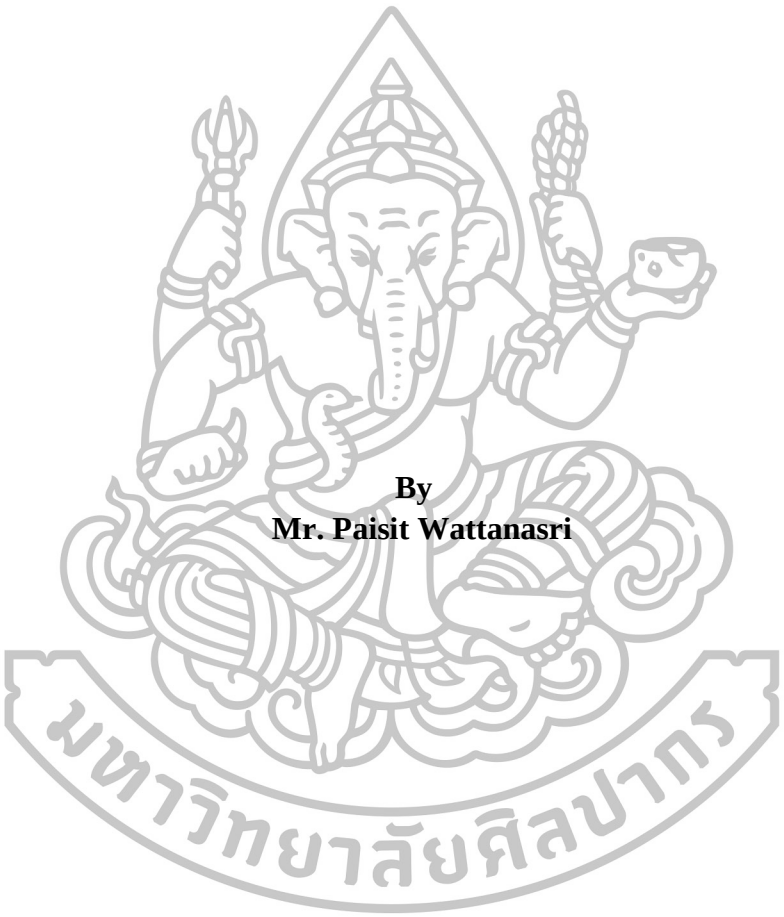
DEVELOPMENT OF MICROEMULSIONS, MICROEMULGELS AND ORGANOGELS FOR TRANSDERMAL DELIVERY OF *Kaempferia parviflora* EXTRACT

By
Mr. Paisit Wattanasri

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree
Master of Pharmacy Program in Pharmaceutical Sciences
Graduate School, Silpakorn University
Academic Year 2016

DEVELOPMENT OF MICROEMULSIONS, MICROEMULGELS AND ORGANOGELS FOR TRANSDERMAL DELIVERY OF *Kaempferia parviflora* EXTRACT



By
Mr. Paisit Wattanasri

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree
Master of Pharmacy Program in Pharmaceutical Sciences
Program of Pharmaceutical Technology
Graduate School, Silpakorn University
Academic Year 2016

การพัฒนาไมโครอิมัลชัน ไมโครอิมัลเจล และออร์แกโนเจลสำหรับนำส่งสารสกัดกระชายดำทางผิวหนัง

โดย

นายไพสิฐ วัฒนศรี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีเภสัชกรรม

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2559

The Graduate School, Silpakorn University has approved and accredited the thesis title of "Development of microemulsions, microemulgels and organogels for transdermal delivery of *Kaempferia parviflora* extract" submitted by Mr.Paisit Wattanasri as a partial fulfillment of the requirements for the degree of Master of Pharmacy in Pharmaceutical Sciences.

……

(Associate Professor Panjai Tantatsanawong, Ph.D.)

Dean of Graduate School

...../...../.....

The Thesis Advisors

1. Associate Professor Praneet Opanasopit, Ph.D.
2. Associate Professor Tanasait Ngawhirunpat, Ph.D.

The Thesis Examination Committee

..... Chairman

(Associate Professor Theerasak Rojanarata, Ph.D.)

...../...../.....

..... Member

(Assistant Professor Warisada Sila-on, Ph.D.)

...../...../.....

..... Member

(Associate Professor Praneet Opanasopit, Ph.D.)

...../...../.....

..... Member

(Associate Professor Tanasait Ngawhirunpat, Ph.D.)

...../...../.....

57364203: MAJOR: PHARMACEUTICAL SCIENCES
KEY WORD: *Kaempferia parviflora* EXTRACT / MICROEMULSION/
MICROEMULGEL / ORGANOGEL / TRANSDERMAL DELIVERY
PAISIT WATTANASRI: DEVELOPMENT OF MICROEMULSIONS, MICROEMULGELS AND ORGANOGELS FOR TRANSDERMAL DELIVERY OF *Kaempferia parviflora* EXTRACT. THESIS ADVISORS: ASSOC. PROF. PRANEET OPANASOPIT, Ph.D. AND ASSOC. PROF. TANASAIT NGAWHIRUNPAT, Ph.D. 107 PP.

The aim of this study was to develop microemulsions (ME), microemulgels and pluronic lecithin organogel (PLO gel) for transdermal delivery of *Kaempferia parviflora* extract (KP extract). The influences of the composition on the characteristics and *in vitro* skin permeation were evaluated. The main methoxyflavone compounds of KP extract contain 5,7-dimethoxyflavone (DMF), 5,7,4'-trimethoxyflavone (TMF) and 3,5,7,3',4'-pentamethoxyflavone (PMF) that were used as markers of KP extract. The ME systems composed of oleic acid, Tween 20, propylene glycol and water were formulated. The formulation consisting of the mixture of surfactant/co-surfactant at the ratio of 1:2 was selected for incorporating 10% KP extract. Limonene was used as the skin enhancer for enhancing the skin permeation of KP extract. 10% limonene incorporating into ME showed the highest total methoxyflavones flux. KP extract-loaded ME with 10 % limonene were selected for incorporating gelling agent to form microemulgels. Among gelling agents, only xanthan gum could form microemulgels. 1.8% w/w xanthan gum had a suitable viscosity for transdermal application. In comparison, the methoxyflavones flux of KP extract-loaded ME was 3.0-fold higher than KP extract-loaded microemulgel. In KP extract-loaded PLO gels, the factors affecting the skin permeation were types of oils, amount of poloxamer 407 and amount of limonene. KP extract-loaded PLO gels containing 10 % KP extract, 10% lecithin, 5% oleic acid, 5% IPM, 5% limonene, 20% poloxamer 407 and 45% water showed the greatest skin permeation flux. In comparison, the skin permeation flux of methoxyflavones was in the order of KP extract-loaded ME with 10 % limonene > KP extract-loaded microemulgels with 10 % limonene > KP extract-loaded PLO gels with 5 % limonene. These formulations showed good stability at room temperature and accelerated condition for 3 months. The results indicated that this ME, microemulgels and PLO gels system had a potential to be the transdermal drug delivery of KP extract.

Program of Pharmaceutical Sciences Graduate School, Silpakorn University
Student's signature..... Academic Year 2016
Thesis Advisors' signature 1. ..... 2. .....

57364203: สาขาวิชาวิทยาการทางเภสัชศาสตร์
คำสำคัญ : ไมโครอิมัลชัน/ไมโครอิมัลเจล/ออร์แกโนเจล/สารสกัดกระชายดำ/นำส่งทางผิวหนัง

ไพสิฐ วัฒนศรี : การพัฒนาไมโครอิมัลชัน ไมโครอิมัลเจล และออร์แกโนเจลสำหรับนำส่งสารสกัดกระชายดำทางผิวหนัง. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : ภญ.รศ.ดร.ปราณีต โอปณะโสภิต และ ภก.รศ.ดร. ธนะเศรษฐ์ ง้าวหิรัญพัฒน์. 107 หน้า.

การวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาตำรับไมโครอิมัลชันไมโครอิมัลเจล และ พลูโรนิค เลซิทิน ออร์แกโนเจล (พีแอลโอเจล) สำหรับนำส่งสารสกัดกระชายดำทางผิวหนัง ศึกษาอิทธิพลของส่วนประกอบในตำรับที่มีต่อคุณลักษณะและการซึมผ่านผิวหนัง โดยสารสกัดกระชายดำมีสารเมทอกซีฟลาโวนเป็นส่วนประกอบหลักประกอบด้วยไดเมทอกซีฟลาโวน (ดีเอ็มเอฟ) ไตรเมทอกซีฟลาโวน (ทีเอ็มเอฟ) และเพนตะเมทอกซีฟลาโวน (พีเอ็มเอฟ) โดยใช้สารเหล่านี้เป็นสารมาตรฐานของสารสกัดกระชายดำ ระบบไมโครอิมัลชันซึ่งประกอบด้วยกรดโอเลอิก ทวีน 20 โพรพิลีนไกลคอล และน้ำ ได้เลือกตำรับที่ประกอบด้วยสารลดแรงตึงผิวและสารลดแรงตึงผิวร่วมที่อัตราส่วน 1:2 เพื่อบรรจุสารสกัดกระชายดำความเข้มข้นร้อยละ10 ลิโมนีนใช้เป็นสารเพิ่มการซึมผ่านทางผิวหนังเพื่อเพิ่มการซึมผ่านของสารสกัดกระชายดำ ไมโครอิมัลชันที่บรรจุสารสกัดกระชายดำและมีลิโมนีนร้อยละ 10 ให้ค่าฟลักซ์ของเมทอกซีฟลาโวนสูงสุด จึงเลือกตำรับนี้มาผสมกับสารก่อเจลเพื่อเตรียมเป็นไมโครอิมัลเจล จากการใช้สารก่อเจลหลายชนิดพบว่ามีเพียงแซนแทนกัมเท่านั้นที่สามารถเตรียมเป็นไมโครอิมัลเจลได้ แซนแทนกัมที่ปริมาณร้อยละ 1.8 มีความหนืดที่เหมาะสมสำหรับใช้ในทางผิวหนัง เมื่อเปรียบเทียบค่าฟลักซ์พบว่าตำรับไมโครอิมัลชันที่บรรจุสารสกัดกระชายดำจะให้ค่าฟลักซ์สูงกว่าตำรับไมโครอิมัลเจลที่บรรจุสารสกัดกระชายดำ 3 เท่า ส่วนสารสกัดกระชายดำในตำรับพีแอลโอเจลนั้นมีปัจจัยที่มีผลต่อการซึมผ่านทางผิวหนังได้แก่ ชนิดของน้ำมัน ปริมาณของพอลอกซาเมอร์ 407 และปริมาณของลิโมนีน ซึ่งสารสกัดกระชายดำบรรจุในพีแอลโอเจลที่ประกอบด้วย สารสกัดกระชายดำร้อยละ 10 เลซิตินร้อยละ 10 ไอโซโพรพิลไมริสเตตร้อยละ 5 กรดโอเลอิกร้อยละ 5 ลิโมนีนร้อยละ 5 พอลอกซาเมอร์ร้อยละ 20 และน้ำร้อยละ 45 ให้ค่าฟลักซ์สูงที่สุด เมื่อเปรียบเทียบค่าฟลักซ์ของทั้ง 3 ตำรับโดยเรียงจากมากไปหาน้อยได้ ดังนี้ สารสกัดกระชายดำบรรจุในไมโครอิมัลชันที่มีลิโมนีนร้อยละ 10 > สารสกัดกระชายดำบรรจุไมโครอิมัลเจลที่มีลิโมนีนร้อยละ 10 > สารสกัดกระชายดำบรรจุในพีแอลโอเจลกับลิโมนีนร้อยละ 5 โดยตำรับเหล่านี้มีเสถียรภาพดีที่สภาวะการเก็บที่อุณหภูมิห้องและสภาวะเร่งในระยะเวลา 3 เดือน การศึกษานี้แสดงให้เห็นว่าตำรับไมโครอิมัลชัน ไมโครอิมัลเจล และพีแอลโอเจลนี้มีศักยภาพในการเป็นระบบนำส่งสารสกัดกระชายดำทางผิวหนังได้

สาขาวิชาวิทยาการทางเภสัชศาสตร์ บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ลายมือชื่อนักศึกษา..... ปีการศึกษา 2559

ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ 1. ..... 2. .....

ACKNOWLEDGEMENTS

I would like to express my deepest sense of gratitude to my advisors, Associate Professor Dr. Praneet Opanasopit for guidance, encouragement and generous help throughout the entire project. She provided me good opportunities and support in various ways. My sincere appreciation also goes to my thesis co-adviser, Assoc. Prof. Dr. Tanasait Ngawhirunpat for his various suggestions and kindness given to me during my study.

I would like to especially thank Mr. Subhachai Saibour, the factory director department manager at Bangkok lab and Cosmetics Co., Ltd. for providing good opportunities and supports my thesis.

I would like to express my sincere gratitude to members in Faculty of Pharmacy, Silpakorn University and especially members of the Pharmaceutical Development of Green Innovation Group (PDGIG) who supported the knowledge, technique, equipment and chemical for my thesis.

I thank my parents and beloved family for all along caring, support and encouragement. Finally, an apology is offered to those whom I cannot mention personally one by one here.

