results of a previous study[17] showing that the vesicle size of temoporfin-loaded invasomes increased with increasing concentrations of terpenes from 0, 0.5, and 1%, respectively. Furthermore, the quadratic term of Lim (X_1^2) was considered to be a strong influence on vesicle size (p values <.05), revealing a nonlinear relationship and reaching a plateau at 1.5% (Figure 1(A)). The response surface of the vesicle size suggested that with the incorporation of Lim up to 1.5%, the vesicle size of the TV may increase more than 180 nm. To formulate the TV formulation using a vesicle size smaller than 100 nm, the Lim percentage should not be more than 1.0%.

The Com percentage provided a negative influence (coefficient = −6.84), suggesting that an increase in the Com percentage leads to a decrease in the vesicle size. This effect can be attributed to the fact that the insertion of Com (as single chain surfactant) into the TV increases the curvature, resulting decreased vesicle size[18]. Moreover, the vesicle size decreases as the percentage of the surfactant increases, thereby leading to crucially stabilized vesicles because the small vesicles have more chances to collide with each other[19]. However, at high Com monomer to PC (Com:PC) weight ratios, vesicles smaller than 50 nm are nearly translucent. Therefore, an increase in Com percentage will not necessarily lead to a dramatic vesicle size reduction but may lead to the presence of mixed-micelles in the formulation. Furthermore, vesicle size can be controlled by the method of preparation, e.g. the ultrasonication process or the drug loading in the formulation, as well as the percentage of surfactant used[20,21]. However, the quadratic term of Com (X_2^2) indicated that the percentage of Com did not evidently influence the vesicle size (p values = .1023) (Figure 1(A)). The response surface of the vesicle size suggested that the incorporation of Com at various percentages between 10 and 30% also resulted in nano-size range of all formulations (no more than 200 nm).

Size distribution response

The only factor that significantly influenced the reduction in the size distribution was the Com percentage in the TV formulation. The Com percentage exerted a negative influence (coefficient = −.013), suggesting that an increase in the Com percentage leads to a decrease in the size distribution (Table 2). However, an

Table 2. Terms of the significant model, regression coefficient value, and analysis of variance (p value) for the response variables.

	Vesicle size		Size distribution		Zeta potential		Entrapment efficiency		Skin permeation flux	
Polynomial term	Coefficient	p values	Coefficient	p values	Coefficient	p values	Coefficient	p values	Coefficient	p values
Model	–	<.0001*	–	.0002*	–	.6280	–	<.0001*	–	<.0001*
Intercept	99.59	–	.24	–	−17.49	–	136.91	–	2.46	–
X_1: Lim	25.80	<.0001	.038	.0001	.83	.1398	−19.98	<.0001	−0.64	<.0001
X_2: Com	−6.84	.0645	−.013	.1344	.10	.8499	2.30	.3678	.07	.4099
X_1X_2	−11.60	.0130	−.036	.0020	.54	.4249	−6.52	.0437	.10	.3229
X_1^2	18.99	.0026	−.026	.0704	.44	.6157	−19.85	<.0001	−0.21	.1218
X_2^2	9.61	.1023	.0046	.7356	−.47	.5901	−6.76	.1048	−0.11	.4142
R^2	.7711		.8192		.1275		.8066		.7306	
Adjusted R^2	.7234		.5353		−.0543		.7663		.6744	
Predicted R^2	.6091		.4363		−.3593		.6908		.6093	
Adequate precision	11.8920		9.2350		2.658		11.1670		9.9290	
Lack of fit	1.99	.1457	2.47	.0904	1.74	.1889	1.40	.2703	.88	.4663

*Significant p values.

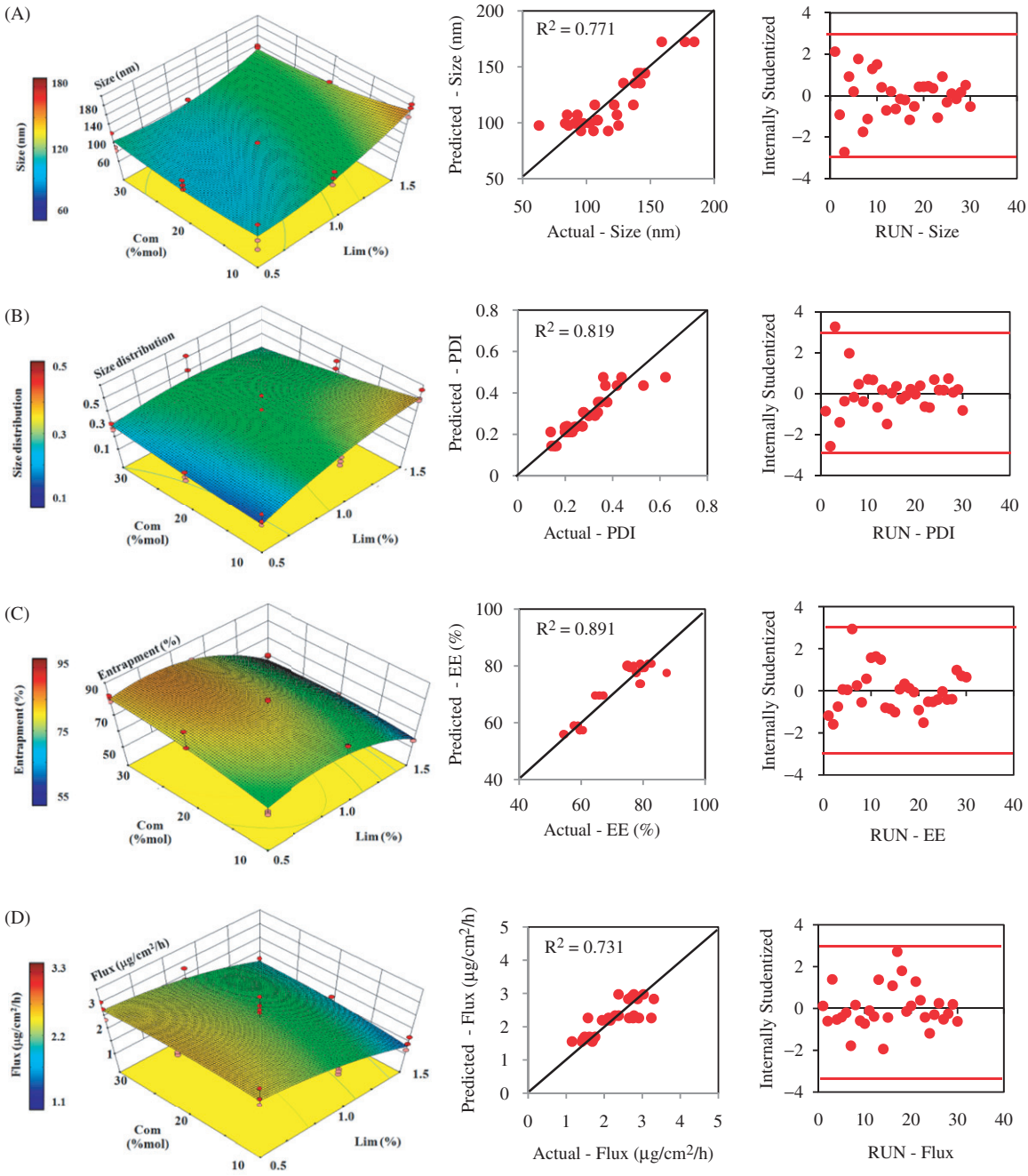

Figure 1. The response surface (left), the linear correlation (middle), and the corresponding residual plot (right) of the (A) size, (B) size distribution, (C) entrapment efficiency, and (D) skin permeation flux of the model transinvasome formulation.

increase in the Lim percentage leads to an increase in the size distribution (Figure 1(B)). Among the quadratic factors (X_1^2 and X_2^2), binary interactions among factors (X_1X_2) and single formulation factors (X_1 and X_2) that determine the feasibility of significantly affecting the size distribution, Lim (X_1) was the most significant, as the p values = .0001. Dragicevic-Curic et al. reported that an increase in the Lim percentage (from 0.5% to 1.0%) leads to an increase in the size distribution, with acceptable homogeneity (PDI <0.3).

Zeta potential response

The quadratic term of the Lim and Com factors (X_1^2 and X_2^2; p values >.05) was not significant because both formulation factors act as penetration enhancers with no intrinsic charge (nonionic compounds). The zeta potential was one of the most important response variables influencing the skin permeability of the vesicle formulation[16]. Therefore, in case of the ionic compound the result may show inversely. This study suggested that the response surface of the zeta potential should be considered case by case. Nevertheless, the TV formulation in this study showed a negative surface charge because of the influence of PC as a zwitterionic compound with an isoelectric point (pI) of approximately 6–7[22]. The experimental condition included a pH of 7.4, which was higher than the pI of the PC carrying a net negative charge[23].

Entrapment efficiency response

The Lim percentage was a strongly significant factor influencing the entrapment efficiency of the CAP-loaded TV formulations (p

values <.0001). The Lim percentage exerted a negative influence (coefficient = −19.98). In addition, the quadratic term of the Lim was also verified as being strongly significant (X_1^2; p values <.0001). An increase in the Lim percentage led to a decrease in the entrapment efficiency of CAP in the formulation. Moreover, the response surface of the entrapment efficiency showed an entrapment efficiency of CAP-loaded TV formulations of more than 80% when the incorporation of Lim was not more than 1% (Figure 1(C)). The increase in the Lim percentage (log P 4.23)[24] increased the lipophilicity in the lipid bilayer of the TV vesicles, which may compete with CAP (as lipophilic drug, log P 3.8)[25] when arranged in the lipid bilayer[26,27] and hence the CAP was excluded as it assembled into the bilayer of the TV vesicles.

Skin permeation flux response

The only factor that had a significant influence on skin permeation flux was the Lim percentage in the TV formulation. The Lim percentage exerted a negative influence (coefficient = −.64), demonstrating that an increase in the Lim percentage leads to a decrease in the skin permeation flux (Table 2). This result might be attributed to the intrinsic property of Lim. As the partition coefficient of Lim (log P) is 4.23[24], Lim strongly exhibited lipophilic property. Therefore, CAP would rather in remain in the TV formulation more than permeate through the release medium. The response surface indicated that for a satisfactory skin permeation flux greater than 2.5 μg/cm^2/h, the Lim should not be more than 1.0%, whereas the percentage of Com should be between 10% and 25% (Figure 1(D)). However, several studies have previously suggested that a ratio of 1% for terpenes is appropriate for enhancing the transdermal delivery of various drugs, e.g. carboxyfluorescein[28], sodium fluorescein[27], and temoporfin[9,17,28]. This result can be attributed to the fact that the addition of Com may simultaneously affect the skin permeability of TV formulations to enhance the transdermal delivery of CAP. Therefore, TV formulations with a 0.5% Lim percentage presented an adequate skin permeation flux in this study. Moreover, Com was not only used as a potential penetration enhancer in the TV formulation but was also utilized to increase the solubility of Lim in TV vesicles instead of ethanol. Ethanol (as solubilizing agent for Lim), which was used in vesicle formulations with Lim or other terpene derivatives (e.g. cineol, methanol, carveol) in previous studies[17], may limit the formulation stability of the vesicle formulation because of the evaporation of ethanol after storage. Thus, Com was used as an alternative solubilizing agent and nonionic surfactant for the vesicle formulation incorporating Lim or terpene derivatives instead of volatile substances, such as ethanol.

The reliability of all response surfaces was confirmed by linear correlation, and the corresponding residual plots between the experimental run and the internally studentized residuals are shown in Figure 1 in the middle and right columns, respectively. These results indicated that the linear correlation of almost all response surfaces was between 0.7 and 0.9, indicating good correlation. Furthermore, under the completely randomized run, the vertical distribution of the internally studentized residuals was within the line from top-to-bottom. This result suggested that all points fell within the limits of a 95% confidence interval (CI).

Optimal TV formulation

The conformity between the adjusted and the predicted R^2 values, as a difference lower than 0.2 of the mathematical model obtained by the RSM, permits the safe estimation of the optimal TV formulation. The numerical optimization identifies an appropriate point that maximizes the desirability index based on pre-established values. The optimization options of a goal can be altered after adjusting the limits, weights, and importance. For all formulation factors and response variables, the goals were intergraded into one desirability index. The desirability of the optimal formulation includes a target function scoring from zero to one (outside of the limits to the target). The desirability index was absolutely dependent on how far the lower and upper limits were set in relation to the genuine optimal formulation.

The criteria focused on the minimization of the vesicle size, size distribution, and zeta potential and the maximization of the entrapment efficiency and skin permeation flux. According to the dataset obtained from the statistical analysis shown in Table 2, the target of the optimization was to optimize the proportion of Lim (X_1) and Com (X_2) because the vesicle formulation that offered relatively high-skin permeability also contained high concentrations of penetration enhancers. The skin irritation indicates that the safety of the penetration enhancers used may restrict the consumption of this formulation[14]. However, the selected penetration enhancers in this study were based on the safety of their intrinsic properties, as described above.

Under the numerical optimization conditions, the integrated desirability index of the optimal TV formulation (OTV) containing 0.5% Lim (X_1) and 20%mol Com (X_2) was 0.771. A desirability index higher than 0.7 indicated that the model was capable of optimizing all of the factors (formulation factors and response variables) simultaneously until an adequate level was reached[29]. The individual desirability index of both Lim and Com predicted by this software was 1.000, indicating that this model was highly competent for the optimization requirements for each of the factors.

To confirm the accuracy and reliability of the OTV formulation predicted using Design Expert®, the experimental TV formulation (ETV), conventional transfersomes (CTS), conventional invasomes (CIV), and conventional liposomes (CLP) prepared and investigated experimentally. The cumulative skin permeation per area and the skin permeation flux of different vesicle formulations were compared with the commercial product of CAP (GoodSense® Capsaicin Arthritis Pain Relief, a product manufactured in the USA), as shown in Figure 2. The concentration of CAP in all formulations was 0.15%. The cumulative skin permeation per area of the different formulations increased linearly after 2 h. The skin permeation flux

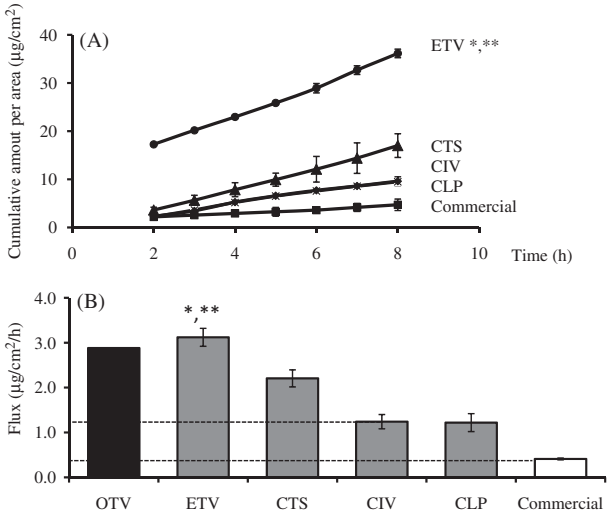

Figure 2. (A) The skin permeation profile and (B) flux of different CAP formulations. * and ** indicate $p < .05$ compared with the commercial product and the conventional transinvasomes, respectively.