Table of Contents

Page

LIST OF TABLES

Tables **Page**

LIST OF TABLES

Tables **Page**

LIST OF FIGURES

Figures **Page**

LIST OF FIGURES

Figures **Page**

LIST OF FIGURES

Figures **Page**

List of Abbreviations

%	percentage
%RH	percent relative humidity
˚C	degree Celsius
<	less than
>	more than
®	registered trademark
cm	centimeter (s)
cm^2	square centimeter
DLS	dynamic light scattering
DMF	5,7-dimethoxyflavone
et al.	and others
etc.	et cetera (Latin); for example, such as
FDA	Food and Drug Administration
g	gram (s)
hr	hour (s)
k	kilo (s)
kg	kilogram (s)
KP	*Kaempferia parviflora* Wall. Ex Baker
L	liter (s)
M	molar (s)
ME	microemulsions
mg	milligram (s)
min	minute (s)
mL	milliliter (s)
mRNA	messenger ribonucleic acid
MW	molecular weight
nm	nanometer (s)
PBS	phosphate-buffered saline
PDI	polydispersity index

pH	potentia hydrogenii (latin); power of hydrogen
PLO gel	pluronic lecithin organogel
PMF	3,5,7,3’,4’-pentamethoxyflavone
rpm	revolutions per minute or rounds per min
R^2	coefficient of determination
SD	standard deviation
S cm^{-1}	siemen per centimeter
TMF	5,7,4’-trimethoxyflavone
UPLC	Ultra Performance Liquid Chromatography
v/v	volume by volume
w/v	weight by volume
w/w	weight by weight
β	beta
µg	microgram(s)
µS/cm	microsiemen per centimeter

CHAPTER 1
INTRODUCTION

1.1 Rational and problem statement

Kaempferia parviflora Wall. Ex Baker (KP), a plant of Zingiberaceae family, is grown in the northern and northeastern region of Thailand. It is commonly referred as “Krachaidum” or “Black Ginger”. This plant is a perennial herb with dark purple to black rhizomes and these colors lead to its name. Its rhizome has been used as a folk medicine for the treatment of a wide variety of illnesses. There are many therapeutic functions of KP that have been reported in the antimicrobial, aphrodisiac effect, anti-gastric ulcer, antidepressant, anticholinesterase activity, anti-obesity effects, vasodilator and antioxidant effects [1, 2]. In addition, KP extract demonstrated the efficacy of anti-inflammatory through the inhibition of nitric oxide and prostaglandin E_2 release in RAW 264.7 macrophage cells and decreased carrageenan-induced rat paw edema [3, 4, 5]. Therefore, KP extract is an interesting choice of herbal medicine product for anti-inflammatory effect.

Chemical constituents in KP are discovered at least 11 methoxyflavones analyzed by using gas chromatography [6]. The main methoxyflavone compounds of KP extract contain 5,7-dimethoxyflavone (DMF), 5,7,4’-trimethoxyflavone (TMF) and 3,5,7,3’,4’-pentamethoxyflavone (PMF) that are used as markers of KP extract. Nevertheless, these compounds are low water solubility, high lipophilicity and low bioavailability (about 1-4%) [7, 8]. The physicochemical properties of methoxyflavones are suitable for developing transdermal delivery in order to improve its therapeutic effects [7, 9]. Therefore, transdermal delivery systems are good choice to increase the effectiveness of KP.

Microemulsions are transparent systems composed of two immiscible phases stabilized by the mixture of surfactant and co-surfactant. They are widely used in lipophilic drugs, thermodynamically stable and high potential for skin permeation

enhancement. Some studies reported the design of transdermal delivery system with improving drug permeation for low water solubility and high lipophilicity [10]. Moreover, the microemulsion significantly enhanced the permeation of drug compared to conventional formulation such as solutions, gels or creams. However, low viscosity of microemulsion and less retention capacity in the skin restrain its application in the pharmaceutical industry. To overcome this disadvantage, gelling agents such as carbopol 940, xanthan gum and poloxamer have been added into the microemulsion for forming microemulgel in order to increase its viscosity which could be suitable for topical application [11].

Tavano L et al. reported that microemulsions were a possible matrix for transdermal delivery of capsaicin. These microemulsions were prepared from a mixture of surfactants (Tween 80 and Span 80) to be applied topically. They were prepared using a particular ratio between surfactants, to obtain systems with a specific HLB (10, 12 and 14). Microemulsion systems were comprised of isopropyl myristate, tween 80/span 80, ethanol and water. *In vitro* skin permeation study was conducted using rabbits. The results demonstrated that microemulsions showed an enhancing effect for transdermal delivery. The capsaicin cumulative permeated amount of microemulsions was 2-fold higher than capsaicin hydroalcoholic solution (60:40) [12]. Fouad SA et al. prepared microemulgel as a topical delivery system for 1.3% diclofenac epolamine. Microemulsions system was composed of 30% Capryol®, 50% Surfactant mixture (Labrasol®/Transcutol®, 1:2 w/w) and 20% water. The results of permeation test *ex vivo* in mice showed that the cumulative amount of diclofenac from microemulsion and microemulgel were all significantly higher than that of the commercial gel [13].

Organogel is a semi-solid system in which an organic liquid phase is immobilized by a three-dimensional network. It is thermodynamically stable, viscoelastic bi-phasic systems comprising of a gelator and a nonpolar phase, with or without the presence of water molecules within the network formed by the gelator system [14]. Lecithin organogel have gained high interest in pharmaceutical science. Lecithin has the ability to form different shapes owing to its amphiphilic structure. Reverse micelles are formed when small amounts of organic solvent are added to

lecithin. Cylindrical reverse micelles start growing, upon the addition of polar solvents until they intertwine to form a gelling network [15]. In recent years, pluronic lecithin organogel (PLO gel) have been attracted particular interest in the design of transdermal delivery system with improving drug permeation through the skin. PLO gel is used for the transdermal delivery of both hydrophilic and lipophilic drugs that is produced when an aqueous solution of Pluronic F127 ™ (Poloxamer 407) was added to the lecithin organogel. PLO gel have been studied for the topical delivery of several other hydrophilic and hydrophobic drugs including anesthetics, antiemetics, muscle relaxants, neuropathy drugs, nonsteroidal anti-inflammatory drugs and system analgesics. The PLO gel system facilitates the delivery of hydrophilic as well as lipophilic drugs owing to the presence of both oil and aqueous phases within the gel system [16, 17].

Therefore, the aim of the study was to develop microemulsions, microemulgels and organogels for transdermal delivery of KP extract. KP extract-loaded microemulsions were formulated with different compositions of oils, surfactants and co-surfactants. The suitable KP extract-loaded microemulsion formulation was selected to formulate KP extract-loaded. KP extract-loaded PLO gels were formulated with different amount of poloxamer and compositions of the lipid matrices of PLO gels. These microemulsions, microemulgels and organogels were prepared and their physicochemical properties, percent content, stability and *in vitro* skin permeation were also investigated.

1.2 Objective of this research

1.2.1 To develop microemulsions, microemulgels and organogels for the transdermal delivery of KP extract.

1.2.2 To investigate the influence of the formulation compositions on physicochemical properties (i.e. droplet size, size distribution, electrical conductivity and viscosity) and *in vitro* skin permeation enhancement.

1.3 The research hypothesis

1.3.1 The microemulsions, microemulgels and organogels can be potentially used for a transdermal delivery of KP extract.

1.3.2 The formulation compositions influence on the physicochemical properties and in vitro skin permeation enhancement.

CHAPTER 2
LITERATURE REVIEWS

2.1 *Kaempferia parviflora*

2.1.1 Pharmacokinetic

2.1.2 Pharmacological effect

2.1.3 Toxicity

2.1.4 Development of KP products

2.2 Microemulsion

2.2.1 Structure of microemulsion

2.2.2 Component of microemulsion

2.2.3 Phase diagram of microemulsion

2.2.4 Advantages of microemulsion

2.2.5 Microemulsion for transdermal drug delivery

2.3 Microemulgel

2.4 Organogel

2.4.1 Classifiaction of organogel

2.4.2 Lecithin organogel

2.4.3 Pluronic lecithin organogel

2.1 *Kaempferia parviflora*

Kaempferia parviflora Ex Baker (KP) or Krachaidam is a traditional herbal from the family Zingiberaceae. This plant is a perennial herb that can reach up to 25 cm, tall. The leaves are 7-20 cm long, 4-9 cm wide thin, rounded at the base, plain green. Flowers are few in a sessile central tuft which has white with purple colors in the middle of labarum. The rhizomes are subglobose to globose and light to dark purple (Figure 2.1) [18]. KP is distributed over an area from India, Myanmar, Thailand and Laos [19].

Traditionally in Thailand, KP is known as an energy enhancer with excellent tonic effect. KP products have been used in different preparations such as fresh or dried rhizomes, dried powder in tea bag and wine. Its rhizome has been used as a folk medicine for the treatment of a wide variety of illnesses. Recently, there are many therapeutic functions of KP that have been reported in the antimicrobial, aphrodisiac effect, anti-gastric ulcer, antidepressant, anticholinesterase activity, anti-obesity, vasodilation effect and antioxidant effect. Traditional medicines of KP is approved by Thai FDA that they include pill, capsule, tablet, powder and essence tincture. Furthermore, there are many dosage forms that are developed including lozenge effervescent tablet, transdermal patch, spray and gel for new dosage forms of KP [1].

Many chemical constituents in KP are discovered such as flavonoids, phenolic glycosides, volatile oil, glyceroglycolipids and sphingoglycolipid. Recently, new glycosides in KP is isolated including kaempferiaosides A-F [20, 21]. The flavonoids are the major component at least 11 methoxyflavones as shown in Figure 2.2. However, the content of methoxyflavones of KP extract is analyzed by using gas chromatography. The main constituents of KP extract contain DMF, TMF and PMF which have high content in this plant [6]. Nevertheless, these constituents have problem about low solubility and low bioavailability [7, 8].

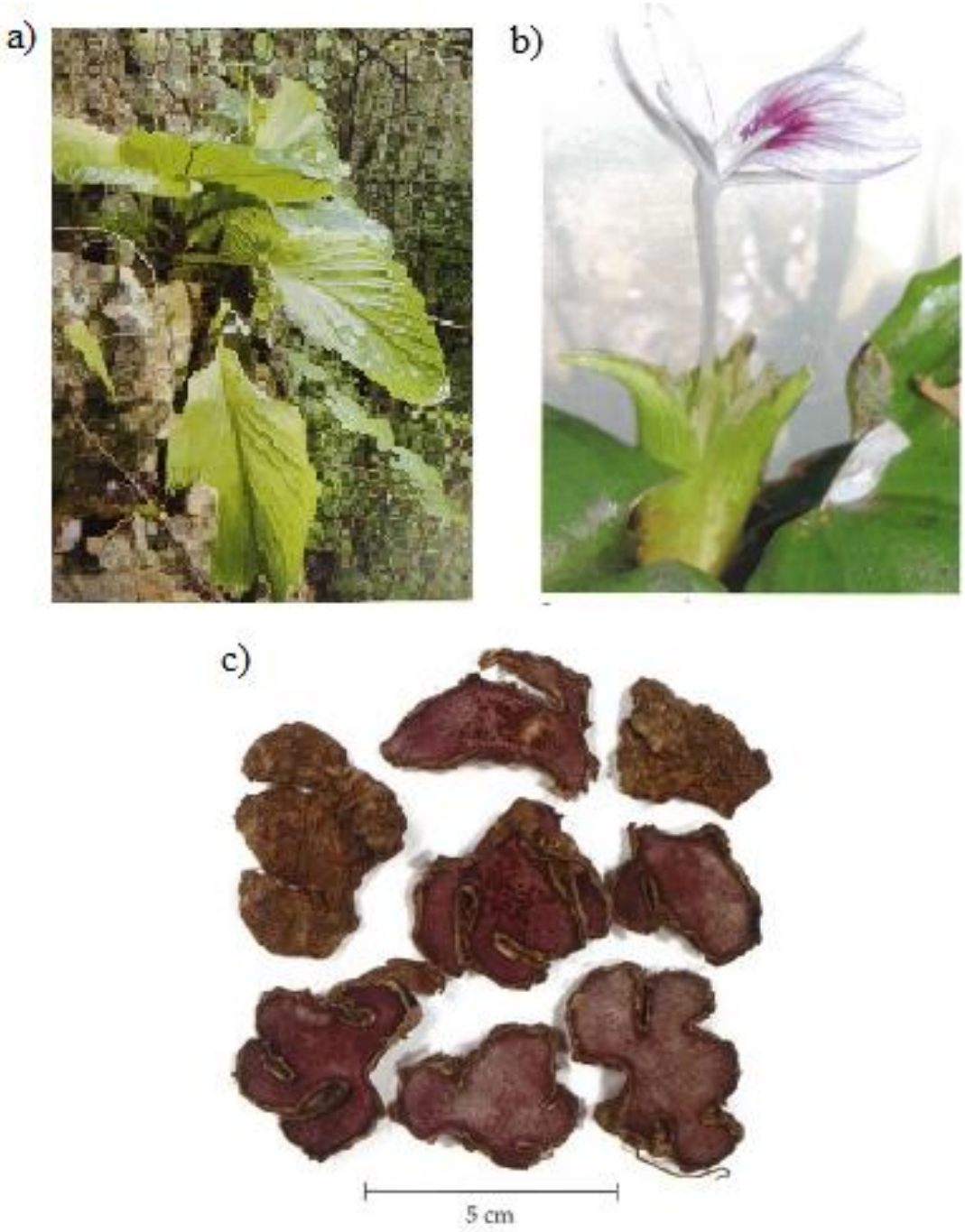
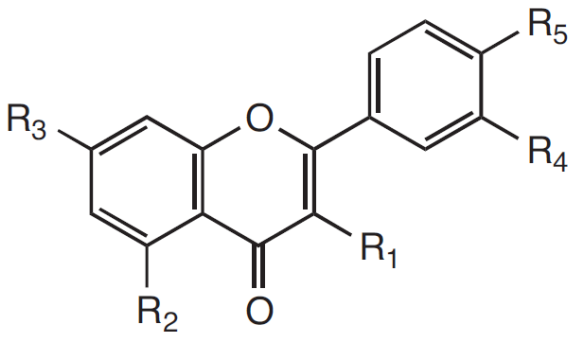


Figure 2.1 *Kaempferia parviflora* Ex Bake.

a) Plant b) Flower c) Rhizomes

Source: Department of Medical Sciences. **Thai Herbal Pharmacopoeia 2009 volume III.** Bangkok : Prachachon Co.,Ltd.



	R1	R2	R3	R4	R5
5-hydroxy-7-methoxyflavone	H	OH	OCH_3	H	H
5-hydroxy-3,7-dimethoxyflavone	OCH_3	OH	OCH_3	H	H
5,7-dimethoxyflavone (DMF)	H	OCH_3	OCH_3	H	H
3,5,7-trimethoxyflavone	OCH_3	OCH_3	OCH_3	H	H
5- hydroxy -3,7,4’-trimethoxyflavone	OCH_3	OH	OCH_3	H	OCH_3
5- hydroxy -7,4’-dimethoxyflavone	H	OH	OCH_3	H	OCH_3
5- hydroxy -3,7,3’,4’-tetramethoxyflavone	OCH_3	OH	OCH_3	OCH_3	OCH_3
5,7,4’-trimethoxyflavone (TMF)	H	OCH_3	OCH_3	H	OCH_3
3,5,7,4’-tetramethoxyflavone	OCH_3	OCH_3	OCH_3	H	OCH_3
5,7,3’,4’-tetramethoxyflavone	H	OCH_3	OCH_3	OCH_3	OCH_3
3,5,7,3’,4’-pentamethoxyflavone (PMF)	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3

Figure 2.2 Chemical structure of KP.

Source: Sutthanut K, Sripanidkulchai B, Yenjai C, Jay M. “Simultaneous identification and quantitation of 11 flavonoids constituents in *Kaempferia parviflora* by gas chromatography.” **Journal of Chromatography A** 1143, 1-2 (March 2): 227–233.

2.1.1 Pharmacokinetic

Mekjaruskul et al. reported pharmacokinetics of methoxyflavones (DMF, TMF and PMF) of KP ethanolic extract including bioavailability, distribution, excretion and identification of metabolites after administration in rats, as shown in Table 2.1. After oral administration, pharmacokinetic studies of KP extract demonstrated low oral bioavailability (about 1–4 %). Three methoxyflavones achieved the maximum concentration within 1–2 h after administration and their $T_{1/2}$ were 3–6 h. The concentration of three methoxyflavones was detected at their highest levels in liver followed by kidney lung, testes, and brain. The major compounds of KP extract were mainly excreted through urine in the form of demethylated, sulfated and glucuronidated products and as demethylated metabolites in the feces. [8].

In addition, the metabolism of KP extract was found to induce several hepatic cytochrome P 450 enzymes on mice including CYP1A1, CYP1A2, CYP2B and CYP2E1. Nevertheless, KP extract did not affect the CYP3A enzyme activity [22].

Table 2.1 Pharmacokinetic parameters and oral bioavailability of methoxyflavones after single oral or intravenous administration of 250 mg/kg KP in rats

PK Parameters	Route	PMF	TMF	DMF
AUC (h ·µg/ml)	Oral	3.65 ± 0.63**,***	6.96 ± 1.11***	7.01 ± 1.37***
	IV	76.77 ± 19.50**	275.66 ± 86.06	233.48 ± 71.57
$t_{1/2}$ (h)	Oral	3.12 ± 1.34	5.04 ± 1.10	5.85 ± 1.72
	IV	2.36 ± 1.89	4.19 ± 1.45	3.75 ± 1.01
K_e (h^{-1})	Oral	0.28 ± 0.17***	0.15 ± 0.04***	0.13 ± 0.03***
	IV	0.61 ± 0.22*	0.32 ± 0.12	0.32 ± 0.11
Cl (mL/h)	Oral	622.85 ± 114.86*	337.00 ± 62.17	367.28 ± 82.35
	IV	21.56 ± 7.18*,***	6.30 ± 2.17***	7.98 ± 2.34***
T_{max} (h)	Oral	1.71 ± 0.36*	0.85 ± 0.40	0.76 ± 0.40
C_{max} (µg/ml)	Oral	0.55 ± 0.05**	0.88 ± 0.11	0.78 ± 0.11
V_d (ml)	Oral	2637.13 ± 846.59	2385.10 ± 364.37	2957.53 ± 458.19
K_a (h^{-1})	Oral	1.23 ± 0.54	8.53 ± 3.64	8.69 ± 2.33
Bioavailability (%)		3.32	1.75	2.10

Data are expressed as mean ± standard deviation. (n = 10). IV, intravenous.

* Polyethylene glycol 400: significant higher than the others in the same route at $p < 0.05$.

** Significance lower than the others in the same route, $p < 0.05$.

*** Significance lower than the other route.

Source: Mekjaruskul C, Jay M, Sripanidkulchai B. "Pharmacokinetics, bioavailability, tissue distribution, excretion, and metabolite identification of methoxyflavones in *Kaempferia parviflora* extract in rats." **Drug Metabolism Disposition** 40, 12 (December): 2342–2353.

2.1.2 Pharmacological effect

In recent, KP has been shown many pharmacological effect**,** such as anti-inflammation, aphrodisiac, anti-obesity, antimicrobial, anti-peptic ulcer and anti-allergic. In currently, several pharmacological effects of KP and their components are proven by scientist.

2.1.2.1 Anti-inflammation

Tewtrakul et al. reported effects of compounds from KP extract on nitric oxide (NO), prostaglandin E_2 (PGE_2) and tumor necrosis factor-alpha (TNF-α) productions in RAW264.7 macrophage cells. 7 compounds of KP extracts were isolated, and the result showed that 5-hydroxy-3,7,3',4'-tetramethoxyflavone exhibited the highest activity against NO release (IC_{50} = 16.1 μM). Furthermore, this compound showed appreciable inhibitory effect on PGE_2 release (IC_{50} = 16.3 μM) but a mild effect on TNF- α (IC_{50} > 100 μM) in RAW 264.7 [3]. Peerapattana et al. reported efficacy of KP gel for reduced rat paw edema. KP gel containing KP ethanolic extract 10 % and 20 % decreased rat paw edema in the efficacy nearby 1% diclofenac and 0.1 % betamethasone [1].

The effects of KP extract on carrageenan-induced rat paw edema and inflammatory mediators were reported by Sae-wong et al. KP extracted with ethanol and further partitioned with hexane, ethyl acetate, chloroform and water fractions decreased paw edema at 3-5 h. The chloroform and hexane fractions showed higher effect on decrease of paw edema than that of indomethacin. Furthermore, KP ethanolic extract and 5-hydroxy-3,7,3',4'-tetramethoxyflavone decreased cellular iNOS mRNA level but inhibitory effect on COX-2 mRNA expression was partly affected [4]. Subsequently, Sae-wong et al. found that DMF, TMF and PMF inhibited the production of NO, TNF-α, and they strongly inhibited expression of iNOS mRNA in a dose-dependent [5].

Horigome et al. found that DMF and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone showed inhibition of the production and mRNA expression of inflammatory mediators such as TNF-α, IL-4 and MCP-1. Furthermore, these compounds inhibited the secretion mechanisms of inflammatory mediators rather than the pathway from Ca^{2+} influx to transcriptional activation of inflammatory mediators [23].

2.1.2.2 Aphrodisiac effect

Sudwan et al. demonstrated that KP ethanolic extracts in the dose of 60, 120 and 240 mg/kg body weight (BW) treated to adult male rats induce significantly high courtship behavior, especially the first 10-minutes period of observation [24]. Chaturapanich et al. reported the ethanol, hexane and aqueous extracts of KP showed no effect on the reproductive organ and sperm motility even in 5 week male rats. This study found that a single oral administration of KP ethanolic extract at a dose of 70 mg/kg significantly decreased mount and ejaculatory latencies and increased blood flow to the testis. Whereas, hexane and water extracts had no influence on sexual behavior parameters [25]. Temkitthawon et al. reported the effect of KP for PDE5 inhibitory activity. They found DMF, TMF, PMF and 3,5,7-trimethoxyflavone showed PDE5 inhibitory activities with IC_{50} values of 10.64, 37.38, 30.41 and 16.32 μM, respectively [26]. Wattanathorn et al. reported sexual performance of the KP ethanolic extract that was observed after treatment rats at dose of 200 mg/kg BW for 2 weeks. They found that rats treated with KP ethanolic extract significantly increased the frequency of mounting, intromission and ejaculation. Nevertheless, the extract decreased the latencies of all sexual behaviors [27].

Recently, Horigome et al. reported effect of DMF on testosterone production in mouse testis-derived tumour cells. They found that DMF enhanced testosterone production via cyclic AMP (cAMP) binding protein signal. In particular, DMF inhibited activation of phosphodiesterase [28].

2.1.2.3 Anti-obesity

Akase et al. reported the efficacy of KP in preventing obesity using Tsumura, Suzuki, Obese Diabetes (TSOD; obesity, glucose/lipid metabolism abnormalities) mice. KP powder-containing at 1.0 and 3.0 % given in TDOD mice for 8 weeks

significantly decreased mice body weight, visceral fat, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and insulin [29]. Similarly, Shimada et al. found KP extract decreased body weight and visceral fat. In addition, KP extract and its component showed an inhibitory effect on pancreatic lipase [30].

Horikawa et al. found that KP extract induced differentiation of 3T3-L1 cells. TMF and PMF of KP strongly promoted differentiation of 3T3-L1 preadipocytes to adipocytes through the transcriptional activation of peroxisome proliferator-activated receptor γ (PPAR γ) without PPAR γ ligand activity in a dose-dependent manner. Furthermore, TMF and PMF induced an increase in the mRNA expression levels of adipose triglyceride lipase and hormone-sensitive lipase that enhanced adipocyte lipolysis in lipid droplets [31].

2.1.2.4 Antimicrobial

KP has recently been reported to possess antiplasmodial, antimycobacterial and antifungal agent (Yenjai et al., 2004). 3,5,7,4'-Tetramethoxyflavone and TMF of KP extract possessed antifungal activity against *Candida albicans* with IC_{50} values of 39.71 and 17.63 mg/ml, respectively. Furthermore, TMF and 5,7,3',4'-tetramethoxyflavone of KP extract exhibited antiplasmodial activity against *Plasmodium falciparum* with IC_{50} values of 3.70 and 4.06 mg/ml, respectively [32]. Kummee et al. found that KP ethanolic extract exhibited strong anti-fungal activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum gypseum* with MIC values of 62.5, 125 and 250 mg/ml, respectively [33].

Chaichanawongsaroj et al. reported anti-*Helicobacter pylori* of KP extract. *Helicobacter pylori* were inhibited by KP extract composing of volatile oil, hexane, ethyl acetate and methanol. All extracts except volatile oil showed significant antibacterial activity. The MICs of hexane, ethyl acetate and methanol were 64, 32 and 64 μg/ml, respectively [34].

2.1.2.5 Anti-allergic

Tewtrakul et al. reported anti-allergic activity of KP compounds. Generally, β-hexosaminidase was used as a marker of degranulation in RBL-2H3 cell. This study

found that KP compounds inhibited β-hexosaminidase released. 5-hydroxy-3,7,3',4'-tetramethoxyflavone possessed the highest anti-allergic activity with an IC_{50} value of 8.0 μM [35].

Kobayashi et al. found that 5-hydroxy-3,7,4'-trimethoxyflavone and 5,3'-dihydroxy-3,7,4',-trimethoxyflavone showed potent inhibitory activities of degranulation in RBL-2H3 cells. These compounds were related to the suppression of degranulation due to Ca^{2+} influx, phosphorylation of Syk and PLCγ1, and translocation of FcεRI to the cell surface [36].

2.1.2.6 Others

Rujjanawate et al. reported anti-gastric ulcer activity of KP ethanolic extract. Oral administration of the KP ethanolic extract at 30, 60 and 120 mg/kg significantly inhibited gastric ulcer formation induced by indomethacin, HCl/ethanol and water immersion restraint-stress in rats. Furthermore, KP ethanolic extract at doses of 60 and 120 mg/kg significantly increased gastric mucus but had no effect the inhibition of gastric acid secretion [37].

Wattanapitayakul et al. reported effect of KP ethanolic extract on endothelial function. KP ethanolic extract at 10 μg/ml significantly enhanced nitric oxide production in human umbilical vein endothelial cells which nitric oxide plays significant role in maintaining normal vascular function, preventing cardiovascular disease and dilation of blood vessel. Furthermore, eNOS mRNA and protein expression were significantly enhanced by KP extract at dose of 1 and 10 μg/ml [38].

Nakao et al. reported anti-hyperuricemia of 4 Zingiberaceae plant extracts on the inhibitory xanthine oxidase. This study found that KP extract showed the most potent activity (38 % Inhibition). Furthermore, KP compounds revealed that PMF was the most potent with IC values of 4.0 mM, followed by 5,7,3'4'-tetramethoxyflavone with IC values of 0.9 mM [39].

2.1.3 Toxicity

Chivapat et al. (2004), the first group who reported acute toxicity and chronic toxicity of KP powder. They showed that LD_{50} of KP powder was more than 13.33 g/kg BW. At this dose, no abnormal histopathological changes were found in various

internal organs. Chronic toxicity study has been performed in male and female rats. KP powder at the doses of 20, 200, 1,000 and 2,000 mg/kg/day BW were orally given for 6 months. The result revealed that all KP powder dose showed no difference on body weight and internal organs when compared with control group. Nevertheless, rats receiving with 2,000 mg/kg BW of KP powder showed significant liver weight gain, lower body weight and less eosinophil. In female rats, cholesterol level significantly increased compared with the control group [40].

Sudwan et al. reported chronic toxicity study of KP ethanolic extract (Ethanol 50%) in rats at doses of 60, 120, and 240 mg/kg BW for 60 days. All dosages had no significant difference of complete blood count, alanine aminotransferase, blood urea nitrogen and creatinine. Nevertheless, there was morphological change in the liver in histopathological study. Hemoglobin of rats receiving doses of 60 mg/kg was significantly lower than the control and the highest-dose groups [24].

In 2010, Chivapat et al. studied chronic toxicity of KP ethanolic extract (Ethanol 95%) for six months in the rats orally given KP extract (doses of 5, 50 and 500 mg/kg/day). The rats obtained KP extract at dose of 500 mg/kg BW showed less food consumption and lower body weight when compared with control group. The increase of heart, lung, liver stomach and kidney related with weight was found in the highest dose–treated male and female rats. Additionally, the decrease of eosinophil was found in both males and females treated with the highest dose; however these alterations of a few hematological parameters were within the normal range. Histopathological study of visceral organs revealed no remarkable lesions related to the toxicity of KP extract [41]

Yorsin et al. (2014) found that no signs of toxicity were reported in the middle-aged rats after having been oral administration with the KP extract (dichloromethane) at 100 mg/kg BW twice a day for 6 weeks. There were no differences in the organ weights or abnormal gross observation of the internal organs between the KP extracts treated groups. Furthermore, the serum liver and kidney enzyme levels were in the normal range [42].

2.1.4 Development of KP products

The main constitutes of KP demonstrate hydrophobic and low oral bioavailability which limits the KP products. Therefore, there are many scientific researches to overcome these limitations.

Suthanut et al. developed solid nanoparticle (SLN) for transdermal of KP extract. The best formulation of KP-SLN was composed of stearyl alcohol (Oil), tocopheryl polyethylene glycol succinate (Surfactant) and PEG6000MS (PEGylating agent) which showed entrapment efficiencies of 87%. In the permeability study, KP-SLN was compared with KP-hydroxypropyl methylcellulose gel. The amount of methoxyfavones through the skin of KP-SLN was significantly more than KP gel. Furthermore, the flux values of DMF, TMF and PMF were greater when incorporated in SLN [7].