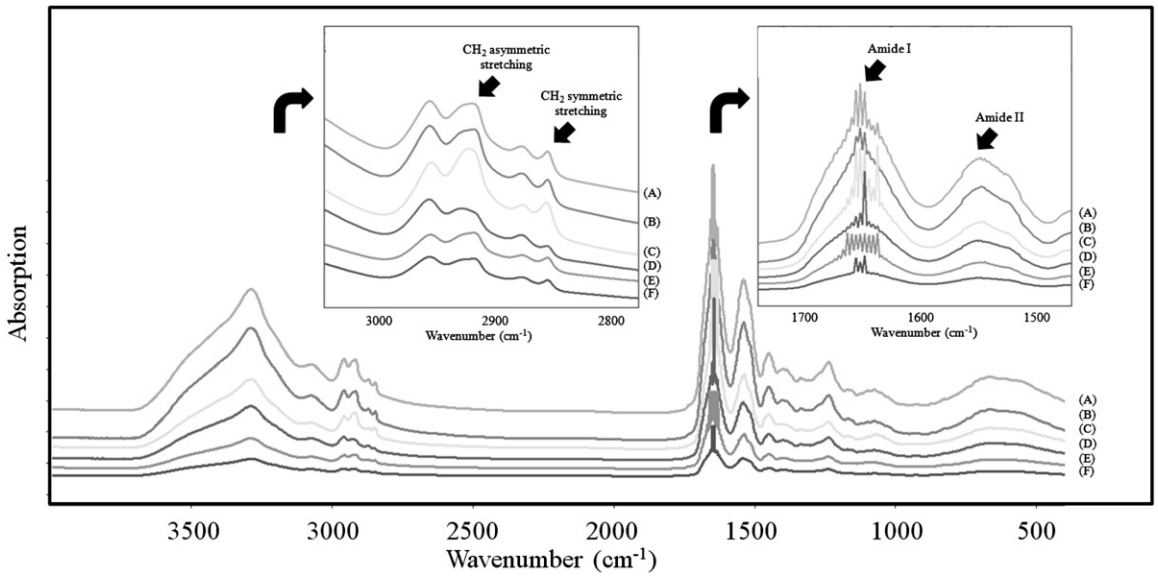

Figure 3. The FT-IR spectra of the shed snakeskin after treatment with different formulations; (A) ETV, (B) commercial product, (C) CTS, (D) CIV, and (E) CLP compared with the skin treated with (F) PBS.

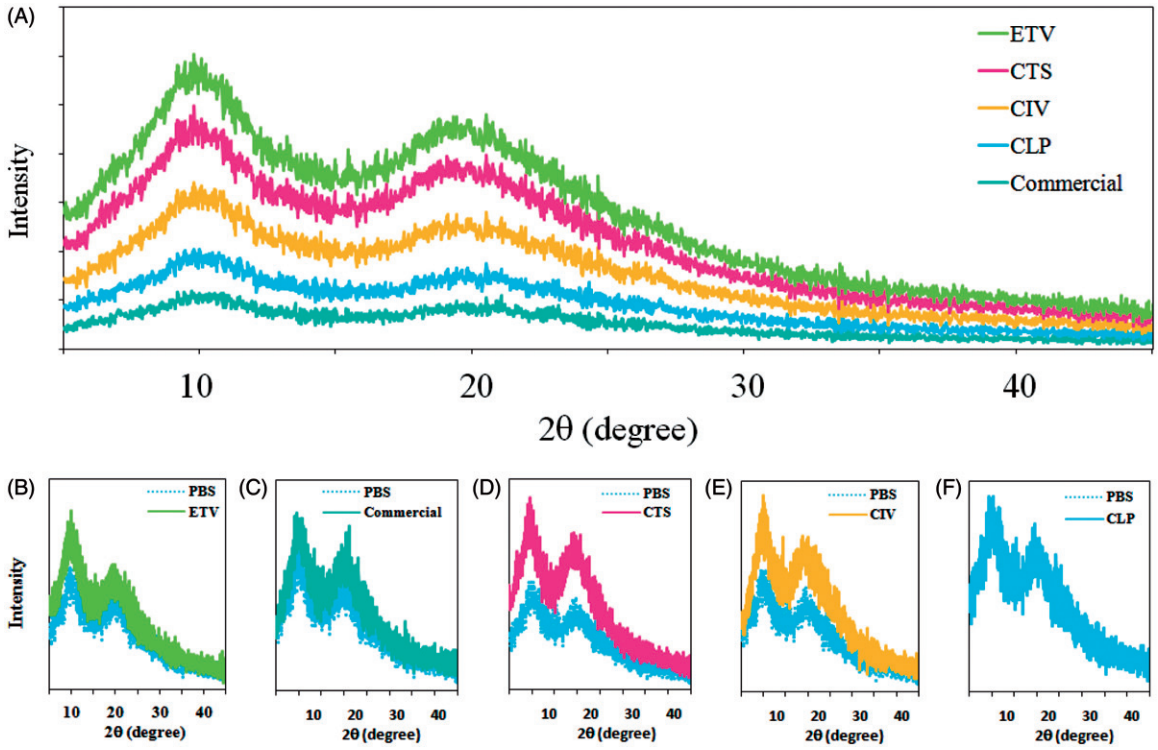

Figure 4. The X-ray diffractogram of the shed snakeskin after treatment with different formulations (A) all diffractograms, (B) ETV, (C) commercial product, (D) CTS, (E) CIV, and (F) CLP compared with the skin treated with PBS.

of CAP through the skin model was defined as the slope of the linear portion of the plot. According to the skin permeation profile, the results showed that the skin permeation profile of ETV was higher than that of the CTS, CIV, CLP, and commercial products (Figure 2(A)). The skin permeation flux of the ETV was significantly higher that of the CLP (p values = .004) and the commercial products (p values = .002), as shown in Figure 2(B). The skin permeation data indicated that the dramatic synergistic permeability-enhancing effect of two different types of surfactants, as in ETV, was greater than that of Com and Lim, as in CTS and CIV, respectively. This result strongly suggested that Lim and Com act synergistically[30] to improve the transdermal delivery of CAP. The synergistic effect of Com and Lim may be due to the combination of different mechanisms of each intrinsic property. The ETV formulation may promote skin permeability by various mechanisms. The possible mechanisms by which this ETV formulation (as elastic liposomes) improved the skin delivery of CAP encompassed the penetration-enhancing mechanism and the vesicle adsorption to and/or fusion with the SC[3,31].

The possible mechanism of action of the different formulations was confirmed by the FT-IR spectra and X-ray diffractograms, as shown in Figures 3 and 4, respectively. The spectrums between

1500 and 1700 cm^{-1}, and 2800 and 3000 cm^{-1} of the FT-IR spectra are due to the amide (I and II) and the CH_2 stretching patterns, respectively. The amide I patterns were split into a doublet (1600–1700 cm^{-1}), whereas the amide II pattern was broad peak (1500–1580 cm^{-1}). The alteration of the peak of the amide I and amide II patterns were used to understand the organization and interaction of hydrogen bonds at the polar interface. The modified shapes of the CH_2 symmetric stretching (2850 cm^{-1}) and asymmetric stretching (2920 cm^{-1}) frequencies were attributed to changes in the conformational order or hydrocarbon chain fluidity and alkyl chain packing[32]. The result indicated that the CH_2 stretching patterns of skin treated with ETV, CLP, and commercial product did not markedly differ from those of intact skin treated with PBS. In contrast, the CH_2 asymmetric stretching peaks of the skin treated with the CTS and CIV shifted from 2920 to 2924 and 2931 cm^{-1}, respectively. Furthermore, the amide I bands of the skin treated with the CTS and CIV changed from the intact skin treated with PBS, as shown in Figure 3. The X-ray diffractograms at $2\theta = 10$ and 20° demonstrated the hexagonal packing of the alkyl chains of

Table 3. The response variables of the optimal formulation.

Response	Actual value	Predicted value	95% Confidence intervals (lower–upper)		Bias[a] (%)
Size (nm)	101.39 ± 2.63	93.90	81.03	−106.76	7.98
Size distribution	0.19 ± 0.02	0.19	0.16	−0.21	0.24
Charge (−mV)	18.80 ± 1.05	18.32	16.95	−19.68	2.62
EE (%)	80.84 ± 1.24	78.47	75.26	−81.68	3.03
Flux (μg/cm^2/h)	3.12 ± 0.20	2.92	2.69	−3.14	6.95

[a]Bias was calculated as {(predicted value − experimental value)/experimental value} × 100%.

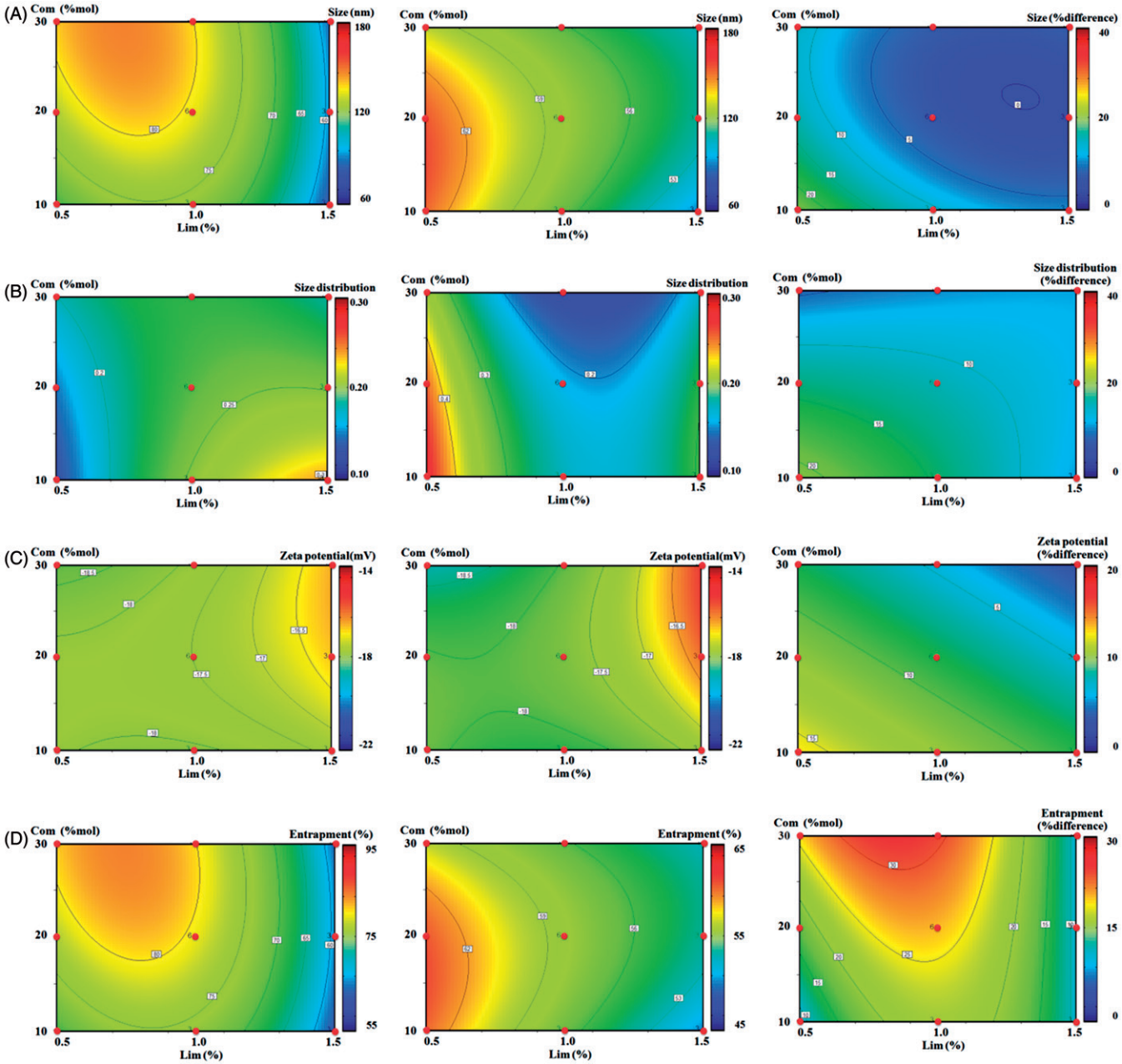

Figure 5. The contour plots of the (A) size, (B) size distribution, (C) zeta potential, and (D) entrapment efficiency of the model formulation of TV at initial preparation (left column), 90 days after preparation (middle column) and the percent difference between days 0 to 90 (right column).

lipids in the skin[33]. The X-ray diffractograms of the skin treated with ETV, CLP, and commercial product did not significantly differ from those of the intact skin (as treated with PBS) as shown in Figure 4(B,C,F), respectively, whereas the skin treated with the CTS and CIV exhibited notable differences, as shown in Figure 4(D,F), respectively. These results revealed that the combination of various types of surfactants may increase safety compared with single penetration enhancers by reducing skin irritation.

The experimental values of the confirmation experiments were close to the predicted values and fell within the 95% CI, as presented in Table 3. These results suggested the good estimation of the model for response analysis and confirmed the good reliability of the established model for the response observed in the RSM design. Furthermore, the reliability of these predicted values was also displayed as the percentage of bias (lower than 10%)[34]. These findings provide useful fundamental information for the development and design of novel vesicle formulations to enhance the transdermal delivery of CAP.

Stability of the TV formulation

The stability evaluation of 10 model formulations of TV was performed after measuring the vesicle size, size distribution, zeta potential, and entrapment efficiency initially and at 90 days after preparation. The TV formulations remained transparent, and no signs of sedimentation at $4 \pm 1\,^{\circ}C$ for 90 days were observed. The physicochemical stabilities of 10 TV formulations incorporating CAP are displayed in Figure 5. The contour plots represent the relationship formulation factors (Lim and Com) on the formulation stability as the initial preparation (left column), 90 days after preparation (middle) and the different values between days 0 and 90 (right column). The contour plots of the percent difference between days 0 and 90 were useful for the estimation of the relationship between the formulation factors and the formulation stability. The small difference in vesicle size, size distribution, and zeta potential at days 0–90 reflected the addition of a high percentage of Lim and Com in the TV formulation, as shown in the blue areas in Figure 5(A–C) (right column). The percent difference in the vesicle size, size distribution, and zeta potential was smaller than 10% after 90 days. In addition, the remaining entrapment efficiency of the TV formulation was approximately 80% after storage, as shown in the green areas in Figure 5(D) (right column). The changes in the fraction of the formulation factors of TVs demonstrated the feasibility of the TV formulations to acquire favorable physicochemical stability[35]. The results revealed the ability of computer software to estimate the formulation stability of the vesicle formulation, which can reduce the number of measurements required to simultaneously determine the effects of both Lim and Com on the physicochemical stabilities of TV formulations.

Conclusion

The attempt to design and develop simultaneous OTV formulations to enhance the transdermal delivery of CAP using computer software presented an advancement challenge as the complicated relationship between various formulation factors and response variables must be considered. The design of experimental and computer software was applied to reduce the number of experimental formulations and to model the optimal TV formulation with proper characteristics. The accuracy and reliability of OTV formulations were experimentally verified by the formulation and evaluation of ETV. The results indicated that the experimental value agreed well with the predicted response variables. Considering the skin permeation data of the ETV formulation, we successfully showed the feasibility of the transdermal delivery of CAP using an ETV formulation. Moreover, the RSM was applied to construct a probabilistic graphical model of the formulation stability pattern and elucidated the complicated relationships between the formulation factors and the response variables.

Disclosure statement

The authors declare no conflicts of interest.

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ผลิตภัณฑ์ต้นแบบเพื่อเป็นสินค้าทดลองใช้ แต่ห้ามจำหน่าย

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เทคโนโลยี เจลพริกนาโนเทคโนโลยี

นักวิจัย : ผู้ช่วยศาสตราจารย์ ดร.สุรีวัลย์ ตวงจิตต์
กลุ่มวิชาเคมีและเทคโนโลยีเภสัชกรรม
คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี

สถานภาพสิทธิบัตร : อยู่ระหว่างยื่นคำขอรับความคุ้มครอง

ที่มา ข้อมูลเบื้องต้น ความสำคัญของปัญหา :

แคปไซซินเป็นสารสำคัญที่พบมากในพริกที่มีฤทธิ์ทางเภสัชวิทยามากมาย เช่น ฤทธิ์ป้องกันหลอดเลือดและหัวใจ ฤทธิ์ต้านมะเร็ง ฤทธิ์ต้านเกล็ดเลือด ฤทธิ์ต้านการอักเสบ ช่วยลดน้ำหนัก ฯลฯ โดยฤทธิ์ที่น่าสนใจอย่างหนึ่งคือ ฤทธิ์ในการบรรเทาอาการปวด อย่างไรก็ตาม ด้วยคุณสมบัติของแคปไซซินคือ มีความระคายเคืองต่อผิวหนังอย่างมาก หากทาผิวหนังโดยตรง ดังนั้นการพัฒนาระบบนำส่งแคปไซซินทางผิวหนังโดยใช้นาโนเทคโนโลยี จะช่วยให้สามารถนำส่งแคปไซซินให้ลึกลงสู่ชั้นผิวหนังได้ และช่วยลดข้อจำกัดของแคปไซซินในการช่วยลดความระคายเคืองต่อผิวหนังได้

สรุปเทคโนโลยี :

สามารถพัฒนาเจลพริกนาโนเทคโนโลยีให้อยู่ในรูปผลิตภัณฑ์สำหรับวางจำหน่ายในท้องตลาด และคาดว่าจะช่วยเพิ่มมูลค่าและคุณค่าของงานวิจัยสมุนไพรไทยที่มีการศึกษาก่อนหน้าให้สามารถประยุกต์ใช้ต่อเนื่องจนสามารถผลิตเป็นผลิตภัณฑ์ที่ประชาชนทั่วไปสามารถเข้าถึงได้

จุดเด่นของเทคโนโลยี :

การใช้ระบบนำส่งยารูปแบบใหม่ในการนำส่งสารสำคัญจากพืชสมุนไพรโดยใช้นาโนเทคโนโลยี เพื่อให้ลดข้อจำกัดของสารสำคัญจากพืชสมุนไพรและเพิ่มประสิทธิภาพในการนำส่งสารสำคัญเข้าสู่ร่างกายทางผิวหนังโดยการเพิ่มการนำส่งสารแคปไซซินที่มีฤทธิ์ในการบรรเทาอาการปวด โดยเจลนาโนเทคโนโลยีจะช่วยลดความระคายเคืองต่อผิวหนังของแคปไซซินและสามารถนำส่งสารสำคัญให้ลึกลงสู่ชั้นผิวหนังได้

เปรียบเทียบลักษณะภายนอกของสูตรตำรับนาโนเทคโนโลยีที่บรรจุแคปไซซินและผลิตภัณฑ์แคปไซซินที่มีในท้องตลาด

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อ่อน
ภสัชกรรม คณะเภสัชศาสตร์

คำขอรับความคุ้มครอง

ประโยชน์ ได้แก่ โพลีแซคคาไรด์ เช่น
pin, cordycepic acid, กรดอะมิโน
สารอาหารสำคัญอื่น ๆ เช่น โปรตีน
ประสงค์ที่จะพัฒนาผลิตภัณฑ์เยลลี่
ยังช่วยซ่อมแซมส่วนที่สึกหรอของ
มดื่มที่ผสมผักโขมและถั่งเช่านั้นยัง
าน

โภชนาการ โดยใช้สารสกัดผักโขมและ
ชัน และองค์ประกอบหรือสารช่วยใน

ผู้บริโภค
มหวาน
ร่างกาย
องเศราะห์)
และการ
าพ เช่น

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งานแสดงสินค้านวัตกรรม
ผลงาน Startup อุบลราชธานี

ผลงานวิจัย

เทคโนโลยี เจลพริกนาโนเทคโนโลยี

นักวิจัย : ผู้ช่วยศาสตราจารย์ ดร.สุรีวัลย์ ดวงจิตต์
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คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี

สถานภาพสิทธิบัตร : อยู่ระหว่างยื่นคำขอรับความคุ้มครอง

ที่มา ข้อมูลเบื้องต้น ความสำคัญของปัญหา :

แคปไซซินเป็นสารสำคัญที่พบมากในพริกที่มีฤทธิ์ทางเภสัชวิทยามากมาย เช่น ฤทธิ์ป้องกันหลอดเลือดและหัวใจ ฤทธิ์ต้านมะเร็ง ฤทธิ์ต้านเกล็ดเลือด ฤทธิ์ต้านการอักเสบ ช่วยลดน้ำหนัก ฯลฯ โดยฤทธิ์ที่น่าสนใจอย่างหนึ่งคือ ฤทธิ์ในการบรรเทาอาการปวด อย่างไรก็ตาม ด้วยคุณสมบัติของแคปไซซินคือ มีความระคายเคืองต่อผิวหนังอย่างมาก หากทาผิวหนังโดยตรง ดังนั้นการพัฒนาระบบนำส่งแคปไซซินทางผิวหนังโดยใช้นาโนเทคโนโลยี จะช่วยให้สามารถนำส่งแคปไซซินให้ลึกลงสู่ชั้นผิวหนังได้ และช่วยลดข้อจำกัดของแคปไซซินในการช่วยลดความระคายเคืองต่อผิวหนังได้

สรุปเทคโนโลยี :

สามารถพัฒนาเจลพริกนาโนเทคโนโลยีให้อยู่ในรูปผลิตภัณฑ์สำหรับวางจำหน่ายในท้องตลาด และคาดว่าจะช่วยเพิ่มมูลค่าและคุณค่าของงานวิจัยสมุนไพรไทยที่มีการศึกษาก่อนหน้าให้สามารถประยุกต์ใช้ต่อเนื่องจนสามารถผลิตเป็นผลิตภัณฑ์ที่ประชาชนทั่วไปสามารถเข้าถึงได้

จุดเด่นของเทคโนโลยี :

การใช้ระบบนำส่งยารูปแบบใหม่ในการนำส่งสารสำคัญจากพืชสมุนไพรโดยใช้นาโนเทคโนโลยี เพื่อให้ลดข้อจำกัดของสารสำคัญจากพืชสมุนไพรและเพิ่มประสิทธิภาพในการนำส่งสารสำคัญเข้าสู่ร่างกายทางผิวหนังโดยการเพิ่มการนำส่งสารแคปไซซินที่มีฤทธิ์ในการบรรเทาอาการปวด โดยเจลนาโนเทคโนโลยีจะช่วยลดความระคายเคืองต่อผิวหนังของแคปไซซินและสามารถนำส่งสารสำคัญให้ลึกลงสู่ชั้นผิวหนังได้

เปรียบเทียบลักษณะภายนอกของสูตรตำรับนาโนเทคโนโลยีที่บรรจุแคปไซซินและผลิตภัณฑ์แคปไซซินที่มีในท้องตลาด

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ผลงานวิจัย

เทคโนโลยี เจลพริกนาโนเทคโนโลยี

นักวิจัย : ผู้ช่วยศาสตราจารย์ ดร.สุรีรัตน์ ดวงจิตต์
กลุ่มวิชาเทคนิคเคมีและเทคโนโลยีเทคนิคกรรม
คณะเทคนิคศาสตร์ มหาวิทยาลัยอุบลราชธานี

สถานภาพสิทธิบัตร : อยู่ระหว่างขั้นตำแหน่งรับความคุ้มครอง

ที่มา ข้อมูลเบื้องต้น ความสำคัญของปัญหา :

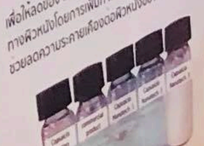
แคปไซซินเป็นสารสำคัญที่พบมากในพริกที่มีกฤทธิ์ทางเภสัชวิทยามากมาย เช่น กฤทธิ์ในการต้าน หลอดเลือดและหัวใจ กฤทธิ์ด้านมะเร็ง กฤทธิ์ด้านเกล็ดเลือด กฤทธิ์ด้านการอักเสบ ช่วยลดไขมัน ที่น่าสนใจอย่างหนึ่งคือ กฤทธิ์ในการบรรเทาอาการปวด อย่างไรก็ตาม ด้วยคุณสมบัติของแคปไซซินดังกล่าว ทำให้ ระคายเคืองต่อผิวหนังอย่างมาก หากทาผิวหนังโดยตรง ดังนั้นการพัฒนารูปแบบนำส่งแคปไซซินจึงมีความ โดยใช้นาโนเทคโนโลยี จะช่วยให้สามารถนำส่งแคปไซซินเข้าสู่ชั้นผิวหนังได้ และช่วยลดการระคายเคือง แคปไซซินในการช่วยลดความระคายเคืองต่อผิวหนังได้

สรุปเทคโนโลยี :

สามารถพัฒนาเจลพริกนาโนเทคโนโลยีให้อยู่ในรูปผลิตภัณฑ์สำหรับการทำหน้าที่ต่อต้านอนุมูลอิสระ และคาดว่าจะช่วยเพิ่มมูลค่าและคุณค่าของวัตถุดิบสมุนไพรไทยที่มีการศึกษาอย่างต่อเนื่องจนสามารถผลิตเป็นผลิตภัณฑ์ที่ประชาชนทั่วไปสามารถหาซื้อได้

จุดเด่นของเทคโนโลยี :

การใช้ระบบนำส่งยารูปแบบใหม่ในการนำส่งสารสำคัญจากพืชสมุนไพรเข้าสู่ผิวหนังได้ เพื่อให้ลดข้อจำกัดของสารสำคัญจากพืชสมุนไพรและเพิ่มประสิทธิภาพในการนำส่งสารสำคัญสู่ชั้นผิวหนัง ทางผิวหนังโดยการเพิ่มการนำส่งสารสกัดแคปไซซินที่มีกฤทธิ์ในการบรรเทาอาการปวด โดยเฉพาะในกลุ่มโรค ช่วยลดความระคายเคืองต่อผิวหนังของแคปไซซินและสามารถนำส่งสารสำคัญสู่ชั้นผิวหนังได้



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Applied Science

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Praneet Opanasopit | *Silpakorn University*
Theerasak Rojanarata | *Silpakorn University*
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Warayuth Sajomsang | *National Nanotechnology Center*
Theerasak Rojanarata | *Silpakorn University*
Tanasait Ngawhirunpat | *Silpakorn University*
Prasert Akkaramongkolporn | *Silpakorn University*
Praneet Opanasopit | *Silpakorn University*

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Prasopchai Tonglairoum | *Silpakorn University*
Theerasak Rojanarata | *Silpakorn University*
Tanasait Ngawhirunpat | *Silpakorn University*
Ruchadaporn Kaomongkolgit | *Naresuan University*
Wanchai Sutananta | *Silpakorn University*
Praneet Opanasopit | *Silpakorn University*

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Weerapath Winotapun | *Silpakorn University*
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YoungJoong Lee | *Agency for Defense Development*
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Pawinee Piyachaturawat | *Mahidol University*
Theerasak Rojanarata | *Silpakorn University*
Tanasait Ngawhiranpat | *Silpakorn University*
Praneet Opanasopit | *Silpakorn University*

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Boonnada Pamornpathomkul | *Silpakorn University*
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Praneet Opanasopit | *Silpakorn University*
Tanasait Ngawhirunpat | *Silpakorn University*

SICEAS-731

Optimization of Solid Lipid Nanoparticles Gels for Transdermal Delivery of Capsaicin by Response Surface Methodology

Sureewan Duangjit | *Ubon Ratchathani University*
Tanasait Ngawhiranpat | *Silpakorn University*

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YoungJoong Lee | *Agency for Defense Development*
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Yi-Ku Ting | *University of Taipei*
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SICEAS-682

Transfection efficiency of novel cationic liposomes with different helper lipids for gene delivery systems in human cervical carcinoma cell line

Supusson Pengnam | *Silpakorn University*
Lalita Leksantikul | *Silpakorn University*
Theerasak Rojanarata | *Silpakorn University*
Tanasait Ngawhirunpat | *Silpakorn University*
Nattisa Niyomtham | *Ramkhamhaeng University*
Boon-Ek Yingyongnarongkul | *Ramkhamhaeng University*
Praneet Opanasopit | *Silpakorn University*

SICEAS-731
Optimization of Solid Lipid Nanoparticles Gels for Transdermal Delivery of Capsaicin by Response Surface Methodology

Sureewan Duangjit [a,*], Tanasait Ngawhirunpat [b]

[a] Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand

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Abstract

The objective of this study was to develop solid lipid nanoparticles (SLN) loaded gel formulations for enhancing transdermal delivery of capsaicin (CAP). Ten model CAP formulations were formulated according to a multilevel categoric design of experiment. The lipid compositions of the CAP loaded SLN formulations were defined as casual factors (X_n) and response variables (Y_n), respectively. CAP formulations containing a constant concentration of 0.15% CAP, cetyl palmitate, transcutol P and butylated hydroxytoluene, carbopol 940, tween 20, tween 80, distilled water and various amount of d-limonene (X_1) and cocamide diethanolamine (X_2) as penetration enhancers were prepared. The physicochemical characteristics, e.g. the vesicle size, size distribution, zeta potential, entrapment efficiency, and skin permeability of the CAP loaded SLN formulations were evaluated. Moreover, the physical characteristics, e.g. physical appearance, pH, viscosity, spreadability of CAP gel formulation were also investigated. The results indicated that the skin permeability of the optimal CAP loaded SLN estimated by the Design Expert® was significantly higher than that of the CAP gel formulation and the commercial products. The response surfaces methodology predicted by the computer software was helpful in understanding the complicated relationship between the causal factors and the response variables of the optimal CAP loaded SLN formulation.