Mekjaruskul et al. developed methoxyflavones (DMF, TMF and PMF) of KP extract by complexation with 2-hydroxypropyl-β-cyclodextrin (HPβCD) and self-microemulsifying drug delivery system (SMEDDS) to increase oral bioavailability. KP- HPβCD was prepared using freeze-dry technique. SMEDD of KP extract composed of polyoxyethylene castor oil (53.3%), propylene glycol (26.7%) and triglyceride of coconut oil (20%). The bioavailability values of PMF, TMF and DMF of KP-HPβCD complex were higher than that of KP solution by 21.63, 34.20, and 22.90-times, respectively. For KP-SMEDDS, the oral bioavailability values were greater than those of KP solution by 25.38, 42.00, and 26.01-times for PMF, TMF, and DMF, respectively [43].

Tuntiyasawasdikul et al. developed monolithic transdermal patch of KP extract using acrylic polymer Durotak® 87-2852 (adhesive polymer). The 13 KP patches were prepared with different ingredient loading and permeation enhancers. The skin permeation was studied using porcine ear skin. Oleic acid could be a suitable enhancer for transdermal delivery of methoxyflavones; whereas menthol shortened the lag time. In this study, KP patch comprising oleic and menthol mixture showed the maximum flux of methoxyflavones and minimum lag time [44].

2.2 Microemulsion

Schulman & Hoar (1943) are the first group who explored a clear single-phase solution by titrating milky emulsion with hexanol. In 1959, Schulman et al. described these systems as “optically isotropic transparent oil and water droplet” [10].

Microemulsion is optically transparent and thermodynamically stable dispersion systems of at least three components including oil, water and surfactant frequently in combination with a co-surfactant which the co-surfactant created sufficiently the interfacial tension between oil and water for almost spontaneous formation of the microemulsion systems. Microemulsion is formed along with various association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion). There are many differences between microemulsion and emulsion such as appearance (emulsions are opaque and microemulsion are transparent), formation, droplet size (about 500 times smaller), interfacial tension (about 100 times lower), surface area and stability (Table 2.3). Furthermore, emulsion is kinetically but microemulsion is thermodynamically stable. This is because the microemulsions interfacial tension is lowered so that the inter-facial energy becomes very small and, the free energy of droplet formation is lower than zero [10, 45].

Table 2.2 Characteristics of emulsion and microemulsion.

Parameter	Microemulsion	Emulsion
Formation	Spontaneous	Mechanical agitation
Stable	Thermodynamically	Kinetically
Droplet size (μm)	0.01–0.10	0.5–50
Appearance	Transparent	Opaque (cloudy)
Surface area	High	Low
Interfacial tension	Ultra low	Low
Surfactant concentration	High	Low

Source: Burguera JL, Burguera M. “Analytical applications of emulsions and microemulsions.” **Talanta** 96: 11-20.

2.2.1 Structure of microemulsion

The structure of microemulsion is classified as three types (Figure 2.3) which are most likely to be formed depending on composition. They are divided into oil-in-water (o/w), water-in-oil (w/o) and bicontinuous microemulsion. In w/o microemulsion, water droplets are dispersed in oil phase. Conversely, o/w microemulsion is formed when oil droplets are dispersed in water phase. In addition, bicontinuous microemulsion is similar between volume of water and oil. All three types of microemulsion, the interface is stabilized by appropriate combination of surfactants and co-surfactants. Therefore, the mixture of oil, water and surfactants is able to form a wide variety of structures and phases depending upon the proportions of the components [10, 45].

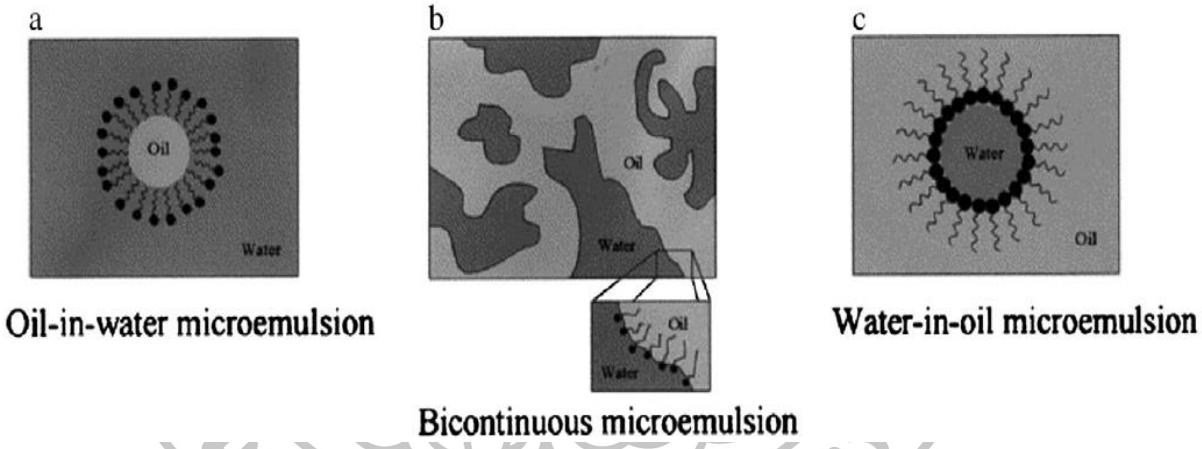


Figure 2.3 The structure of microemulsion:
(a) oil-in-water, (b) bicontinuous and (c) water-in-oil microemulsion.

Source: Lawrence MJ, Rees GD. "Microemulsion-based media as novel drug delivery systems." **Advanced Drug Delivery Reviews** 64, Supplement (December): 175–193.

2.2.2 Component of microemulsion

A large number of materials are available which can be used as components of microemulsion systems but their toxicity and irritation are the limitation. Therefore, researchers have to choose materials that are biocompatible, non-toxic and clinically acceptable [46].

2.2.2.1 Oil Phase

The oil phase should be selected appropriately, since it governs the selection of the other ingredients for the microemulsion. There are two main factors to be considered before selecting the appropriate oil phase. At first, the solubilizing potential of the oil for the selected drug, in order to assure maximum solubilization of the drug. At second, the oil is chosen, such that the microemulsion forming region is enhanced. In addition, Flangan and Singh et al. found that long-chain oils were difficult to be used in producing microemulsion. Due to long chain oils had low polarity, high interfacial tension and high viscosity. Therefore, short chain oils, such as medium-chain triglycerides or medium chain mono- and di-glycerides are easier to form microemulsion compared with oils with long hydrocarbon chains. The criterion for selecting the oil is that the drug should have high solubility in microemulsion. This will minimize the amount of the formulation to deliver the therapeutic dose of the drug.

2.2.2.2 Surfactant

Surfactants are molecules consisting of a hydrophilic head group attached to a hydrophobic tail. They can reduce interfacial tension between the oil and water in sufficiently concentrations and surfactants that form a monolayer, with the hydrophobic tails of the surfactant oriented towards the oil phase and the hydrophilic head groups towards the aqueous phase. Therefore, criterion for selecting the surfactant is that surfactant is able to decrease the interfacial tension to a very small value which facilitates dispersion process during the preparation of the microemulsion and provide a flexible film that can readily deform around the droplets and the appropriate lipophilic character to provide the correct curvature at the interfacial region. In addition, the hydrophilic-lipophilic balance (HLB) of the surfactant can be taken into account to try to rationalize the surfactant’s behavior. It is generally accepted that a surfactant with HLB from 3-6 will favor the formation of w/o microemulsion, whereas surfactants with HLB from 8-18 are preferred for o/w microemulsions.

2.2.2.3 Co-surfactant

Sometimes, co-surfactant is required to be used for the formation of microemulsion. Generally, co-surfactant has some functions of (a) reducing the interfacial tension (b) increasing the flexibility and fluidity of the interfacial tension (c) optimizing the ratio of dispersed to continuous phase viscosity (d) supporting solubilize poorly soluble compounds (e) adjusting the curvature of the interfacial film. Ethanol is very common co-surfactant in among formulations and its addition is enhanced the flux of several drugs. Furthermore, it has a significant effect on interfacial tension, with short-chain alcohols increasing interfacial tension (flexibility). However, toxicity concerns limit the use of effective co-surfactants. Therefore, appropriate combination of surfactant and co-surfactant in microemulsion must be selected.

2.2.2.4 Aqueous phase

The aqueous phase can have both preservatives and hydrophilic active ingredients. Many studies use the buffer solutions as the aqueous phase. The most commonly used aqueous phase is water.

2.2.3 Phase diagram of microemulsion

The formation of microemulsion requires an adequate amount of the components (oil, surfactant, co-surfactant and water). Determination of these proper amount and compositions are the important problem to obtain the microemulsion. In 1960, Ekwall et al found pseudo ternary phase diagram for microemulsion preparation that was used to identify the microemulsion regions and optimize the concentration of oil, surfactant and co-surfactant (Figure 2.4) [10, 47].

The common technique construct pseudo-ternary phase diagram is titration method. The diagram consists of oil, surfactant and/or co-surfactant and water at each corner of the diagram represents 100% of vehicle. This technique can be constructed by mixing the oil component with surfactant and co-surfactant components. Aqueous components can be added gradually to the mixture of oil containing surfactant and co-surfactant components. Pseudo-ternary phase diagram is often constructed to find the different zones including microemulsion zone for microemulsion preparation [47].

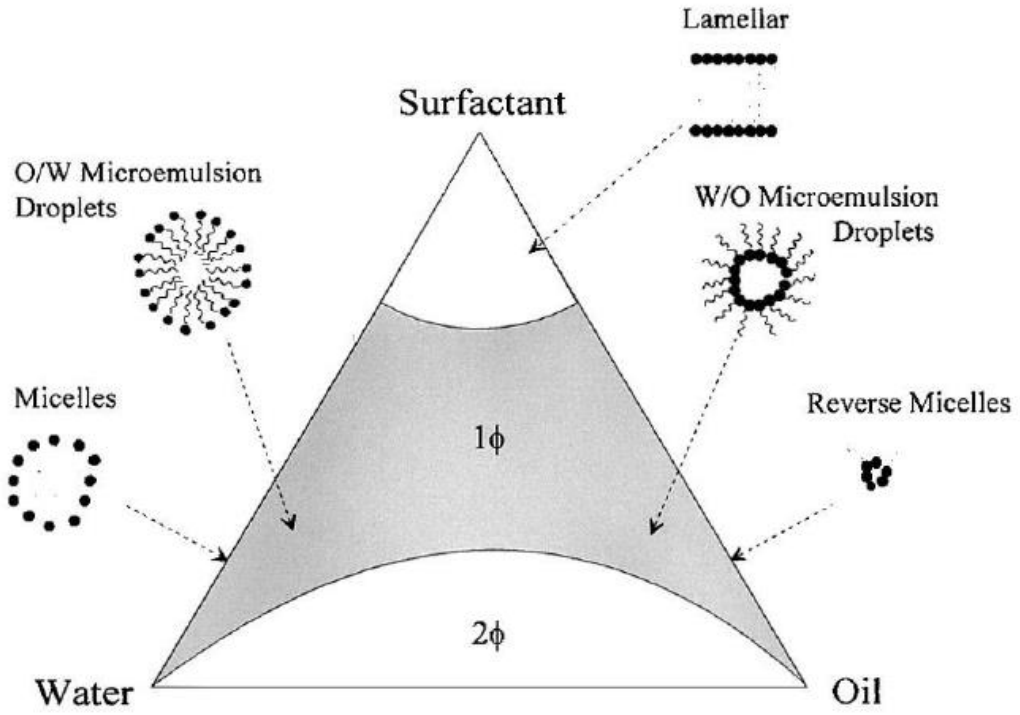


Figure 2.4 Pseudo-ternary phase diagram of oil/surfactant (and co-surfactant)/water system with microemulsion and emulsion phases. Within the phase diagram, existence fields are shown where conventional micelles, reverse micelles or w/o microemulsions and o/w microemulsions are formed along with the bicontinuous microemulsions.

Source: Lawrence MJ, Rees GD. "Microemulsion-based media as novel drug delivery systems." **Advanced Drug Delivery Reviews** 64, Supplement (December): 175–193.

2.2.4 Advantages of microemulsion

The basic property of microemulsion is developed for many applications such as the delivery of food, cosmetic, pharmaceutical, as well as in petrochemical recovery. For pharmaceutical application, microemulsions have a higher solubilization capacity for both hydrophilic and hydrophobic drug. Microemulsions have shown great potential in the area of pharmaceuticals. They can be applied to a wide variety of dosage forms including oral, topical, ocular, parenteral, periodontal, buccal, and nasal formulations. Because of the ease of microemulsion preparation,

drugs are easily incorporated without the risk of degradation [48]. In drug delivery, microemulsions increase the surface area of drugs, which improves their solubilization and permeation behavior. They are shown to increase solubility and bioavailability of low solubility and low permeability [46, 49]. Plasma concentration profiles and drug bioavailability have been shown to be more reproducible in microemulsion formulations [10, 49].

2.2.5 Microemulsion for transdermal drug delivery

Transdermal drug delivery offers many advantages over other routes of drug delivery. These include avoidance of the hepatic first pass metabolism, ease of administration with a good control over the rate of drug delivery. In addition, it allows for immediate termination of therapy when needed. Nevertheless, the barrier nature of the skin makes it difficult for most drugs to be delivered into and through it. Stratum corneum is made up of keratin-rich dead cells embedded in a lipid matrix that makes it highly impermeable. Microemulsions provide another promising alternative for dermal and transdermal delivery of both hydrophilic and lipophilic drugs that allow for high loading capacities. Microemulsions have high solubilizing power, which significantly affect this layer of the skin, allowing for increased drug permeation [46, 50]. The microemulsion components can possibly enter into the skin as monomers [51], increasing the solubility of the drug in the skin. Moreover, the microstructure of the system provides high surface area for drug transfer and diffusion through in the skin [52].

Components of the transdermal microemulsions have many ingredients that were defined as permeation enhancers for increase of permeability of drug in microemulsions. Saturated and unsaturated fatty acids serving as an oil phase are used as permeation enhancers. The most popular enhancer is oleic acid. Other enhancers commonly used in transdermal formulations are isopropyl myristate, isopropyl palmitate, d-limonene and medium chain triglycerides. The most popular among the enhancing permeability surfactants are phospholipids that have been shown to enhance drug permeation in a different constituent. The phosphatidylcholine are in a fluid state may diffuse into the stratum corneum and enhance dermal and transdermal drug penetration [46]. Other very commonly used surfactants are tween 20, tween 80,

span 20 and Trancutol® P. For co-surfactants commonly used polyethylene glycol, ethanol and propylene glycol. Furthermore, Che et al. showed synergistic effect of microemulsion combined with HPβCD complex. They found that the combination exhibited significantly synergistic effect on deposition and penetration through the skin [53].

2.3 Microemulgel

Emulgel has combined advantages of gel and microemulsion. Generally, gel is composed of high cross-linkage networks based, containing hydrophilic drugs that are affected for preparation highly hydrophobic drugs. Emulsion provides better stability and bioavailability for hydrophobic drugs. They also have a high ability to penetrate the skin. Subsequently, both microemulsion and gel are used in combination dosage forms the prepared formulations called microemulgel. Microemulgel has the advantages of both gel and microemulsion. It provides high surface area for drug absorption and increases the bioavailability by improving permeability of drugs [54].

Generally, microemulsion exhibits low viscosity and less retention capacity in the skin. Therefore, many studies use gelling agents such as carbomer, hydroxypropyl methylcellulose (HPMC), poloxamer and gums to increase the appropriate viscosity of microemulsion. Spiclin et al. showed the rheological behavior of gelling agents for the increase of viscosity of microemulsion. This study found that xanthan gum and colloidal silicon dioxide could increase viscosity of microemulsions. Nevertheless, the gelling agents: HPMC, methyl cellulose and sodium alginate characterized phase separation and ethyl cellulose affected sedimentation after observed for 24 h [55]. Bachhav et al. evaluated sodium alginate, HPMC and carbopol ETD 2020 for their ability to be microemulsion base gel. They found that sodium alginate separated the oily phase in the microemulgel. HPMC was unable to form gels. Only carbopol ETD 2020 could form clear gel without disturbing the microstructure of the microemulsion. Furthermore, microemulgel in this study showed significantly higher *in vitro* bioadhesion and anti-fungal activity as compared to that of commercial market gel [56].

In addition, microemulgels incorporated microemulsion systems enhanced solubility of both hydrophilic and lipophilic drugs when compared to conventional

gel. Therefore, they can enhance skin permeation and increase retention capacity in the skin for improvement of transdermal drug delivery. Fouad et al. reported that diclofenac epolamine loaded microemulsion based gel has sustained release capability and enhanced skin permeation [13]. Patel et al. reported clobetasol propionate loaded microemulsion based gel showed better minimal irritation potential than microemulsion and commercial market formulation. Subsequently, they demonstrated higher retention after topical application of microemulsion based gel when compared with gel and market formulation containing the same drug in the rat skin [11, 57]. Wan et al. reported *in vitro* and *in vivo* studies between pseudolaric acid B gel and microemulgel. *In vitro* permeation investigation showed that microemulgel significantly enhanced drug permeation through and into the skin. For *in vivo* dermatopharmacokinetics study using microdialysis confirmed that microemulgel significantly improved the dermal bioavailability compared with the gel [58].

2.4 Organogel

Gels are defined as semi-solid formulations comprising of an external solvent phase, either polar or non-polar in nature, that is immobilized within the spaces of a three dimensional network. Organogel has been included into formulations with various applications in pharmaceutical, food and cosmetic. Generally, gels are basically classified into two types depending upon the nature of the solvent phase as organogel and hydrogel [14].

Hydrogels are three-dimensional hydrophilic polymer networks that can absorb large quantities of water. In pharmaceutical, hydrogels have a wide range of applications that especially use for drug carriers. They show a resemblance to natural living tissues in terms of their water content and soft texture due to the high water content of hydrogel contributing to their biocompatibility. Furthermore, hydrogels are widely used as contact lenses, membranes for biosensors, linings for artificial hearts, materials for artificial skin, and as drug delivery devices [59].

Organogels are thermodynamically stable, visco-elastic bi-phasic systems comprising of a gelator and a nonpolar phase, with or without the presence of water molecules within the network formed by the gelator system. They have a lower degree of hydration, when compared with hydrogels. The organogel systems exhibit

morphological and rheological properties similar to solids even though they are composed of large amounts of liquid. The thermodynamic and kinetic stability of these systems can be attributed to the opposing forces which are operating and are associated with the organogelator solubility in the external phase. The physicochemical properties of gel components and their resulting interactions govern the formation of the gelling matrix. Organogels are potential drug delivery systems using in transdermal application and drug reservoir in a transdermal patch. Advantages of this organogel include the capacity to accommodate hydrophilic and lipophilic drugs, thermoreversibility, high degree of stability to moisture and temperature and the ability to control drug release [60].

2.4.1 Classifiaction of organogel

Generally, gels can be classified based on the properties of gelator, solvent and intermolecular interactions which can occur in gels as shown in Figure 2.5. Organogels can be distinguished from hydrogels by their predominantly organic external phase and can be further subdivided based on the molecular weight of the gelator including low molecular weight organogelator (LMWO) and polymeric organogelator. Polymers immobilize the organic solvent by forming a network of either cross-linked or entangled chains for chemical and physical gel [14].

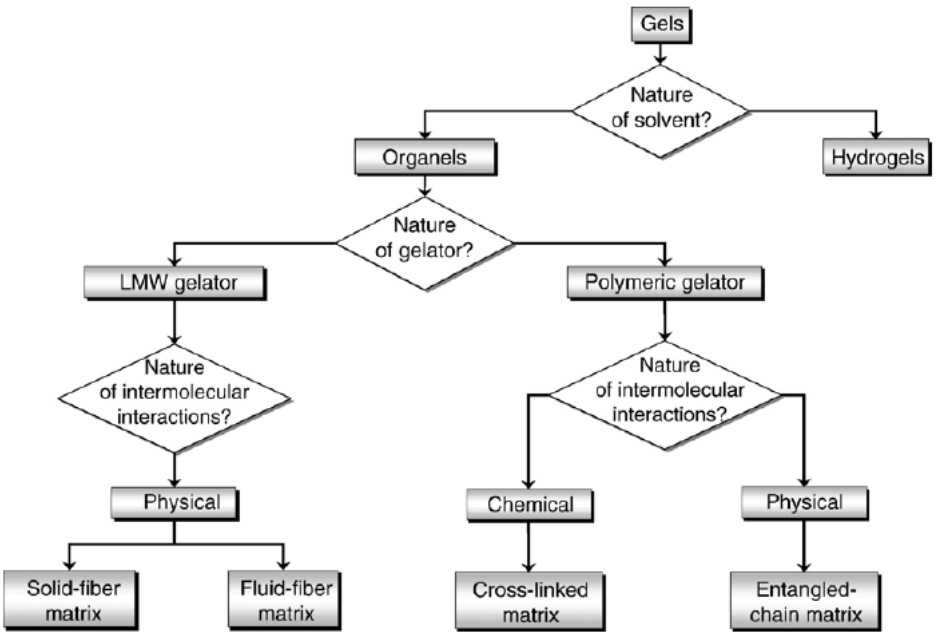


Figure 2.5 Organogel classifications.

Source: Vintiloiu A, Leroux JC. “Organogels and their use in drug delivery.” **Journal of Controlled Release** 125, 3 (February 11): 179-192.

2.4.1.1 Low molecular weight organogelator

LMWO have been defined as organic molecules, with molecular weight lower than 3,000 g/mol that self-assemble in certain solvents forming three-dimensional networks via non covalent bonds such as hydrogen bonds, van der Waals interactions, π-stacking, electro-static interactions. The self-assembly of LMWO depends on physical interactions for the formation of aggregates which are sufficiently long to overlap and induce solvent gelation. The important distinction of LMWO is made between those composed of entangled fiber networks of solid and fluid matrix organogel.

2.4.1.1.1 Solid-matrix organogel

Solid-matrix organogels can form solid network exhibiting a solid-like, viscoelastic, mechanical behavior as a result of their thermoreversibility or the existence of T_{gel} and T_{melt} (melting temperature or gel-to-sol phase transition temperature). Their gelators are L-alanine fatty acid derivatives, 12-hydroxystearic acid, and hexatriacontane. They are prepared by the dissolution of a gelator in a hot solvent. As the temperature falls, the affinity between the gelator and the solvent molecules decreases. The gelator assembles itself into solid aggregates by intermolecular interactions. Solid-matrix organogels are more robust due to their permanent solid-like networks in which the junction points are relatively strong structure of organogel as shown in Figure 2.6. [14, 61].

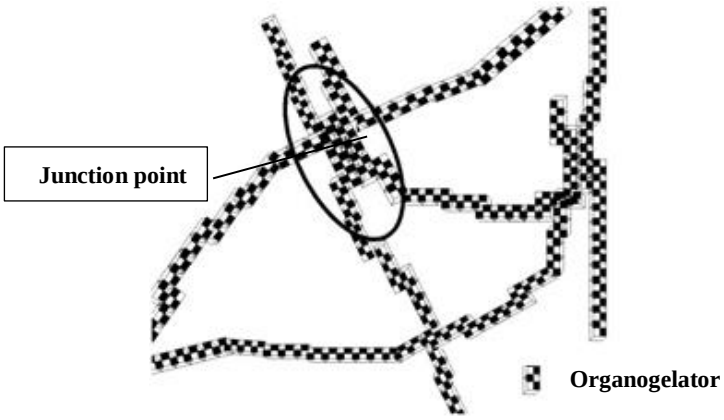


Figure 2.6 Solid-matrix organogels.

Source: Vintiloiu A, Leroux JC. “Organogels and their use in drug delivery.”
Journal of Controlled Release 125, 3 (February 11): 179-192.

2.4.1.1.2 Fluid-matrix organogel

These are thermo reversible gels which may be transparent or opaque. They are made of transient three dimensional networks exhibiting a liquid-like viscoelastic behavior. Fluid matrices are formed as a result of the reorganization of surfactant molecules into mono- or bilayer cylindrical aggregates upon the incorporation of polar solvents into organic solutions of surfactants. The transient networks are characterized by the continuous breaking and recombination of the constituent rods, and are also referred to as “worm-like” or “polymer-like” networks as shown in Figure 2.7. Some gelators of this organogel are lecithin and sorbitan monostearate organogels that have wide range of pharmaceutical applications [14, 62].

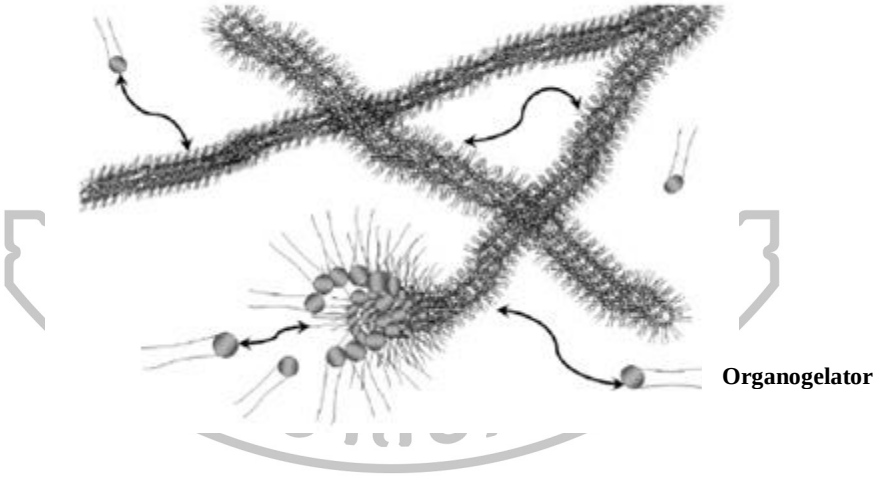


Figure 2.7 Fluid-matrix organogels.

Source: Vintiloiu A, Leroux JC. “Organogels and their use in drug delivery.”
Journal of Controlled Release 125, 3 (February 11): 179-192.

2.4.1.2 Polymeric organogel

Polymeric organogels are high molecular weight molecules of organogelators that are capable of gelling organic solvent by the formation of crosslinking. They can be of different shapes including linear to hyper branched and star-shaped, with similar

characteristics. Most commonly used polymeric gelators include poly (ethylene) organogels, copolymers of methacrylic acid and methyl methacrylate, etc. Poly (ethylene) organogels are generally used as ointment bases, while the later materials are used in the preparation of organogels and sustained release formulations for rectal administration [14].

As lecithin organogels are effective and safety for transdermal delivery system, they have gained an increasing interest in pharmaceutical. Therefore, this literature review is focusing on lecithin organogel.

2.4.2 Lecithin organogel

The first description of lecithin organogel was given in an article published by Scartazzini and Luisi in 1988. They investigated the suitable conditions for soy lecithin to form reverse micelles. In these experiments, water was added to various organic solutions of purified soybean lecithin. It was observed that addition of trace amounts of water into organic solution of soy lecithin caused immediate increase in viscosity, producing a transition of the initial non viscous solution into a gel or jelly-like state. Subsequently, lecithin organogels have been studied extensively in many laboratories worldwide with regard to their various aspects lecithin organogel and have also been proposed for transdermal drug delivery [15].

Lecithin organogels are thermodynamically stable, clear, viscoelastic, biocompatible and isotropic gels. They can form as gel-like reverse micellar system showing three dimensional networks of entangled micelles, which mobilizes the external phase. Lecithin organogels are very promising in transdermal delivery owing to their amphiphilic and solubilizing properties, possibility of dissolution of both hydrophilic and lipophilic drugs as well as their permeation enhancing properties. Furthermore, they are able to control the delivery of drugs, minimize toxic effects, and are particularly appropriate for the treatment of cutaneous pathologies [63].

2.4.2.1 Composition of lecithin organogel

Lecithin organogel is composed of lecithin, which acts as a gelling agent and non-polar organic solvent as external phase and polar agent (usually water). Lecithin

is well known as the phospholipids, which are biocompatible and biodegradable emulsifier of lipids, and a cellular component. It mainly consists of phosphatidyl choline, phosphatidyl thanolamine, phosphatidyl serine and phosphatidyl inositol combined with different amounts of triglycerides and fatty acids. Lecithin can self-assemble to build up the microstructure of organogel. It contributes in the development of reverse micelle structures and furthermore converts these micelles to three-dimensional networks. In addition, the purity of lecithin plays a critical role in the organogel formation. Poorly purified lecithin does not possess gel-forming properties, and it has been demonstrated that lecithin should contain at least 95% phosphatidylcholine content for the preparation of organogels.