Keywords: Solid lipid nanoparticle, capsaicin, response surface method, d-limonene, experimental design

1. Background/ Objectives and Goals

In the design and development of the topical and transdermal drug delivery systems, it is important to optimize the pharmaceutical formulations having appropriate skin efficacy, skin safety and formulation stability. The previous study suggested that the skin permeation, skin irritation, and stability of formulation are major influenced by the formulation. Consequently, only specially designed products have been shown to facilitate topical and transdermal drug

delivery systems. Since the original research article report the effectiveness of the experimental design and the response surfaces methodology which was helpful in understanding the complicated relationship between the causal factors and the response variables was published. The novel optimal pharmaceutical formulations such as hydrogels, liposomes, microemulsions have been developed and introduced. Considering the skin permeability of the drugs, the application of the response surface methodology was successfully showed the feasibility of the transdermal drug delivery including meloxicam (Duangjit et al., 2012), ketoprofen (Duangjit et al., 2014) and capsaicin (Duangjit et al., 2016a).

The objective of this study was to develop solid lipid nanoparticles (SLN) loaded gel formulations for enhancing transdermal delivery of capsaicin (CAP) using response surface methodology (RSM). SLN are the first generation of lipid nanoparticles, reported that can increase skin permeation, increase skin safety and decrease formulation degradation of drugs (Charoenputtakun et al., 2014; Muller et al., 2002). CAP loaded SLN gel containing a constant concentration of 0.15% CAP, cetyl palmitate, transcutol P and butylated hydroxytoluene as oil phase, and carbopol 940, tween 20, tween 80 and distilled water as water phase, and various amount of d-limonene (X_1) and cocamide diethanolamine (X_2) as penetration enhancers were formulated. The physicochemical characteristics, e.g. the vesicle size (Y_1), size distribution (Y_2), zeta potential (Y_3), entrapment efficiency (Y_4), and skin permeability (Y_5) of the CAP loaded SLN formulations were evaluated. Moreover, the physical characteristics, e.g. physical appearance, pH, viscosity, spreadability of CAP gel formulation were also determined. The RSM was utilized to understand the relationship between causal factor and response variables, and the optimal CAP loaded SLN was subsequently predicted using Design Expert® software.

2. Methods

Ten model CAP formulations were prepared according to the SLN obtained from the previous study (Duangjit et al., 2016b). The SLN containing a constant amount of 0.15% CAP, cetyl palmitate (CP), transcutol P (Trans P), butylated hydroxytoluene (BHT), carbopol 940 (C940), tween 20 (T20), tween 80 (T80) and distilled water (DI water) and various amount of d-limonene (X_1: 0.5, 1.0 and 1.5%) and cocamide diethanolamine (X_2: 0.015, 0.030 and 0.045%) as penetration enhancers were prepared. CAP loaded SLN formulations were evaluated for physicochemical properties (e.g. vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability. Limonene (Lim) and cocamide diethanolamine (Com) used in SLN formulations defined as causal factors (X_1 and X_2, respectively), while the physicochemical properties and skin permeability were defined as response variables (Y_n). CAP loaded SLN formulations were prepared by the sonication method, as shown in Table 1.

Table 1: The composition of model CAP loaded SLN formulations

SLN	Oil phase (% w/w)						Water phase (% w/w)		
	CAP	CP	Trans P	BHT	C940	Lim	Com	T20:T80	DI water
1	0.15	30	1.2	0.002	0.5	0.5	0.015	10	to100
2	0.15	30	1.2	0.002	0.5	0.5	0.030	10	to100
3	0.15	30	1.2	0.002	0.5	0.5	0.045	10	to100
4	0.15	30	1.2	0.002	0.5	1.0	0.015	10	to100
5	0.15	30	1.2	0.002	0.5	1.0	0.030	10	to100
6	0.15	30	1.2	0.002	0.5	1.0	0.045	10	to100
7	0.15	30	1.2	0.002	0.5	1.5	0.015	10	to100
8	0.15	30	1.2	0.002	0.5	1.5	0.030	10	to100
9	0.15	30	1.2	0.002	0.5	1.5	0.045	10	to100
10	0.15	30	1.2	0.002	0.5	1.0	0.030	10	to100

Abbreviation: capsaicin (CAP), cetyl palmitate (CP), transcutol P (Trans P), butylated hydroxytoluene (BHT), carbopol 940 (C940), limonene (Lim), cocamide diethanolamine (Com), tween 20 (T20), tween 80 (T80), distilled water (DI water)

The preparation of carbopol 940 gels was followed as described in previous study (Patel et al., 2012). The gel formulation was composed of CAP loaded SLN (2%) and carbopol 940 (0.5, 1.0 and 2%). Carbopol 940 was dispersed over CAP loaded SLN formulation under constant stirring with magnetic stirrer, taking care to avoid the lumps. The dispersion was neutralized using triethanolamine. The SLN gel formulations were allowed to hold overnight to remove entrapped air.

The vesicle size, size distribution and zeta potential of CAP loaded SLN were measured by photon correlation spectroscopy (Zetasizer Nano series, Malvern Instrument, UK). Ten µl of the sample were diluted with the appropriate amount of DI water. All CAP loaded SLN formulations were performed at least three in dependent samples, at room temperature (25 ºC). The concentration of CAP in the SLN formulation was analyzed by HPLC after disruption of the CAP formulation 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.

The pH of various SLN gel formulations was determined by using Digital pH meter (Mettler Toledo™ FE20 FiveEasy™ Benchtop pH Meter, Fisher Scientific, USA). The measurement of viscosity of prepared SLN gels was carried out with the HAAKE™ MARS™ Rheometers (Thermo Fisher Scientific Inc, USA). The spreadability represents the extent of area to which the SLN gels readily spread on application to skin. The SLN gel formulation was placed over one of the slides. The other slide was placed on the top of the gels that the gels was sandwiched between the two slides in an area occupied by the distance of 10 cm of the slide. The weight of 125 g was placed upon the upper slides for 30 sec then the weight was removed. The distance of the spread gel was measured and calculated for the spreadability. The measurement of pH, viscosity and spreadability of each sample was done in triplicate.

Due to the similarity of shed snake skin to human skin in lipid content and permeability, the shed snake skin from the Siamese cobra (*Naja kaouthia*) was used as a model membrane in our study. 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 CAP formulation 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 CAP in the aliquot was analyzed using a HPLC.

The concentration of CAP in all samples 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 utilized. The mixture of acetonitrile and 0.01% phosphoric acid (50:50) was used as the mobile phase. A UV detector was set at 227 nm for capsaicin detection at ambient temperature. The flow rate was 1.0 ml/min and the injection volume was 20 µL. The calibration curve for CAP was in the range of 1-100 µg/ml with a correlation coefficient of 0.999. The data were reported as mean ± S.D. (n=3) and statistical analysis of the data was carried out using paired t-test. A p-value of less than 0.05 was considered to be significant.

3. Results

3.1 Physicochemical Characteristics of CAP Loaded SLN Formulation

The physicochemical characteristics i.e., vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability of CAP loaded SLN are shown in Table 2. The physical appearance of CAP loaded SLN was white creams as same as milky solution, when the SLN was added in gel formulation, the SLN gel was white emulsions as same as emulgels.

The vesicle size of CAP loaded SLN formulation was varied depending on the ratio of penetration enhancer (Lim and Com), but also within 220 nm under sonication method. The results indicated that the ratio of penetration enhancer was not affected the vesicle size and size distribution in this study, because the method of preparation used in this application of technique can prepared the homogeneous and narrow size distribution of SLN formulation. However, the difference in Lim concentration of SLN formulations may affect the energy barriers required for stabile dispersion systems (Charoenputtakun et al., 2014). All CAP loaded SLN formulations had a negative zeta potential. The previous study suggested that the compositions of formulation may contribute to the negative charge of the lipid vesicle formulations. Several SLN compositions used in this formulation (i.e., trans P, Com, T20, T80 and) were nonionic compounds. Hence, the CAP, CP, Lim may affected the total net charge of SLN formulation. Kedmi et al. reported that using SLN formulation with negative surface charge was less toxic and have high stability than SLN formulation with positive surface

charge (Kedmi et al., 2010). The CAP incorporated in SLN formulation was very close to 100% as shown in Table 2.

Table 2: The causal factors (X_n) and response variables (Y_n) of model CAP loaded SLN formulations

SLN	causal factors (X_n)		Response variables (Y_n)				
	X_1	X_2	Y_1	Y_2	Y_3	Y_4	Y_5
1	-1	-1	196.58±1.38	0.17±0.02	-27.28±14.89	99.99±0.01	0.51±0.12
2	-1	0	189.10±10.44	0.18±0.03	-24.49±1.82	99.98±0.10	0.49±0.10
3	-1	1	193.43±9.46	0.19±0.04	-26.18±1.74	99.97±0.09	0.40±0.16
4	0	-1	201.04±8.93	0.20±0.03	-24.84±0.99	99.89±0.02	0.53±0.03
5	0	0	212.48±15.80	0.21±0.06	-24.83±0.80	99.95±0.03	0.46±0.12
6	0	1	183.19±7.06	0.16±0.05	-22.34±1.35	99.72±0.03	0.43±0.07
7	1	-1	203.96±13.39	0.19±0.04	-26.51±0.10	98.90±0.30	0.35±0.15
8	1	0	192.88±10.05	0.18±0.04	-23.16±3.01	99.99±0.20	0.50±0.05
9	1	1	191.26±12.18	0.17±0.04	-26.50±0.68	98.87±0.10	0.44±0.08
10	0	0	197.33±1.53	0.14±0.01	-23.89±0.78	99.99±0.04	0.39±0.05

The skin permeation of all CAP loaded SLN was significantly higher than that of CAP loaded SLN without penetration enhancer (data not show). These results were agreed well with the previous studies that the incorporation of limonene can enhance the skin permeation flux of various drugs (i.e. all trans retinoic acid, sodium fluorescein) because terpene can increase drug partitioning into the stratum corneum (Charoenputtakun et al., 2014; T. Subongkot et al., 2012). The optimal CAP loaded SLN estimated using the Design Expert® was SLN composed of 1% Lim and 0.015% Com. The optimal CAP loaded SLN predicted by the computer program was coincided well with the experimental formulation in this study. Therefore, the CAP loaded SLN containing 1% Lim and 0.015% Com was selected as the optimal CAP loaded SLN for incorporating in gel formulation in further study.

3.2 Physical Characteristics of CAP Loaded SLN Gel Formulation

The SLN gel formulation was successfully prepared by sonication method. The physical characteristics of SLN gel formulation are presented in Table 3. The increase in concentration of C940 resulted in significantly increase in viscosity and spreadability of SLN gel formulations.

Table 3: Physical Characteristics of CAP loaded SLN Gel Formulation

Gel	C940 (%)	pH	Viscosity (Pa·s)	Spreadability (g·cm/sec)*
1	0.5	7.52±0.02	213.13±16.54	0.0733±0.002
2	1.0	7.62±0.01	474.30±14.89	0.0772±0.001
3	2.0	7.93±0.03	528.70±5.37	0.0812±0.001

* spreadabilty = (weight of gel x Length of the glass)/ time taken in seconds

3.3 Skin Permeation Study

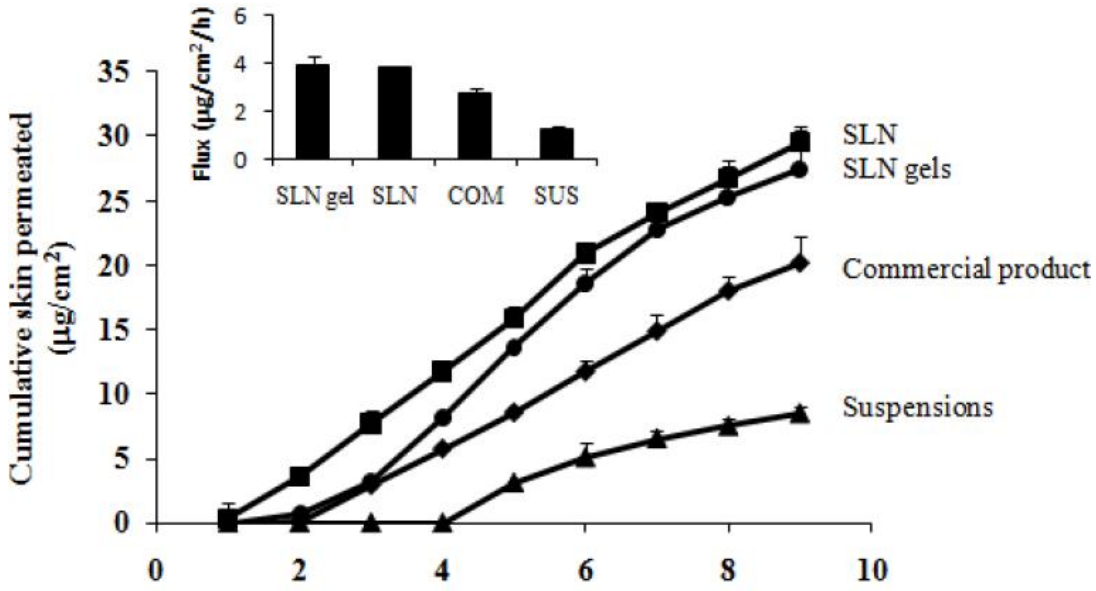

Fig. 1: The skin permeation flux of different CAP formulations.