Organic solvent demonstrated in organogel uses for solubilizing drug as well as lecithin, and enhancing skin penetration. Many organic solvents are available for lecithin organogel including ethyl laureate, ethyl myristate, isopropyl myristate, isopropyl palmitate, cyclopentane, cyclooctane, n-pentane, n-hexane, n-hexadecane and tripropylamine.

Polar agents act as a structure forming and stabilizing agent have a very crucial role to play in the process of gelling. Water is the most commonly employed polar agent, although some other polar solvents such as glycerin, ethylene glycol and formamide have also been found to possess the capability of transferring an initial nonviscous lecithin solution into a jelly-like state of organogel [15, 63].

2.4.2.2 Mechanism gelation of lecithin organogel

The gelation of the lecithin solution in organic solvent is induced as a result of the incorporation of a polar agent (usually water). Lecithin tends to be self-assemble into spherical reverse micelles in organic solvent. The enormous micelle growth of these spherical reverse micelles was formed and subsequently transformed into tubular or cylindrical micelle aggregates triggered by the addition of small and critical amounts of polar agent. This formation is resulted from hydrogen bonds of polar solvent and phosphate group of lecithin molecules. After reaching a critical length, these extended micelles begin to overlap, entangle themselves, and build up a transient three-dimensional network in lecithin organogel as shown in Figure 2.8 [15].

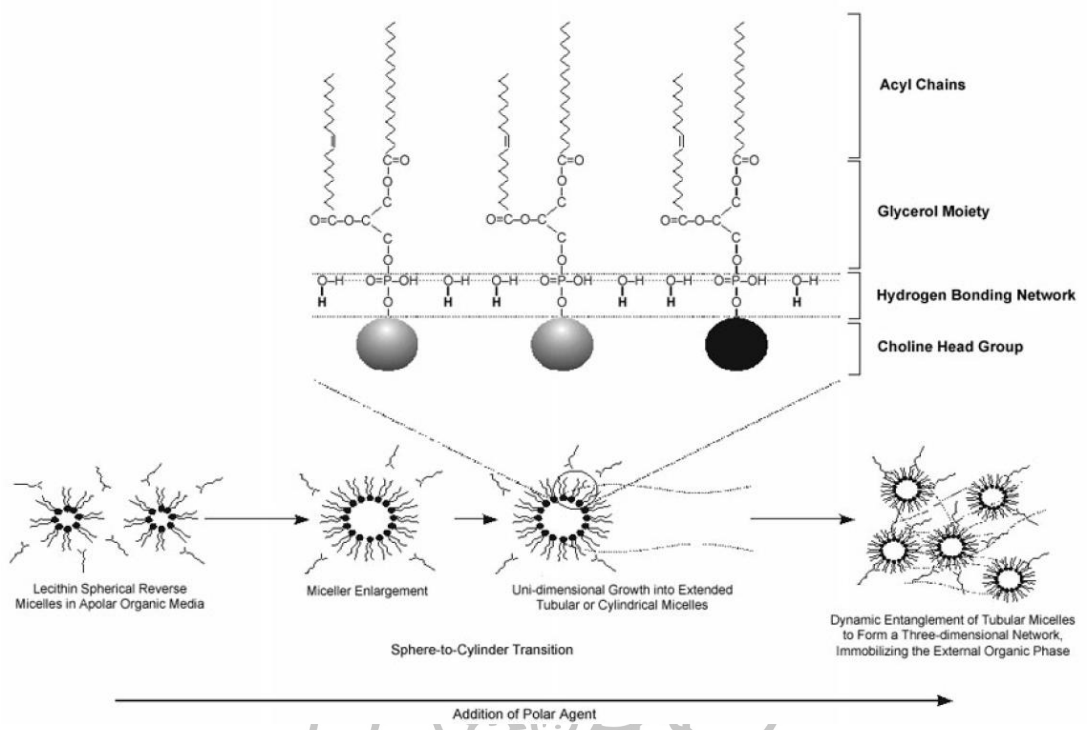


Figure 2.8 Formation of a three-dimensional network of lecithin organogel.

Source: Kumar R, Katare OP. "Lecithin Organogels as a potential phospholipid-structured system for topical drug delivery: A review." **American Association of Pharmaceutical Scientists** 6, (October): 298-309.

2.4.2.3 Lecithin organogel for transdermal drug delivery

Lecithin organogels are particularly interesting due to their ability to solubilize drug with different physiochemical properties, their thermodynamic stability and their biocompatibility. They can be used in pharmaceutical and cosmetic applications such as vitamins, hormones, NSAIDS, peptides, amino acids, local anesthetics and antifungal agents that can be efficiently carried for transdermal delivery. In addition, the pemeation enhancer property of lecithin brought lecithin organogel to be a potential as transdermal delivery, and to promote skin permeation and partitioning of the drug into the skin layers. New lecithin organogel called pluronic lecithin organogel (PLO gel) for improving skin permeation and stabilizing the formulation has been recently reported in the literature [15, 63].

2.4.3 Pluronic lecithin organogel

PLO gel was introduced by Jones and Kloesel (1990). It is a biphasic system consisting of an oil phase (lecithin dissolved in isopropyl myristate or isopropyl palmitate in a 1:1 ratio) and a water phase containing 20-30% pluronic F127. It is a thermodynamically stable, visco-elastic system, which is non-irritating, odorless and biodegradable [17, 64].

Pluronic F127 or poloxamer 407 is a copolymer of polyoxyethylene and polyoxypropylene which forms a thermoreversible gel in concentrations between 15-30% w/v. Poloxamer exists in a liquid state at refrigerated conditions ($4^{o}C$) and forms a gel at room or body temperature. Water plays the role of a structure-forming agent and stabilizes the process of gel formation as it solubilizes the poloxamer and other hydrophilic drugs [65].

Agrawal et al. reported the comparison of skin permeation between lecithin organogel and PLO gel of sumatriptan. They showed that drug permeated in PLO gel was more than lecithin organogel, because poloxamer in PLO gel disrupts lipid layers of the stratum corneum [66].

Boddu et al. reported permeation between transdermal eyelid delivery of PLO gel and commercial eye drop. They reported that the efficiency of PLO gel was significantly higher than commercial eye drop. Furthermore, they found PLO gel also used for sustained release drug delivery [67].

In addition, PLO gels have been studied for the transdermal delivery of several hydrophilic and hydrophobic drugs including anesthetics, antiemetics, antipsychotic, calcium channel blockers, hormones and opoids. The PLO gel system facilitates the delivery of hydrophilic as well as lipophilic drugs owing to the presence of both oil and aqueous phases within the gel system [16, 17].

CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

3.2 Equipments

3.3 Methods

3.3.1 Preparation of KP extract

3.3.2 UPLC analysis

3.3.3 Preparation of KP extract-loaded microemulsions

3.3.3.1 Solubility studies of KP extract

3.3.3.2 Construction of pseudo-ternary phase diagrams

3.3.3.3 KP extract-loaded microemulsions

3.3.4 Preparation KP extract-loaded microemulgels

3.3.5 Preparation KP extract-loaded PLO gels

3.3.6 The percentage of total methoxyflavones content in formulations

3.3.7 Physicochemical characterization

3.3.7.1 Electrical conductivity measurement

3.3.7.2 The average droplet sizes measurement

3.3.7.3 pH measurement

3.3.7.4 Rheological measurement

3.3.8 Skin permeation studies

3.3.9 Stability evaluation

3.3.10 Statistical analysis

3.1 Materials

3.1.1 Acetronitrile HPLC grade (LabScan, Bangkok, Thailand)

3.1.2 Butylene glycol (KH Neo ChemCo., Ltd, Japan)

3.1.3 Caprylic/capric triglyceride (KLK Oleo, Malaysia)

3.1.4 Carbomer (Carbopol 940; Lubrizol, USA)

3.1.5 Ethoxydiglycol (Transcutol® CG; Getteffosse, France)

3.1.6 Formic acid (Thermo Fisher Scientific, Leicester, UK)

3.1.7 Hydroxyethyl cellulose (Dow Chemical, USA)

3.1.8 Hydroxypropyl methyl cellulose (Methocel™ 15V; Dow Chemical, China)

3.1.9 Isopropyl palmitate (Nikkol, Singapore)

3.1.10 Isopropyl myristate (KLK Oleo, Malaysia)

3.1.11 Lecithin (Emulmetik® 900; Lucas Meyer Cosmetics, France)

3.1.12 Oleic acid (Sigma Aldrich, MO, USA)

3.1.13 Poloxamer 407 (Kolliphor® P 407 BASF, Germany)

3.1.14 Polyoxyethylene castor 35 oil (Cremophor EL®; BASF, Germany)

3.1.15 Polyoxyethylene castor 40 oil (Cremophor RH 40®; BASF, Germany)

3.1.16 Polysorbate 20 (Lonza, USA)

3.1.17 Porcine skin (obtained from the new born porcines that died naturally from porcine farm in Ratchaburi)

3.1.18 Propylene glycol (SKC Co.,Ltd, Korea)

3.1.19 d-limonene (97%) (Sigma Aldrich, MO, USA)

3.1.20 Reverse osmosis (RO) water

3.1.21 Triethanolamine (Ajax Finechem, Australia)

3.1.22 Xanthan gum (Danisco Landerneau, France)

3.1.23 Water HPLC grade (RCI Labscan, Thailand)

3.2 Equipments

3.2.1 Analytical balance (Sartorious CP224S and CP3202S, Thailand)

3.2.2 Beaker (Pyrex, USA)

3.2.3 Brookfield Programmable DVIII+ Digital Rheometer (Brookfield Engineering Laboratories, USA)

3.2.4 Column UPLC (ACQUITY UPLC BEC C18 analytical column 5 mm, 2.1 mm x 100 mm, 1.7 µm, Waters, USA)

3.2.5 Electrical conductivity meter (Metler Toledo, Sevencompact S230)

3.2.6 Filter set and filter membrane 0.22 µm

3.2.7 Franz diffusion cell

3.2.8 Magnetic stirrer (Framo, Germany) and Magnetic bar

3.2.9 Microcentrifuge tube 1.5 ml (Eppendorf® tubes)

3.2.10 Micropipette (20-200 µl, 100-1000 µl, 1-5 ml) and micropipette tip

3.2.11 pH meter (Metler Toledo, Sevencompact S220)

3.2.12 Refrigerator 4 °C and -20 °C

3.2.13 Shaking incubator (GFL 3031)

3.2.14 Ultra-Performance Liquid Chromatography (UPLC) WATERS®

3.2.15 Vortex mixer (Labnet, USA)

3.2.16 Zetasizer Nano ZS (Malvern Instruments, Malvern, UK)

3.2 Methods

3.3.1 Preparation of KP extract

The dried rhizomes of KP were purchased from Loei province, Thailand. The rhizomes were grounded and passed through a sieve until powder was obtained. KP rhizome powder was extracted by percolation with ethanol. The solvent was removed under reduced pressure at 50 ºC until the KP extract was obtained. The amount of methoxyflavones in KP extract was measured by using Ultra-Performance Liquid Chromatography (UPLC).

3.3.2 UPLC analysis

The total methoxyflavones (Sum as: DMF, TMF and PMF) concentration was analyzed by UPLC. All samples were stored at 4 ºC until analyzed. The UPLC system was composed of ACQUITY UPLC Core system (Waters Corporation, Milford, USA) binary solvent management and 2 switching solvent/DEGAS/ACQUITY TUV

Detector and column heater. The UPLC column was ACQUITY UPLC BEC C18 analytical column, 2.1 mm x 100 mm, 1.7 µm (Waters®).

Chromatographic condition:

3.1 Mobile phase	:	Acetronitrile : 0.5 % formic acid in water
3.2 Flow rate	:	0.5 ml/min
3.3 Injection volume	:	2.0 µL
3.4 UV detector	:	335 nm
3.5 Column temperature	:	38 ºC
3.6 Sample temperature	:	25°C

3.3.3 Preparation of KP extract-loaded microemulsions

3.3.3.1 Solubility studies of KP extract

The solubility of KP in various oils (Caprylic/capric triglyceride, isopropyl myristate (IPM), isopropyl palmitate (IPP) and oleic acid), surfactants (Tween 20, polyoxyethylene castor 35 oil (Cremophor® EL) and polyoxyethylene castor 40 oil (Cremophor® RH40)) and co-surfactants (Propylene glycol (PG), ethoxydiglycol and butylene glycol) was studied in order to screen the suitable compositions of microemulsions. Each experiment was performed in triplicate. An excess amount of KP extract was added to 5 mL of water, oils, surfactants or co-surfactants. The mixtures were shaken at 30±2°C for 48 h. Then the mixtures were centrifuged (9,000 rpm, 15 minutes) and supernatants were collected. The concentrations of PMF, TMF, and DMF in supernatants were quantified by using UPLC.

3.3.3.2 Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature to obtain the concentration range of the components for microemulsions. The microemulsion system was composed of oil, surfactant, co-surfactant and water. The surfactant/co-surfactant weight ratio was 1:1, 2:1, 1:2 and 1:3, respectively. For each phase diagram, the mixtures of oil and surfactant/co-surfactant were prepared at volume ratios of 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10, respectively. Water was added drop by drop, under gentle

magnetic stirring, to each oily mixture. After being equilibrated, the mixtures were assessed visually and determined as being microemulsions.

After the microemulsion regions in the phase diagram were identified, the microemulsion vehicles at different component ratio were selected in order to use in the preparation of microemulsions containing KP extract and to study the effect of oil, mixture of surfactant and water ratios on the characteristic of microemulsions.

3.3.3.3 KP extract-loaded microemulsions

According to the microemulsion regions in the pseudo-ternary phase diagrams, the potential formulations having the volume of water in microemulsion approximately 35 % v/v was selected to develop KP extract-loaded microemulsions. The formulations were prepared by mixing surfactant mixture, oil and water by volume ratio using magnetic stirrer at ambient temperature. KP extract-loaded microemulsions with or without limonene containing a controlled amount of 10 % w/w KP extract and various amount of limonene (1, 5 and 10 % w/w) were added, following by stirring with magnetic stirrer at ambient temperature.

3.3.4 Preparation KP extract-loaded microemulgels

KP extract-loaded microemulgels were prepared using the formulation giving the highest skin permeation of KP extract-loaded microemulsions with limonene, and added with various gelling agents namely hydroxyethyl cellulose (HEC), poloxamer 407, hydroxypropyl methyl cellulose (HPMC), carbopol 940 and xanthan gum to be the gel matrix. Each gelling agent was mixed with selected microemulsions under continuous stirring. After that, the clear microemulgel was obtained. In case of carbopol 940, the dispersion was neutralized by using triethanolamine to obtain the viscous gel.

3.3.5 Preparation KP extract-loaded PLO gels

KP extract-loaded PLO gels containing a controlled amount of 10 % w/w KP extract, 10 % w/w lecithin, various types of 10 % w/w oil (IPM, IPP, IPM/oleic acid and IPP/oleic acid) and various amount of limonene (1, 5 and 10 % w/w) were prepared. The oil phase was prepared by dissolving lecithin in oil. Then KP extract

with or without limonene was added and mixed until a clear solution was obtained. The aqueous phase was prepared by dissolving poloxamer 407 (15, 20 and 25 % w/w) in cold water, followed by mixing the oil phase and the aqueous phase at ambient temperature. The effect of various types of oils, amount of poloxamer 407 and amount of limonene on skin permeation of KP extract-loaded PLO gels was determined.

3.3.6 The percentage of total methoxyflavones content in formulations

The amount of total methoxyflavones (Sum as: DMF, TMF and PMF) incorporated into the formulations (microemulsions, microemulgels and PLO gels) were determined by UPLC. All samples were calculated using the following equations:

$$\% \text{ DMF content} = \frac{DMF_1}{DMF_2} \times 100$$

$$\% \text{ TMF content} = \frac{TMF_1}{TMF_2} \times 100$$

$$\% \text{ PMF content} = \frac{PMF_1}{PMF_2} \times 100$$

$$\% \text{ Total methoxyflavones content} = \frac{(DMF_1 + TMF_1 + PMF_1)}{(DMF_2 + TMF_2 + TMF_2)} \times 100$$

Where DMF_1, TMF_1 and PMF_1 are the amount of DMF, TMF and PMF in the formulations and DMF_2, TMF_2 and PMF_2 are the amount of DMF, TMF and PMF of KP extract in the formulations.

3.3.7 Physicochemical characterization

Microemulsions, microemulgels and PLO gels were characterized before and after loading with KP extract as followed:

3.3.7.1 Electrical conductivity measurement:

The electrical conductivity of the microemulsion formulations was determined by using conductivity meter (Metler Toledo, Sevencompact S230) at 25ºC. The measurements were performed in triplicate.

3.3.7.2 The average droplet sizes measurement:

The average droplet sizes and polydispersity index were determined by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern, UK) using a helium-neon gas laser with beam wavelength 632.8 nm. Sample was loaded into 1 cm^3 disposable zeta cell. Measurement angles were monitored at 12.8° and 175° and fixed temperature at 25°C.

3.3.7.3 pH measurement:

The pH is determined using pH meter (Metler Toledo, Sevencompact S220) at temperature 24-26°C. The measurements are performed in triplicate.

3.3.7.4 Rheological measurement:

The rheological properties of microemulgels and PLO gels were measured using a Brookfield rheometer (Brookfield Engineering Laboratories, USA). The measurements were performed in triplicate at 25 °C. The equilibrium time before every measurement was 5 min and the sample weight used approximately 3 grams.

3.3.8 Skin permeation studies

The skin permeation of methoxyflavones from KP extract-loaded microemulsions, KP extract-loaded microemulgels, KP extract-loaded PLO gels and KP extract in water was determined by using Franz diffusion cell with a penetration area of 2.31 cm^2. The model skin was porcine abdominal skin. The skins were washed with phosphate buffer pH 7.4 and stored at -10°C prior to use. After thawing, the skins were mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the other side facing downwards into the receptor compartment of the Franz diffusion cell (approximately 6.0 mL volume and 2.3 cm^2 effective diffusion areas) with a water jacket connected to a water bath at 32°C. Each sample (Approximately 1 g) was filled into the donor compartment. The receptor compartment was filled with 6 mL of PBS (pH 7.4) and stirred with a magnetic bar. At the predetermined times of 15, 30 min, 1, 2, 4, 8 and 24 h, 0.8 mL of receiver medium was withdrawn, and KP content was determined by UPLC. The

same volume of PBS was added into the receiver compartment to maintain a constant volume. Each sample was analyzed in triplicate.

3.3.9 Stability evaluation

The formulations resulting in highest skin permeation of KP extract-loaded microemulsions, KP extract-loaded microemulgels, and KP extract-loaded PLO gels were stored at room temperature and accelerated (40 ± 2^{o} C, 75 ± 5 %RH) for 3 months. Both the chemical and physical stability of the methoxyflavones were evaluated. The appearance was assessed by visual inspection. The pH, droplet size, size distribution, electrical conductivity and viscosity were determined on initial, 1, 2 and 3 months. The methoxyflavones concentration remaining in the formulations was determined by UPLC on initial, 1, 2 and 3 months.

3.3.10 Statistical analysis

All experimental measurements were triplicate performed. Result values were expressed as mean value ± standard deviation (SD). Statistical significance of differences between formulations was examined by using analysis of variance (ANOVA) followed by LSD post hoc test. For skin permeation studies of the formulation, independent T-test was used to compare the difference formulations. The value of $p < 0.05$ was considered statistically significant.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Preparation of KP extract

4.2 Preparation of KP extract-loaded microemulsions

- 4.2.1 Solubility studies of KP extract
- 4.2.2 Construction of pseudo-ternary phase diagrams
  - 4.2.2.1 Screening of co-surfactants
  - 4.2.2.2 Screening of surfactants
  - 4.2.2.3 Screening surfactant and co-surfactant mixture ratio
- 4.2.3 KP extract-loaded microemulsions
- 4.2.4 KP extract-loaded microemulsions with limonene

4.3 Preparation KP extract-loaded microemulgels

4.4 Preparation KP extract-loaded PLO gels

- 4.4.1 Effect of various types of oils
- 4.4.2 Effect of amount of poloxamer
- 4.4.3 Effect of amount of limonene

4.5 Stability evaluation

4.1 Preparation of KP extract

The dried rhizomes of KP were grounded and passed through a sieve until powder was obtained. KP rhizome powder was extracted by percolation with ethanol. KP rhizomes, KP powder and KP crude ethanolic extract are shown in Figure 4.1. Its dried rhizomes had dark purple color. The KP extract was brown and provided a 9.71% yield. The methoxyflavones (DMF, TMF, and DMF) of KP extract were isolated using column chromatography. The amount of DMF, PMF and TMF in KP extract was 10.39, 9.18, and 10.13%, respectively.

Figure 4.1 *Kaempferia parviflora* Ex Bake:

a) KP rhizomes b) KP powder c) KP crude extract

4.2 Preparation of KP extract-loaded microemulsions

4.2.1 Solubility studies of KP extract

The microemulsion formulations consisted of oils, surfactants, co-surfactants, water and total methoxyflavones (DMF, TMF and PMF) in KP extract should be a clear and isotropic liquid at ambient temperature. It is very important to find out an appropriate solvent to dissolve methoxyflavones, since it is an important role for skin permeability of methoxyflavones. In order to screen appropriate solvents for the preparation of microemulsions, the solubility of methoxyflavones in various oils, surfactants, co-surfactants and water was measured and the results are shown in Figure 4.2.

The solubility of total methoxyflavones in water was found to be extremely low (1.115±0.050 mg/mL). The solubility of total methoxyflavones in surfactants was the increasing order of Tween 20 > Cremophor® EL > Cremophor® RH40. The solubility of total methoxyflavones in co-surfactants was in the order of ethoxydiglycol > PG > BG, and in oils was in the order of oleic acid > caprylic/capric triglyceride > IPM > IPP. The solubility of total methoxyflavones in oleic acid showed the highest value. As oleic acid is an excellent skin enhancer in transdermal delivery, it was selected as the oil phase for preparation of microemulsions [46, 68, 69].

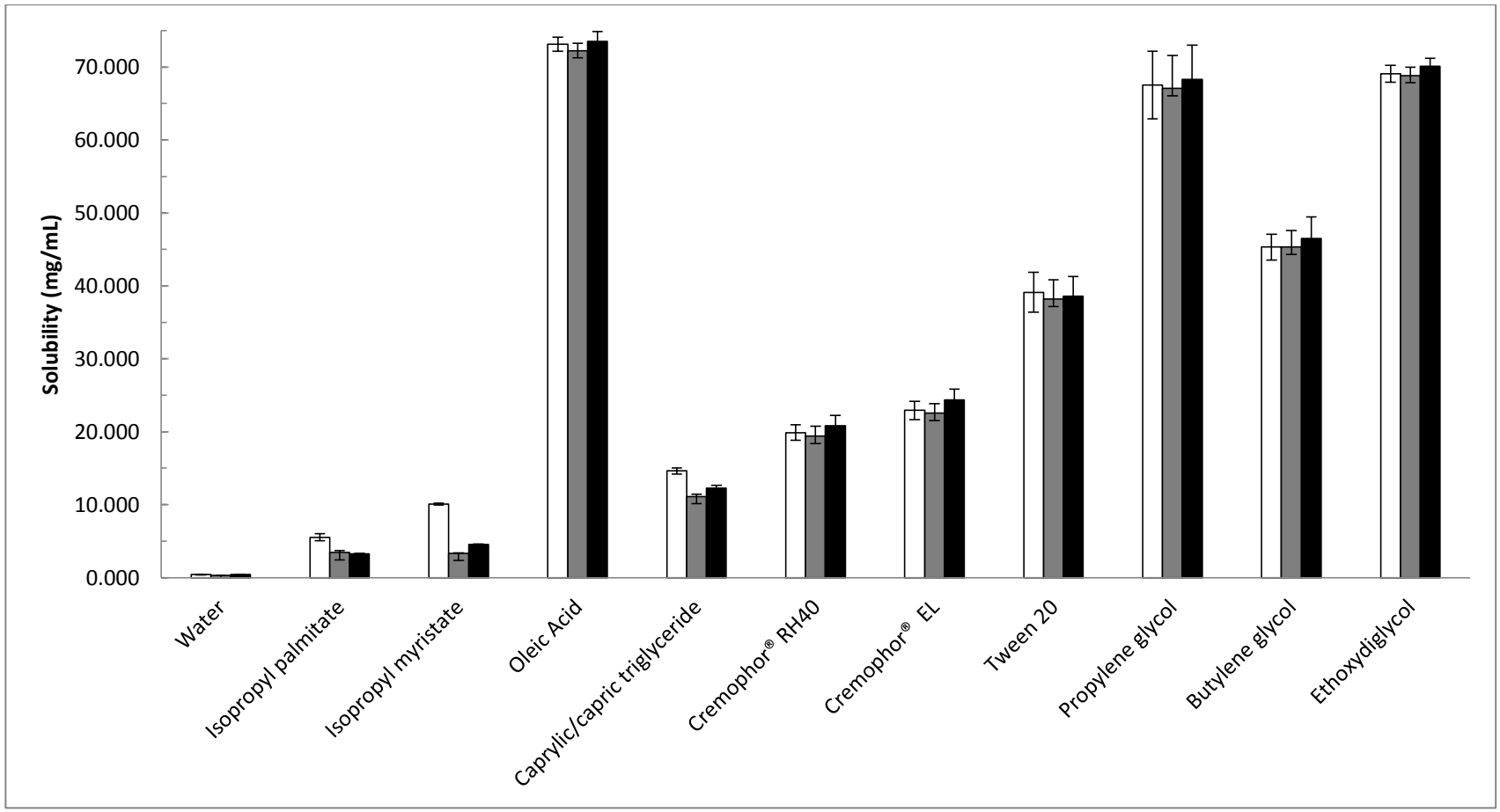


Figure 4.2 The solubility of (□) DMF, (■) TMF and (■) PMF in KP extract at 30±2°C in water, oils, surfactants and co-surfactants.

4.2.2 Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams enable the determination of the components and their concentration range needed to form microemulsions at ambient temperature. In order to find out suitable pseudo-ternary phase diagram, various surfactants and co-surfactants were studied by using the water titration method.

4.2.2.1 Screening of co-surfactants

In many cases, surfactants alone are incapable to reduce the interfacial tension to produce stable microemulsion. Co-surfactants are added to achieve microemulsion systems at low surfactant concentration [46]. To select the co-surfactant, the sizes of the microemulsion region in the phase diagrams were compared at a fixed surfactant/co-surfactant (S_{mix}) ratio (1:1) by using Tween 20 as a surfactant and by varying the co-surfactants (BG, PG and ethoxydiglycol). The results of pseudo-ternary phase diagrams are shown in Figure 4.3-4.5. The gray area of the pseudo-ternary phase diagram indicated microemulsion regions while the outside area referred to the emulsion regions.

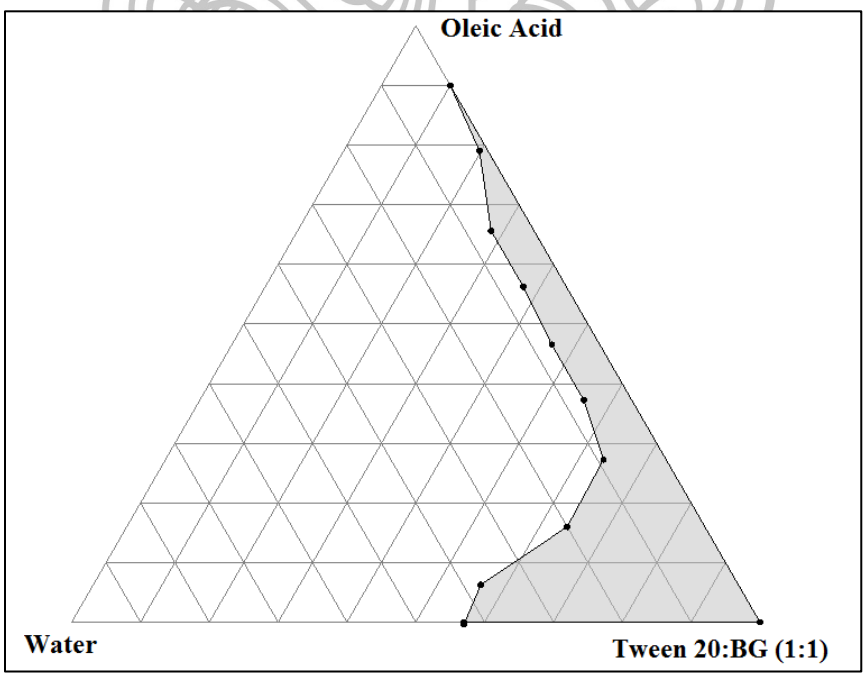


Figure 4.3 Pseudo-ternary phase diagram containing oleic acid, Tween 20:BG (1:1) and water.