Figure 1 illustrated skin permeability of the cumulative skin permeation per unit area and the steady-state flux of various CAP formulations over an incubation period of 1–9 hours. The skin permeation profile and the flux of SLN gel formulation, SLN formulation, commercial product (COM) and suspension (SUS) were significantly different. According to the skin permeation profile, the results showed that the skin permeation profile of SLN gel and SLN formulation was significantly higher than the COM and SUS. These skin permeation data indicated that the dramatic synergistic permeability-enhancing effect of SLN gel formulation, as in SLN gel formulation was greater than that of SLN formulation, COM and SUS, respectively. This result strongly suggested that Lim and Com act synergistically to improve the transdermal delivery of CAP. The synergistic effect of SLN and gel may be due to the combination of different mechanisms of each intrinsic property (Davidsen et al., 2002). The SLN formulation may promote skin permeability by various mechanisms. The possible mechanisms by which this SLN formulation (as nanoparticles) improved the skin delivery of CAP encompassed the penetration-enhancing mechanism (Lim and Com) and the vesicle adsorption to and/or fusion with the stratum corneum. The gel formulation may enhance the skin permeation via skin hydration mechanism. However, the possible mechanism of action of the different formulations should be confirmed by the X-ray diffractograms and/or FT-IR spectra in further study (Tang et al., 2016).

An effort to design and develop simultaneous SLN gel formulations to enhance the transdermal delivery of CAP using response surface method presented an advancement challenge as the complicated relationship between various causal factors and response variables must be

considered. The accuracy and reliability of optimal SLN formulations were experimentally verified by the experimental formulation and evaluation. The results indicated that the experimental value agreed well with the predicted skin permeability values. Considering the skin permeability of CAP loaded SLN and CAP loaded SLN gel formulation, we successfully showed the feasibility of the transdermal delivery of CAP.

3.4 Acknowledgments and Legal Responsibility

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Development of Lipid Nanoparticles for Transdermal Delivery of Capsaicin: Optimization and Characterization

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Abstract

Introduction: A response surface method (RSM) was employed in the development and optimization of several pharmaceutical preparations. Using RSM, the complicated relationships between causal factors and response variables can be easily understood. Moreover, a stable and reproducible simultaneous optimal formulation was obtained. These statistical approaches were powerful in the design and development of an appropriate pharmaceutical preparation. The aim of this study was to design and develop the solid lipid nanoparticles (SLNs) for transdermal delivery of capsaicin (CAP) using the RSM. **Methods:** The lipid compositions of the SLN incorporated CAP were a constant concentration of 0.15% CAP, cetyl palmitate, transcutol P and butylated hydroxytoluene, tween 20, tween 80, distilled water and various amount of terpene (X_1) and surfactant (X_2) as penetration enhancers. SLNs were prepared by sonication method. The physicochemical characteristics (vesicle size, size distribution, zeta potential, entrapment efficiency) and skin permeability of SLN incorporated CAP were investigated. **Results:** The vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability were selected as response variables (Y_n). The optimal SLN formulation was estimated using RSM. The resulted indicated that the experimental values (e.g. The vesicle size, size distribution, zeta potential, entrapment efficiency and skin permeability) of SLNs was very close to the values estimated by the computer programs. **Conclusion:** Considering the experimental values and the values predicted by the computer programs of the optimal SLN formulation, we were successful in showing the feasibility of transdermal delivery of SLN incorporated CAP.

Keywords: Terpene, Capsaicin, Solid lipid nanoparticle, Response surface method

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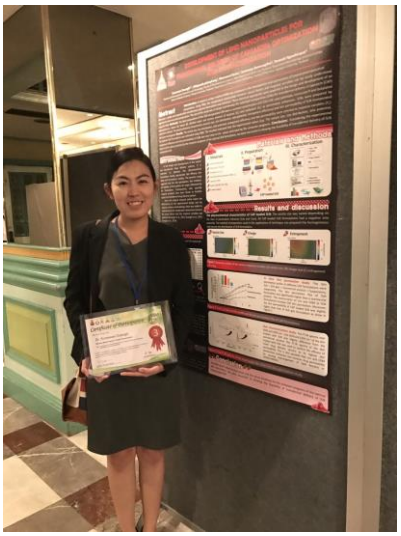
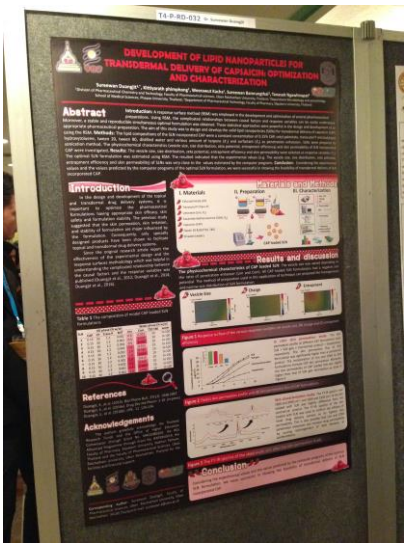
อาจารย์คณะเภสัชศาสตร์ รับเกียรติบัตร Bronze Medal Award (Poster presentation)
นำชื่อเสียงสู่มหาวิทยาลัยอุบลราชธานี





อาจารย์เภสัชกรหญิง ดร.สุรีวัลย์ ดวงจิตต์ อาจารย์ประจำ คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี ได้รับรางวัลนำเสนอผลงานวิจัยแบบโปสเตอร์ Bronze Medal Award ระดับ Scientists ในการประชุมระดับนานาชาติ The 2nd International Conference on Herbal and Traditional Medicine (HTM2017) จัดที่โรงแรมเอเชีย พญาไท กรุงเทพมหานคร จัดโดย คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น ระหว่างวันที่ 25-27 มกราคม 2560

อาจารย์คณะเภสัชศาสตร์ รับเกียรติบัตร Bronze Medal Award (Poster presentation) นำชื่อเสียงสู่มหาวิทยาลัยอุบลราชธานี

อาจารย์เภสัชกรหญิง ดร.สุรีวัลย์ ดวงจิตต์ อาจารย์ประจำ คณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี ได้รับรางวัลนำเสนอผลงานวิจัยแบบโปสเตอร์ Bronze Medal Award ระดับ Scientistes เรื่อง Development of Lipid Nanoparticles for Transdermal Delivery of Capsaicin: Optimization and Characterization ในการประชุมระดับนานาชาติ The 2nd International Conference on Herbal and Traditional Medicine (HTM2017) จัดที่โรงแรมเอเชีย พญาไท กรุงเทพมหานคร จัดโดย คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น ระหว่างวันที่ 25-27 มกราคม 2560

ผลงานวิจัยดังกล่าวประกอบด้วยคณะผู้ร่วมวิจัย ได้แก่ อ.ดร.สุรีวัลย์ ดวงจิตต์ นางกฤตติญารันต์ พิมพ์พงษ์ และนายวีระวุท กะชา จากคณะเภสัชศาสตร์ มหาวิทยาลัยอุบลราชธานี อ.ดร. สุรีวัลย์ บำรุงไทย อาจารย์ประจำคณะวิทยาศาสตร์การแพทย์ มหาวิทยาลัยพะเยา และ รศ.ดร. ธนะเศรษฐ์ ง้าวหิรัฐพัฒน์ อาจารย์ประจำคณะเภสัชศาสตร์ มหาวิทยาลัยศิลปากร โดยเป็นงานวิจัยเกี่ยวกับสมุนไพรไทยคือสารสกัดจากพริก (capsaicin) ซึ่งเป็นสมุนไพรพื้นบ้านของจังหวัดอุบลราชธานี และพัฒนาเป็นผลิตภัณฑ์โดยอาศัยเทคโนโลยีใหม่คือ การนำสารสกัดพริกมาบรรจุในระบบนำส่งยาชนิดอนุภาคไขมันของแข็งขนาดนาโนเมตร (Solid Lipid Nanoparticles) เพื่อเพิ่มคุณภาพและประสิทธิภาพในการออกฤทธิ์ของสารสกัดพริกในการบรรเทาอาการปวดอักเสบข้อเข่าหรืออาการปวดบริเวณต่างๆ ของร่างกาย โดยงานวิจัยนี้อยู่ในระยะต้นเพื่อการหาสูตรที่เหมาะสมที่สุดและการอธิบายคุณลักษณะของสูตรตำรับที่ได้ จุดมุ่งหมายของงานวิจัยนี้เพื่อพัฒนาผลิตภัณฑ์รูปแบบใหม่จากสมุนไพรไทยที่มีประสิทธิภาพและมีความปลอดภัย ตลอดจนสามารถพัฒนาเพื่อเป็นผลิตภัณฑ์สมุนไพรไทยที่สามารถจำหน่ายได้ และเพื่อการพัฒนาเศรษฐกิจและอุตสาหกรรมสมุนไพรไทยให้เจริญก้าวหน้าต่อไป

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Characterization of Nano-Vesicular Containing Tomato Extract for Dermal Delivery: Liposomes, Transfersomes and Niosomes

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Abstract

Lycopene is a powerful phyto-antioxidant from tomato extract (TOM). Due to its intrinsic properties as a very poor solubility in water compound, the permeation through the outer most of the skin the stratum corneum is not allowed. The objective of this study was to design and develop a nano-vesicular for dermal delivery of tomato extract. The physicochemical properties and the formulation stability of liposomes (LP), transfersomes (TS), niosomes (NS) and niosomes-penetration enhancer (NS-PE) containing tomato extract were also investigated. All vesicular formulations were prepared by thin film hydration method. The physicochemical characteristics, i.e., vesicle size, size distribution, zeta potential of the TOM loaded vesicular formulations, and the formulation stability were measured. The physicochemical characteristics of all vesicular formulations were compared at the initial and after storage at 4 °C for 90 days. The vesicle size of all vesicular formulation was in nanosize range (less than 200 nm) with the narrow distribution and negative charge. The vesicle size and zeta potential of TOM-loaded vesicular formulations (LP, TS, NS and NS-PE) at the initial and 90 days after incubation period showed significantly different. On the other hand, the size distribution was not significantly different. Considering overall results, our study indicated that the TS were the candidate vesicular formulation that can be used as transdermal delivery carriers for tomato extract.

Keywords: Tomato extract, Lycopene, Liposomes, Transfersomes, Niosomes

1. Introduction

Lycopene is a phyto-antioxidant from tomatoes. A powerful antioxidant property of lycopene has been reported superior to those of β-carotene [1]. Although several researches suggest that lycopene has been showed in relation to its potential biological and physiological activity, more research and development about the formulation containing lycopene is needed

to support this hypothesis. Because of the biological and physiological activity of lycopene, several attempts have been conducted to formulate it in suitable dosage forms for human use. Lycopene is much more sensitive to light, air, and heat in its pure compound [2]. The utilization of the extraction of lycopene from tomatoes could thus be a good alternative [3].

According to the previous study, the application of topical formulation via dermal delivery of lycopene is more efficient in preventing oxidative damage [4]. Therefore, the lycopene dermal delivery may provide the productive effect in terms of photodamage protection. Unfortunately, lycopene is strongly lipophilic compound (log P ~15), which the possible to penetrate across the stratum corneum into viable epidermis layers was a difficult task. Lycopene tended to remain in the stratum corneum more than diffused in to the deep skin layers. To improve the dermal delivery of lycopene and/or tomato extract in to the deep skin region, a nanotechnology is an ingenious strategies.

The nanotechnology as nanocarriers (such as liposomes, niosomes, microemulsions and nanoparticles) have been studied in wide research as dermal and transdermal delivery systems for antioxidants to improve their beneficial effects in the treatment of anti-aging.

In this recent study, the design and development of a nano-vesicular for topical delivery of tomato extract were investigated. The physicochemical properties (e.g., vesicle size, size distribution, zeta potential) and the formulation stability of liposomes (LP), transfersomes (TS), niosomes (NS) and niosomes-penetration enhancer (NS-PE) containing tomato extract were evaluated.

2. Materials and Methods

2.1 Materials

Phosphatidylcholine (Phospholipon 90G, Lipoid; PC) was supplied as a special gift from LIPOID GmbH (Cologne, Germany). Liquid extraction of tomato was supplied as a special gift from the Global Medical (Thailand) Co., Ltd. Cholesterol (CHOL) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Polysorbate-20 (Tween 20VR , T20) was purchased from the NOF Corporation (Osaka, Japan). Oleic acid was purchased from Sigma-Aldrich (St. Louis, MO).

2.2 Vesicular preparation

The LP, TS, NS and NS-PE were prepared by thin film hydration method (Table 1). The lipid vesicle formulations of LP and TS composed of 10 mM PC, 1 mM of CHOL and various amount of 1 mM oleic acid (as penetration enhancer; PE). Whiles, the NS and NS-PE composed of a constant amount of 10 mM Tween® 20 (T20), 10 mM CHOL and various amount of 1 mM PE. TOM was added in lipid phase before the preparation of the lipid thin film. The dried lipid film containing TOM was hydrated with phosphate buffer solution (PBS; pH 7.4). All vesicular formulations were subsequently sonicated for two cycles using a bath- and probe-type sonicator (5510J-DTH Branson Ultrasonics, Danbury, U.S.A.). The TOM loaded vesicular formulations were freshly prepared and stored in airtight containers at 4°C prior to use.

2.3 Vesicle size, size distribution and zeta potential measurement

Average vesicle size size distribution and zeta potential of the vesicular were measured by photon correlation spectroscopy (PCS) (Zetasizer Nano series, Malvern Instruments, U.K.). All samples were investigated at room temperature (25 °C), after diluting the vesicle formulations. Twenty microlitres the sample formulations were diluted with 1480 µL of deionized water. At least three independent samples were taken, and the vesicle size, size distribution and zeta potential were measured at least three times.

2.4 Stability study

The physicochemical stabilities of the LP, TS, NS and NS-PE formulations were evaluated by monitoring the formulations for at least 90 days after their initial preparation. Various vesicular formulations were stored in glass bottles with plastic plugs at 4±1°C for 90 days to determine the stability of the formulations. The physicochemical stabilities of vesicular formulations were evaluated by visual inspection for sedimentation. The vesicle size, size distribution and zeta potential were determined by PCS.

2.5 Data analysis

The data are reported as the means ± standard deviation (SD) (n=3). A *p*-value of less than 0.05 was considered to be significant.

Table 1 The composition of different vesicular formulations

Formulation	Lipid composition (%W/V)					Buffer qs to
	PC	Chol	PE	T20	TOM	
LP	0.76	0.04	-	-	15%	100 mL
TS	0.76	0.04	0.10	-	15%	100 mL
NS	-	0.40	-	0.40	15%	100 mL
NS-PE	-	0.40	0.10	0.40	15%	100 mL

3. Results and discussion

3.1 Physicochemical characteristics of vesicular formulation

The physicochemical characteristics of nano-vesicular containing tomato extract are shown in Fig.1. The vesicle size of all TOM-loaded vesicular formulations was smaller than 200 nm (Fig.1a). The nano-size of the vesicular that less than 120 has been reported to be an appropriate size for transdermal drug delivery carriers as compared to the large ones [5]. Considering the vesicle size, the lipid composition (PE, T20) significant affected the vesicle size of all TOM-loaded vesicular formulations. The permeability of the vesicular bilayer can be controlled by inducing conformational organizing of the hydrocarbon chains of the bilayer core [6]. Thus, the incorporation of PE into the vesicular composition resulted in the decreased of vesicle size. Moreover, the difference in the vesicular composition is predominately between PC (LP, TS) and T20 and CHOL (NS, NS-PE), resulting in the difference in the vesicle size. The size distribution of all formulations was less than 0.2 (Fig.1b), thus the composition and the method of preparation were appropriate for the further study. These results could be

indicated that the composition and the method of preparation in our study were suitable for formulation the nano-vesicular containing tomato extract with narrow size dispersion.

The zeta potential of all TOM-loaded vesicular formulations was negative zeta potential (-16 to -32 mV) as shown in Fig.1c due to the intrinsic properties of vesicle compositions (PC and oleic acid) and total net charge. The TOM maybe a factor that affect the total net charge of the nano-vesicles. PC is a zwitterionic compound (an isoelectric point was 6 - 6.7). Thus, under the experimental pH (7.4), in which the pH was higher than the isoelectric point, PC vesicles had an overall negative charge. Moreover, oleic acid is a fatty acid with pKa 5.2 that under pH 7.4 of the experimental condition, the negative charge was a major species. The potential of negatively charged vesicles in the precious study suggested that the skin permeation rate of various drugs (e.g., betamethasone, betamethasone dipropionate, melatonin, betahistine, ethosuximide) was significantly higher than that of positively charged ones [7, 8]. Thus, nano-vesicular containing TOM with negative charge may be desirable for dermal delivery.

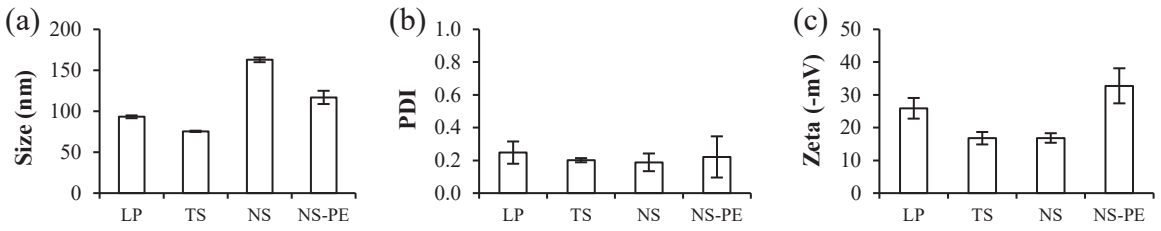

Fig. 1 (a) Vesicle size, (b) size distribution and (c) zeta potential of different vesicular formulation at the initial

3.2 Stability study

The stability evaluation of All TOM-loaded vesicular formulations was performed after kept at 4 °C for 90 days. The nano-vesicular formulation remained transparent, and no signs of sedimentation at 4 ± 1 °C for 90 days were observed. The comparative of the physicochemical properties at the initial and 90 days are displayed in Fig.2. The vesicle size of the formulations at 90 days was significantly increased comparing to those of the initial. The great difference in the vesicle size of LP, TS, NS and NS-PE was 2.7, 2.1, 2.7 and 1.8 times, respectively. A significant difference in the decrease in zeta potential was observed between the initial and at 90 days. The high zeta potential (over -30 mV) has been reported a beneficial to vesicular physical stability as the prevention of aggregation between the vesicles owning to electrostatic repulsion [9, 10]. While the initial showed a zeta potential of -16 to -32 mV, at 90 days the zeta potential decreased to -3 to -11 mV. The similar pattern was also observed from LP, NS and NS-PE. However, the physicochemical properties of TS at the initial and 90 days were not significant difference. The vesicle size at 90 days was still lower than 200 nm. From the above results it is clear that TS was the proper vesicular that could be a carrier for dermal delivery of

TOM. The recommended storage conditions for the TS vesicular formulation is at 4°C and not over 90 days.

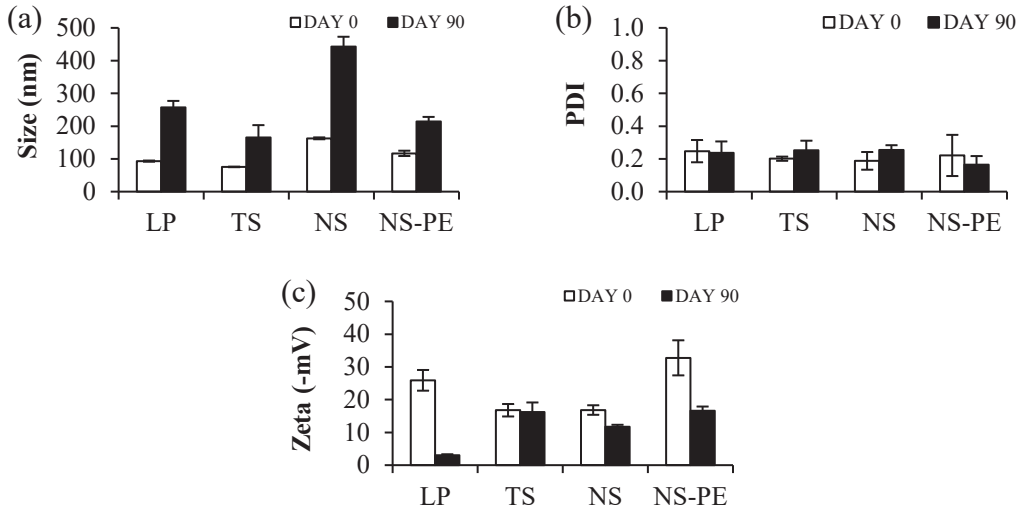

Fig. 2 (a) Vesicle size, (b) size distribution and (c) zeta potential of different vesicular formulation at the initial and at 90 days after incubation period

4. Conclusion

Considering physicochemical properties of all nano-vesicular containing TOM, the results suggested that the TS were the candidate vesicular formulation that can be used as transdermal delivery carriers for tomato extract.

Acknowledgements

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Development of Topical Gel-Based Formulation for Enhancing Transdermal Delivery of Capsaicin: Physical properties characterization

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Keywords: Capsaicin, Gels, Carbopol, Carbomer, Topical gel

Introduction

Capsaicin extract are known for its various biological and physiological activities including physiological and pharmacological effects on the cardiovascular system and gastrointestinal track, anti-oxidants, anti-cancers and anti-inflammatories. In pharmaceutical research fields, the more the capsaicin, the hotter the pepper, and the higher the antioxidant level have been suggested.(1). However, the potential applications of capsaicin were restricted by its strong pungency and irritation.(2) The proper strategy to drive the research for capsaicin as drug delivery systems was desirable.

The research and development of novel drug delivery systems were still interested and attracted increasing. Our previous research have shown that the capsaicin can be delivered into the skin via various novel drug delivery systems such as microemulsions,(3, 4) solid lipid nanoparticles (SLN),(5) liposomes.(6) To date, novel formulation such as microemulsions, liposomes, SLNs loaded capsaicin was not available as commercial product. The primary consideration in the development of all pharmaceutical products, the efficacy, the safety and the stability of basic topical formulation (gels- and creams-based) should be concerned. The intrinsic properties of basic topical formulation may improve the *activity of capsaicin by* promoting the efficacy, the safety and the stability of the novel *formulations*.

The topical gels-based of carbopol have been not only to enhance the stability of liposome formulation, but also serve the *efficacy* of the liposome *loaded* paracetamol *formulation*.(7) Due to carbopol may provide the key mechanical strength to the vesicle formulation. The aim of this study was to develop gel-based formulation for enhancing transdermal delivery of capsaicin. The gel-based formulation was performed to fine the optimal type and concentration of gelling agent to enhance the stability and to prove the efficacy of the novel formulations in promoting the activity of capsaicin. The 0.5-2.0 %w/w of gelling agent were varied. The physical appearance, pH, spreadability, rheology and drug content of capsaicin gel formulation were investigated.

Materials and Methods

Preparation of capsaicin gel formulation

Preparation of capsaicin gel formulation was two steps. Firstly, an aqueous dispersion of gelling agents (e.g., carbopol 934, carbopol 940, carbopol 1342, carbopol ultrez 10, carbopol ultrez 21 and carbopol ETD 2020) in deionized water, propylene glycol 400, preservative and ethanol was prepared. The gelling agent was varied at 0.5, 1.0, 1.5 and 2.0 %w/w. To this mixture 3%w/w of capsaicin resin (0.0125 %w/w capsaicin) in oil phase (vitamin E, methyl salicylate and various types of terpens) was dropped wise along with constant agitation on magnetic stirrer. Secondly, the dispersion was neutralized using triethanolamine for pH adjusting and viscosity enhancement.

Characterization of capsaicin gel formulation

The capsaicin gel was characterized for pH, spreadability, rheology, and drug content through standard methods. The measurement of pH, spreadability, rheology, and drug content of each sample was done at least in triplicate.

***pH* and rheology**

The pH values of the gel was measured by a Digital pH meter (Mettler Toledo™ FE20 FiveEasy™ Benchtop pH Meter, Fisher Scientific, USA). The measurement of viscoelastic properties of prepared gels was carried out with the HAAKE™ MARS™ Rheometers (Thermo Fisher Scientific Inc, USA).

Spreadability

The spreadability represents the extent of area to which the gels readily spread on application to skin. The spreadability measurement was followed as described in previous study. [8] Briefly, the gel formulation was placed over one of the slides. The other slide was placed on the top of the gels that the gels was sandwiched between the two slides in an area occupied by the distance of 10 cm of the slide. The weight of 125 g was placed upon the upper slides for 30 sec then the weight was removed. The distance of the spread gel was measured and calculated for the spreadability.

$$S = \frac{U \times L}{T}$$

Where U = weight tied to upper slide, L = length of glass slides, T = time taken to separate the slides

Drug content

The concentration of capsaicin in all gel samples was analyzed using a HPLC (*Thermo*Scientific™ *Dionex*™ UltiMate 3000 LC systems, Thermo Fisher Scientific Inc, USA). After disruption of the capsaicin formulations with methanol, the samples were centrifuged at 10,000 rpm at 25 ºC for 20 min. The supernatant was filtered with a 0.45 *µ*m nylon syringe filter. A Luna Omega C18 reversed-phase column (Phenomenex®, Phenomenex distributor, Thailand) with dimensions of 5 µm, 4.6×250 mm was utilized. The mixture of acetonitrile and 0.01%w/w phosphoric acid (50:50) was used as the mobile phase. A UV detector was set at 227 nm for capsaicin detection at ambient temperature. The flow rate was 1.0 mL/min and the injection volume was 20 µL. The calibration curve for CAP was in the range of 1-100 µg/mL with a correlation coefficient of 0.999. The data were reported as mean ± SD (n=3) and statistical analysis of the data was carried out using paired t-test. A p-value of less than 0.05 was considered to be significant.

Results and discussion

The appearances of all capsaicin carbopol gels are orange as the color of capsaicin resin. The 0.5%w/w of all carbopol capsaicin gels were homogenous. The appearances of the capsaicin gels were more viscous when the gelling agent concentration was increased. The pH of all capsaicin carbopol gels was ranging from 3.72±0.01 to 7.52±0.02 (Figure 1A), which the pH of gels about 4.0-7.0 was found to be acceptable for the skin. [9] The spreadability of all capsaicin carbopol gels was in the range of 3.07±0.12 to 5.03±0.12 g.cm/sec (Figure 1B). The 0.5%w/w of all gelling agents was more high spreadability indicated the gels had low viscosity and could easily apply to skin. The efficacy of the capsaicin gels depends on their spread. The gel spreading assisted in the uniform application to the skin, therefore the prepared gels must have a good spreadability and fulfill the ideal quality in topical application.

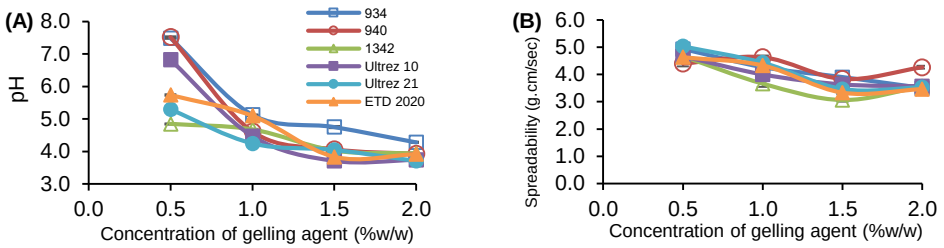

Figure 1. pH (A) and spreadability (B) of the capsaicin gel formulations: Carbopol 934, Carbopol 940, Carbopol 1342, Carbopol Ultrez 10, Carbopol Ultrez 21 and Carbopol ETD 2020. (the bars represent the standard deviations of three replicates)

The viscoelastic properties such as storage modulus (G') and loss modulus (G") of these capsaicin carbopol gels were measured using the HAAKE™ MARS™ Rheometers. The viscoelastic properties of the capsaicin carbopol gels at different types and concentrations of gelling agents are shown in Figure 2. It was observed that G" increased with an increase gelling agent concentration. For 0.5%w/w, the elastic modulus (G') of carbopol 940, carbopol 1342, carbopol ultrez 21 and carbopol ETD 2020 were greater than loss modulus (G") with a good distance between them, indicating strong thickening or solidifying behavior. A crossover of G' and G" was starting at the frequency about 10 rad/sec, the capsaicin gels started to break down, the lower frequency was a desirable feature since it simulated the onset of spreading of gel on the skin. Some capsaicin carbopol gels did not cross between G' and G" until the frequency about 70 rad/sec, indicating that the structures of gels were strong thus the greater frequency was needed to spread the gels. However, the higher frequency was not suitable in topical preparation. Drug content of the capsaicin gels was nearly one-hundred pencatage by HPLC analysis. The incorporation of ethanol resulted in the successful in the loading of capsaicin resin in carbopol gel formulation.

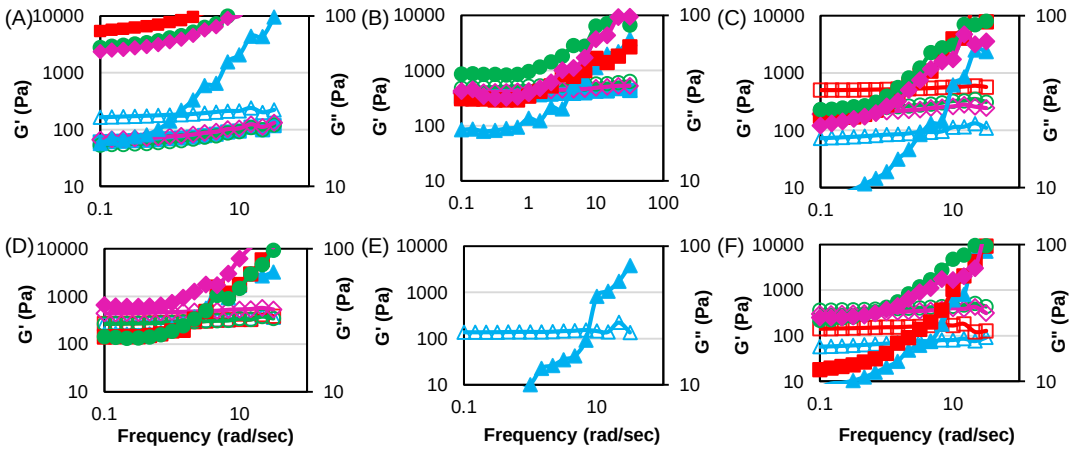

Figure 2. Angular frequency dependence of storage modulus (G', open symbols) and loss modulus (G", closed symbols) for the capsaicin gels: (A) Carbopol 934, (B) Carbopol 940, (C) Carbopol 1342, (D) Carbopol Ultrez 10, (E) Carbopol Ultrez 21 and (F) Carbopol ETD 2020 at different concentrations (0.5%w/w, triangles; 1.0%w/w, squares; 1.5%w/w, circles; 2.0%w/w, diamonds).

Conclusion

The physical structure characterization of the carbopol gel based was investigated using physical appearance, pH, spreadability, rheology and drug content. The suitable gelling agent concentration of all cabopol types were 0.5-1.0 %w/w. These properties could serve as a suitable pre-formulation for enhancing transdermal delivery of capsaicin. To evaluate the potential of optimal gel-based formulation formulation (such as microemulsions, liposomes, SLNs) loaded in optimal gel based formulation should be performed in further study.

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Development of Tomato Extract Loaded Nano-Vesicles For Anti-aging Cosmetics: Physicochemical Characterization, Skin Permeation and Stability Evaluation

Sureewan Duangjit[1,*], Warisada Sila-On[1], Utsana Puapempoonsiri[1], Kusuma Jitseang[1], Tanasait Ngawhirunpat[2], Sureewan Bumrungthai[3], Tuddao Chuchoyte[4]

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Abstract

Lycopene (LYC) is a potential antioxidant commonly occurs in tomato, watermelon, pink grapefruit, apricot and orange. In this study, we focused on the LYC in tomato extract. However, the use of LYC in topical cosmetics is often limited due to its instability, very low water solubility and very low permeability. In order to overcome these limitations, nano-vesicles as liposomes and niosomes are being interested. The objective of this study was to develop the tomato extract loaded nano-vesicles (liposomes, transfersomes and niosomes and niosomes with oleic acid) for anti-aging cosmetic. The nano-vesicles were prepared by the thin film hydration method. The physicochemical characterizations, skin permeation and stability evaluation of tomato extract loaded nano-vesicles were investigated. The stability of formulations was evaluated and monitoring for 120 days. The vesicle sizes of all tomato extract loaded nano-vesicles were less than 200 nm. The polydispersity index of all formulations was 0.2-0.4. The incorporation of tomato extract in nano-vesicles was at least 100 μg/mL. The skin permeation profile of transfersomes was significantly higher than those of niosomes with oleic acid, liposomes and niosomes, respectively. The physicochemical characteristics of the nano-vesicles at the initial and 120 days after incubation time illustrated significantly different. Considering the physicochemical characterizations, skin permeation and stability of all nano-vesicle formulation, we were successful in showing the feasibility of transdermal delivery of tomato extract loaded nano-vesicles for anti-aging cosmetic. The further study is required to confirm the potential of antioxidant activity of tomato extract loaded nano-vesicles.

Introduction

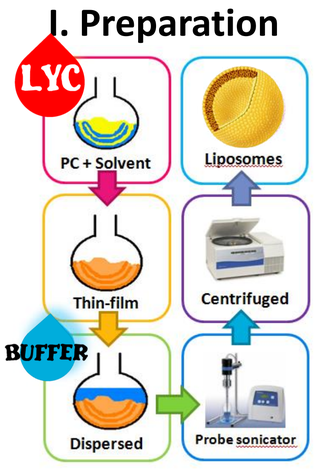
Lycopene (LYC) is a potential antioxidant commonly occurs in tomato, watermelon, pink grapefruit, apricot and orange. In this study, we focused on the LYC in tomato extract. However, the use of LYC in topical cosmetics is often limited due to its instability, very low water solubility and very low permeability. In order to overcome these limitations, nano-vesicles as liposomes and niosomes are being interested. Liposome technology is a powerful technique as its non-toxic, biocompatible, biodegradable. Liposomes provide the possibility of carrying hydrophilic and hydrophobic compounds. Incorporation of LYC into liposomes has been reported that slowed the oxidation of LYC. To improve the stability of LYC, a nano-vesicles is an ingenious strategies. The nano-vesicles such as liposomes and niosomes have been widely studied as cosmetics. In this recent study, the design and development of LYC loaded nano-vesicular for anti-aging cosmetics were investigated. The physicochemical properties (e.g., vesicle size, size distribution, zeta potential, LYC entrapment, skin permeation and stability of liposomes (CLP), transfersomes (TFS), niosomes (NS) and niosomes with oleic acid (as penetration enhancer) (NS-OA) containing tomato extract were evaluated.

Objectives

The objective of this study was to develop the tomato extract loaded nano-vesicles (liposomes, transfersomes and niosomes and niosomes with oleic acid) for anti-aging cosmetic.

Methods

I. Preparation



II. Characterization

- Vesicle size
- Size distribution
- Zeta potential

- Entrapment efficiency

$$\%EE = \left(\frac{C_L}{C_i}\right) \times 100$$

- DPPH assay

III. Skin permeation

- Skin permeability

IV. Stability study

Table 1 The composition of different liposome formulations

Forms	Lipid composition (%W/V) PC	Chol	T20	OA	LYC	ABS Ph 5.5 qs to
CLP	0.77	0.04	-	-	0.10	100 mL
TFS	0.77	0.04	-	0.10	0.10	100 mL
NS	-	0.38	0.38	-	0.10	100 mL
NS-OA	-	0.38	0.38	0.10	0.10	100 mL

Phophatidylchloine (PC), Cholesterol (Chol), Oleic acid (OA), Tween 20 (T20), Lycopene (LYC), Liposome (CLP), Transfersomes (TFS), Niosome (NS), Acetate biffer solution (ABS)

Results & Discussion

Physicochemical characteristics and stability of vesicle formulations: The vesicle size of tomato extract loaded nano-vesicles was smaller than 200 nm. The size distribution was 0.2, thus the composition and the method of preparation were appropriate for the further study. The zeta potential of all nano-vesicles was negative zeta potential (-16 to -32 mV) due to the intrinsic properties of vesicle compositions (PC, Chol, T20 and OA) and total net charge. The entrapment efficiency was 60-90% depended on their compositions.

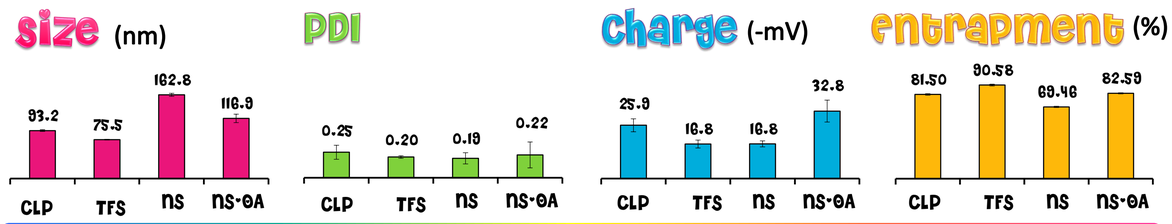


Figure 1 The physicochemical characteristics of tomato extract loaded nano-vesicles

Stability study: The nano-vesicle remained transparent, and no signs of sedimentation at 4 ± 1°C for 120 days were observed. The vesicle size of the formulations at 120 days was significantly increased comparing to those of the initial. However, the vesicle size at 90 days was still lower than 200 nm.

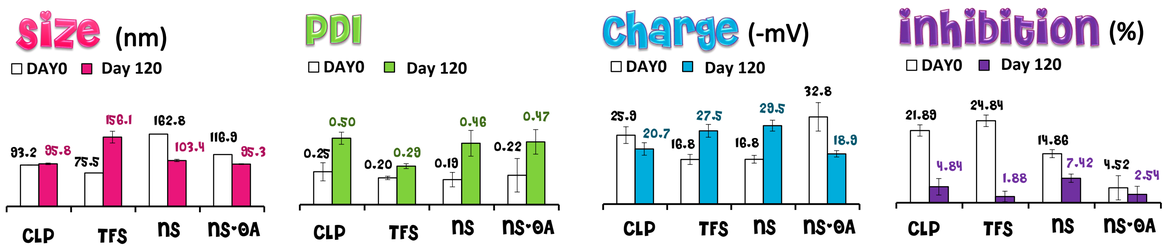


Figure 2 The physicochemical stability of tomato extract loaded nano-vesicles

Skin permeation study: The FTIR spectra of TFS was significantly different from other nano-vesicles.

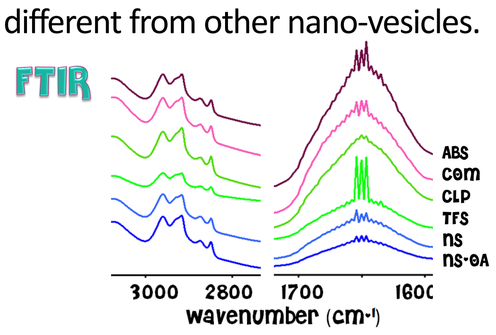


Figure 3 FTIR spectra of treated skin

Antioxidant activity: The %inhibition of TFS was higher than that of CLP, NS and NS-OA, respectively.

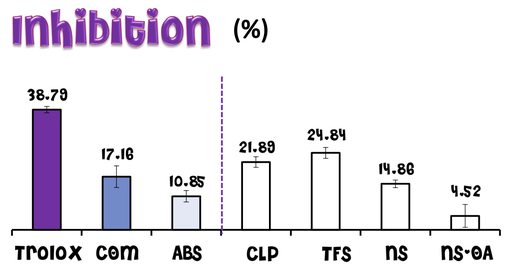


Figure 4 Antioxidant activity

Conclusion

Considering the physicochemical characteristics, skin permeation and stability and the antioxidant activity of tomato extract loaded nano-vesicles, the results suggested that TFS and CLP should be promoted as anti-aging cosmetic. The skin permeation and the skin permeation mechanism of tomato extract loaded nano-vesicles should be confirmed in further study.

Acknowledgements

The authors gratefully acknowledge the Thailand Research Funds and the Office of Higher Education Commission through Grant No. MRG5980260, and RAP59K0019; the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand; the LIPOID GmbH (Cologne, Germany), School of Medical Sciences, University of Phayao and the Global Medical (Thailand) Co., Ltd. for the financial, facilities and chemical support.

Development of Tomato Extract Loaded Nano-Vesicles for Anti-Aging Cosmetic: Physicochemical Characterization, Skin Permeation and Stability Evaluation

Sureewan Duangjit[1,], Warisada Sila-On[1], Utsana Puapempoonsiri[1], Kusuma Jitsaeng[1], Tanasait Ngawhirunpat[2], Sureewan Bumrungthai[3], Tuddao Chuchote[4]*

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Introduction

Lycopene (LYC) is a potential antioxidant commonly occurs in tomato, watermelon, pink grapefruit, apricot and orange. In this study, we focused on the LYC in tomato extract. However, the use of LYC in topical cosmetics is often limited due to its instability, very low water solubility and very low permeability. In order to overcome these limitations, nano-vesicles as liposomes and niosomes are being interested. Liposome technology is a powerful technique as its non-toxic, biocompatible, biodegradable. Liposomes provide the possibility of carrying hydrophilic and hydrophobic compounds. Incorporation of LYC into liposomes has been reported that slowed the oxidation of LYC. The objective of this study was to develop the tomato extract loaded nano-vesicles (liposomes, transfersomes and niosomes and niosomes with oleic acid) for anti-aging cosmetic. The nano-vesicles were prepared by the thin film hydration method. The physicochemical characterizations, skin permeation and stability evaluation of tomato extract loaded nano-vesicles were investigated. The stability of formulations was evaluated and monitoring for 120 days. The vesicle sizes of all tomato extract loaded nano-vesicles were less than 200 nm. The polydispersity index of all formulations was 0.2-0.4. The incorporation of tomato extract in nano-vesicles was at least 100 μg/mL. The skin permeation profile of transfersomes was significantly higher than those of niosomes with oleic acid, liposomes and niosomes, respectively. The physicochemical characteristics of the nano-vesicles at the initial and 120 days after incubation time illustrated significantly different. Considering the physicochemical characterizations, skin permeation and stability of all nano-vesicle formulation, we were successful in showing the feasibility of transdermal delivery of tomato extract loaded nano-vesicles for anti-aging cosmetic. The further study is required to confirm the potential of antioxidant activity of tomato extract loaded nano-vesicles.

Keywords: Tomato extract, Lycopene, Liposomes, Transfersomes, Niosomes

บรรยากาศงานประชุมวิชาการ The 1st International Conference on Natural Medicine (ICNM 2017). 2017 Aug 5-6. The Sukosol Hotel Bangkok, Thailand

Antioxidant Activity of Lycopene Extract Loaded Nano-Vesicles as Antiaging Cosmetics

Sureewan Duangjit[1,*], Warisada Sila-On[1], Utsana Puapempoonsiri[1], Kusuma Jitseang[1], Tanasait Ngawhirunpat[2], Sureewan Bumrungthai[3], Tuddao Chuchoyte[4]

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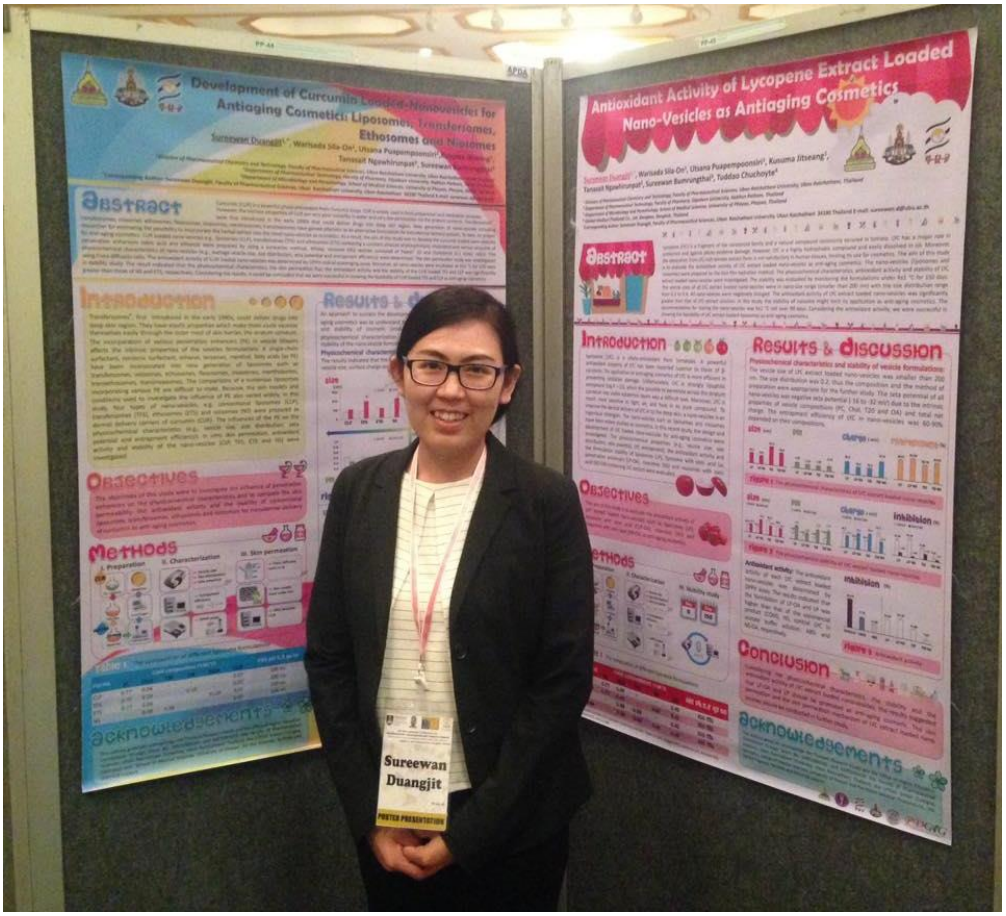
Keywords: Tomato extract, Lycopene, Liposomes, Transfersomes, Niosomes

Lycopene (LYC) is a fragment of the carotenoid family and a natural compound commonly occured in tomato. LYC has a major role in protection and against photo-oxidative damage. However, LYC is a highly hydrophobic compound and easily dissolved in oil. Moreover, the absorption from LYC-rich tomato extract form is not satisfactory in human tissues, limiting its use for cosmetics. The aim of this study is to evaluate the antioxidant activity of LYC extract loaded nano-vesicles as anti-aging cosmetics. The nano-vesicles (liposomes and niosomes) were prepared by the thin film hydration method. The physicochemical characteristics, antioxidant activity and stability of LYC extract loaded nano-vesicles were investigated. The stability was evaluated by monitoring the formulations under 4±1 °C for 150 days. The vesicle sizes of all LYC extract loaded nano-vesicles were in nano-size range (smaller than 200 nm) with the size distribution range from 0.2 to 0.4. All nano-vesicles were negatively charged. The antioxidant activity of LYC extract loaded nano-vesicles was significantly greater than that of LYC extract solution. In this study, the stability of noisome might limit its application as anti-aging cosmetics. The recommendation for storing the nano-vesicles was 4±1 °C not over 90 days. Considering the antioxidant activity, we were successful in showing the feasibility of LYC extract loaded liposomes as anti-aging cosmetics.

บรรยากาศงานประชุมวิชาการ The 5th International Conference on Pharmaceuticals, Nutraceuticals and Cosmetic Science (IPNaCS 2017). 2017 Aug 3-5. The Tawana Hotel, Bangkok, Thailand.

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Antioxidant Activity of Lycopene Extract Loaded Nano-Vesicles as Antiaging Cosmetics
Abstract
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Toshiro Fukami
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Abtract

October 19-20, 2017
Ubon Ratchathani University

Contents

Effect of Penetration Enhancers on Transdermal Delivery of Clotraimazole

Sureewan Duangjit[1,*], Sureewan Bumrungthai[2], Jongjan Mahadlek[3], Naparin Phinphueak[3], Naraporn Sanguanjai[3], Pratanporn Chomsupang[3], Rosawan Thanasomboonpol[3] and Tanasait Ngawhirunpat[3]
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Abstract

It this well known that surfactant, ethanol, terpenes, urea, pyrrolidone and azone can be used as the penetration enhancers for transdermal delivery of several drugs. The objective of this study was to develop antifungal drug-loaded liposome formulation using various types of penetration enhancers. Model formulation of liposomes composed of a constant amount of 0.05% clotrimazole, 10 mM phospholipid, 1 mM cholesterol, 2% Tween® 20 and various amounts of ethanol and terpenes were prepared. The physicochemical properties i.e., vesicle size, size distribution, zeta potential and entrapment efficiency, skin permeation study and antifungal activity of liposomes was evaluated. The snake skin was used a model membrane under 32±1 °C. The standard microbes used in this study were Candida albican. The average vesicle size of liposome formulations was range from 30-140 nm with narrow size distribution 0.1-0.3. The zeta potential was negative (-9 to -26 mV), the entrapment efficiency was up to 90%, the skin permeation flux was 14 to 40 μg/cm^2/h. The antifungal activity was comparable to the commercial product (creams). It could be concluded that antifungal drug-loaded liposome formulation can be successful utilized as transdermal drug delivery.

Keywords: Liposome, Nanovesivles, Ethanol, Antifungal Activity

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ที่อยู่ 85 ถนนสถลมาร์ค
ตำบล/แขวง ตำบลเมืองศรีไค อำเภอ/เขต วารินชำราบ จังหวัด อุบลราชธานี รหัสไปรษณีย์ 34190 ประเทศ ไทย
อีเมล paew2527@yahoo.co.th
☐ เลขประจำตัวประชาชน ☐ เลขทะเบียนนิติบุคคล ☑ เลขประจำตัวผู้เสียภาษีอากร 0 9 9 4 0 0 0 3 3 6 5 0 1 ☐ เพิ่มเติม (ดังแนบ)
ในกรณีที่กรมฯ สื่อสารกับท่าน ท่านสะดวกใช้ทาง ☑ อีเมลผู้ขอ ☐ อีเมลตัวแทน

3.1 สัญชาติ ไทย
3.2 โทรศัพท์ 0 4543 3456
3.3 โทรสาร 0 4543 3456

4. สิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร
☐ ผู้ประดิษฐ์/ผู้ออกแบบ ☑ ผู้รับโอน ☐ ผู้ขอรับสิทธิโดยเหตุอื่น

5. ตัวแทน (ถ้ามี)
ชื่อ
ที่อยู่
ตำบล/แขวง อำเภอ/เขต จังหวัด รหัสไปรษณีย์ ประเทศ
อีเมล
เลขประจำตัวประชาชน ☐ เพิ่มเติม (ดังแนบ)

5.1 ตัวแทนเลขที่
5.2 โทรศัพท์
5.3 โทรสาร

6. ผู้ประดิษฐ์/ผู้ออกแบบผลิตภัณฑ์ ☐ ชื่อและที่อยู่เดียวกันกับผู้ขอ
ชื่อ นางสาวสุรีวัลย์ ดวงจิตต์
ที่อยู่ 5 ถ.สนามจันทร์
ตำบล/แขวง สนามจันทร์ อำเภอ/เขต เมือง จังหวัด นครปฐม รหัสไปรษณีย์ 73000 ประเทศ ไทย
อีเมล sureewan.d@ubu.ac.th
เลขประจำตัวประชาชน 1 3 4 9 8 0 0 0 0 1 2 9 3 ☐ เพิ่มเติม (ดังแนบ)

7. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม
ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ในวันเดียวกับคำขอรับสิทธิบัตร
เลขที่ .... วันยื่น .... เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ
☐ คำขอเดิมมีการประดิษฐ์หลายอย่าง ☐ ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ ☐ ขอเปลี่ยนแปลงประเภทของสิทธิ

หมายเหตุ ในกรณีที่ไม่อาจระบุรายละเอียดได้ครบถ้วน ให้จัดทำเป็นเอกสารแนบท้ายแบบพิมพ์นี้โดยระบุหมายเลขกำกับข้อและหัวข้อที่แสดงรายละเอียดเพิ่มเติมดังกล่าวด้วย

สำหรับเจ้าหน้าที่

จำแนกประเภทสิทธิบัตร/อนุสิทธิบัตร
☐ กลุ่มวิศวกรรม
สิทธิบัตรการประดิษฐ์ (วิศวกรรม)
สิทธิบัตรการประดิษฐ์ (ไฟฟ้า)
สิทธิบัตรการประดิษฐ์ (ฟิสิกส์)
☐ กลุ่มเคมี
สิทธิบัตรการประดิษฐ์ (เคมีเทคนิค)
สิทธิบัตรการประดิษฐ์ (ปิโตรเคมี)
สิทธิบัตรการประดิษฐ์ (เทคโนโลยีชีวภาพ)
สิทธิบัตรการประดิษฐ์ (เภสัชภัณฑ์)

สิทธิบัตรการออกแบบ
☐ สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 1)
☐ สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 2)
☐ สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 3)

อนุสิทธิบัตร
☐ อนุสิทธิบัตร (วิศวกรรม)
☐ อนุสิทธิบัตร (เคมี)

8. การยื่นคำขอนอกราชอาณาจักร ☐ PCT ☐ เพิ่มเติม (ดังแนบ)

วันยื่นคำขอ	เลขที่คำขอ	ประเทศ	สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	สถานะคำขอ
8.1				
8.2				
8.3				

8.4 ☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอสิทธิให้ถือว่าได้ยื่นคำขอนี้ในวันที่ได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรในต่างประเทศเป็นครั้งแรกโดย
☐ ได้ยื่นเอกสารหลักฐานพร้อมคำขอนี้ ☐ ขอยื่นเอกสารหลักฐานหลังจากวันยื่นคำขอนี้

9. การแสดงการประดิษฐ์หรือการออกแบบผลิตภัณฑ์ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรได้แสดงการประดิษฐ์ที่หน่วยงานของรัฐเป็นผู้จัด
วันแสดง ..... วันเปิดงานแสดง ..... ผู้จัด .....

10. การประดิษฐ์เกี่ยวกับจุลชีพ

10.1 เลขทะเบียนฝากเก็บ	10.2 วันที่ฝากเก็บ	10.3 สถาบันฝากเก็บ/ประเทศ

11. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอยื่นเอกสารภาษาต่างประเทศก่อนในวันยื่นคำขอนี้ และจะจัดยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ที่จัดทำเป็นภาษาไทยภายใน 90 วัน นับจากวันยื่นคำขอนี้
โดยขอยื่นเป็นภาษา
☐ อังกฤษ ☐ ฝรั่งเศส ☐ เยอรมัน ☐ ญี่ปุ่น ☐ อื่นๆ .....

12. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้อธิบดีประกาศโฆษณาคำขอรับสิทธิบัตร หรือรับจดทะเบียน และประกาศโฆษณาอนุสิทธิบัตรนี้ หลังจากวันที่ .....
☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอให้ใช้รูปเขียนหมายเลข ..... ในการประกาศโฆษณา

13. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ประกอบด้วย

ก. แบบพิมพ์คำขอ			3	หน้า
ข. รายละเอียดการประดิษฐ์ หรือคำพรรณนาแบบผลิตภัณฑ์			8	หน้า
ค. ข้อถือสิทธิ			2	หน้า
ง. รูปเขียน	3	รูป	2	หน้า
จ. ภาพแสดงแบบผลิตภัณฑ์ ☐ รูปเขียน		รูป		หน้า
☐ ภาพถ่าย		รูป		หน้า
ฉ. บทสรุปการประดิษฐ์			1	หน้า

14. เอกสารประกอบคำขอ
☑ เอกสารแสดงสิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร
☐ หนังสือรับรองการแสดงการประดิษฐ์/การออกแบบผลิตภัณฑ์
☐ หนังสือมอบอำนาจ
☐ เอกสารรายละเอียดเกี่ยวกับจุลชีพ
☐ เอกสารการขอนับวันยื่นคำขอในต่างประเทศเป็นวันยื่นคำขอในประเทศไทย
☐ เอกสารขอเปลี่ยนแปลงประเภทของสิทธิ
☐ เอกสารอื่นๆ

15. ข้าพเจ้าขอรับรองว่า
☑ การประดิษฐ์นี้ไม่เคยยื่นขอรับสิทธิบัตร/อนุสิทธิบัตรมาก่อน
☐ การประดิษฐ์นี้ได้พัฒนาปรับปรุงมาจาก .....

16. ลายมือชื่อ
☐ ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ☐ ตัวแทน
(รองศาสตราจารย์นงนิตย์ ธีระวัฒนสุข)
อธิการบดีมหาวิทยาลัยอุบลราชธานี

หมายเหตุ บุคคลใดยื่นขอรับสิทธิบัตรการประดิษฐ์หรือการออกแบบผลิตภัณฑ์ หรืออนุสิทธิบัตร โดยการแสดงข้อความอันเป็นเท็จแก่พนักงานเจ้าหน้าที่ เพื่อให้ได้ไปซึ่งสิทธิบัตรหรืออนุสิทธิบัตร ต้องระวางโทษจำคุกไม่เกินหกเดือน หรือปรับไม่เกินห้าพันบาท หรือทั้งจำทั้งปรับ