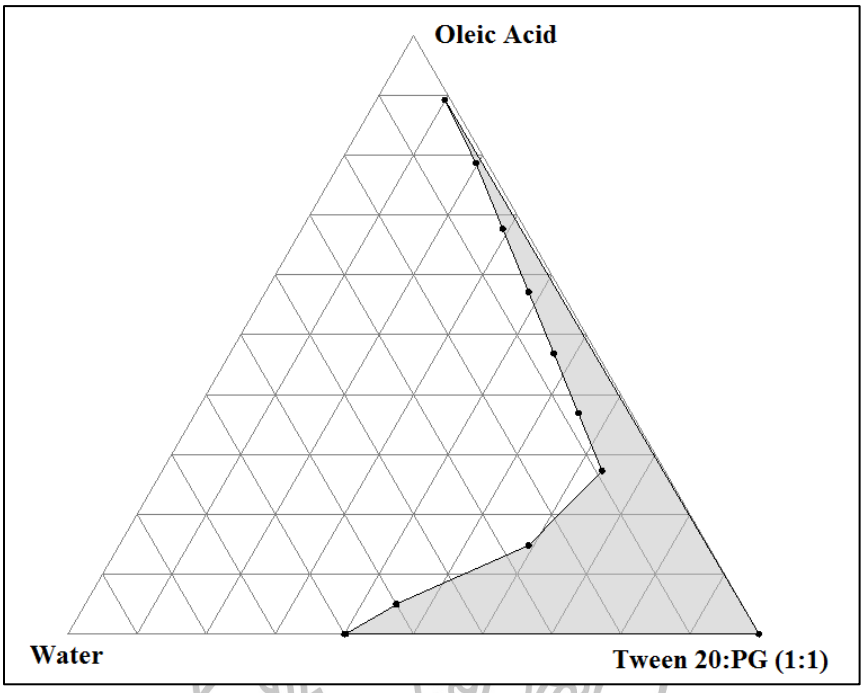


Figure 4.4 Pseudo-ternary phase diagram containing oleic acid, Tween 20:PG (1:1) and water.

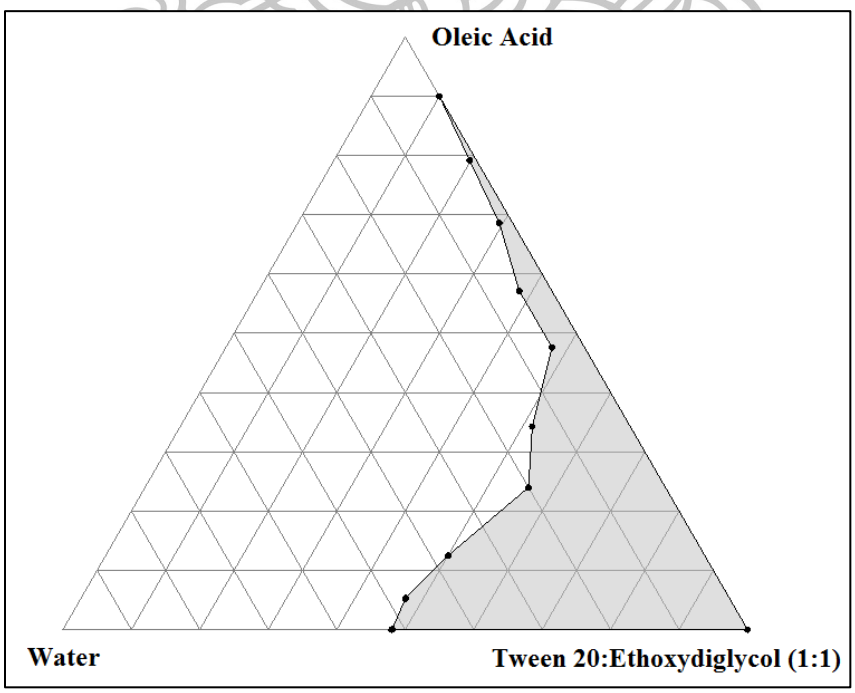


Figure 4.5 Pseudo-ternary phase diagram containing oleic acid, Tween 20: ethoxydiglycol (1:1) and water.

Among co-surfactants, PG and ethoxydiglycol found to provide stable and large microemulsion area (Figure 4.3 and 4.4). The microemulsion area decreased when the co-surfactant of the system was BG (Figure 4.5). Moreover, microemulsion area of Tween 20:PG and Tween 20:ethoxydiglycol at S_{mix} ratio 1:1 were similar regions. Therefore, PG and ethoxydiglycol were selected as co-surfactant for the microemulsion formulation in this study.

4.2.2.2 Screening of surfactants

From solubility and screening of co-surfactant, the microemulsion system composed of oleic acid as oil phase, PG and ethoxydiglycol as co-surfactants and purified water as water phase with different surfactants were performed. The surfactant selected for this study was Tween 20 and Cremophor® EL. Therefore, pseudo-ternary phase diagrams prepared by two surfactants and two co-surfactants at the 1:1 ratio were constructed as shown in Figure 4.4-4.7.

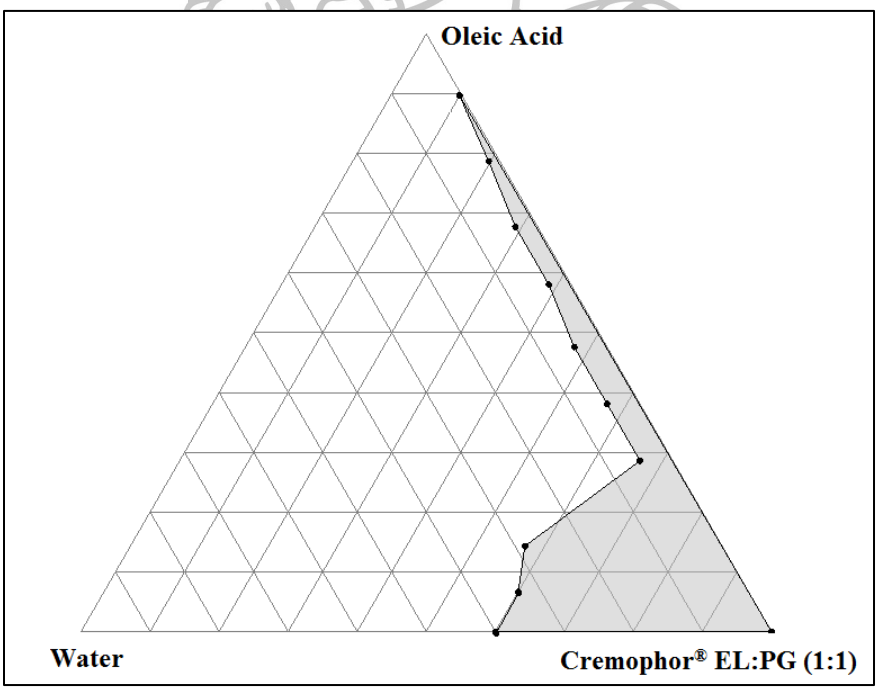


Figure 4.6 Pseudo-ternary phase diagrams containing oleic acid, Cremophor® EL:PG (1:1) and water.

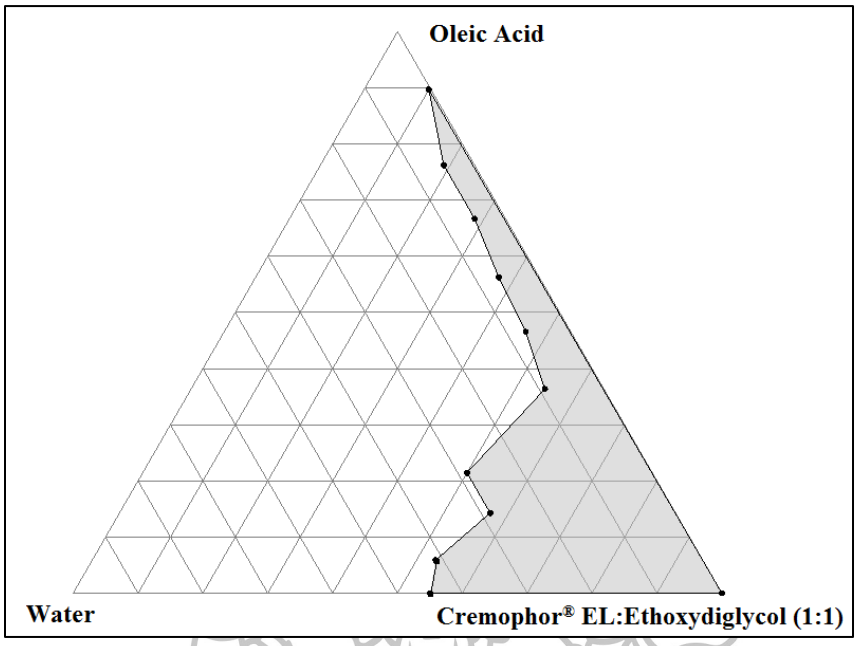


Figure 4.7 Pseudo-ternary phase diagrams containing oleic acid, Cremophor® EL: ethoxydiglycol (1:1) and water.

In this study, the microemulsion systems composed of Tween 20 as surfactant showed larger area than Cremophor® EL in both PG and ethoxydiglycol as co-surfactants (Figure 4.4-4.7). Therefore, Tween 20 was selected as surfactant for formulating the microemulsions.

4.2.2.3 Screening surfactant and co-surfactant mixture ratio

In this study, pseudo-ternary phase diagrams were constructed using two systems composed of oleic acid as oil phase, Tween 20 as surfactant, PG or ethoxydiglycol as co-surfactant and water phase. In order to obtain the appropriate concentration ranges of the components of microemulsion, the pseudo-ternary phase diagrams were constructed at different S_{mix} ratio 1:1, 2:1, 1:2 and 1:3 (Figure 4.8 and 4.9). The S_{mix} ratio provided stable and clear microemulsions were selected for further study.

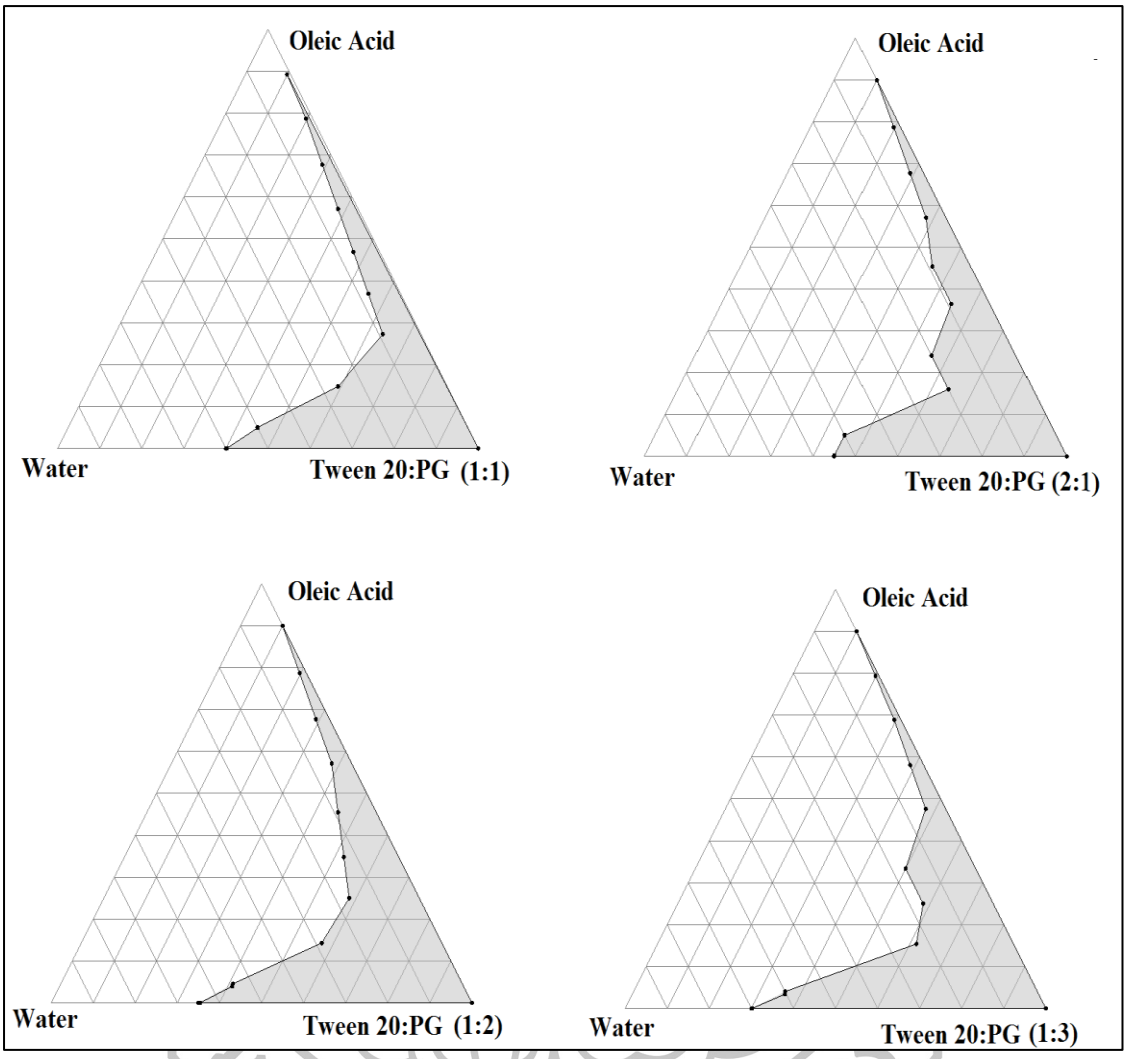


Figure 4.8 Pseudo-ternary phase diagrams containing oleic acid, S_{mix} (Tween 20:PG) and water at various S_{mix} ratios.

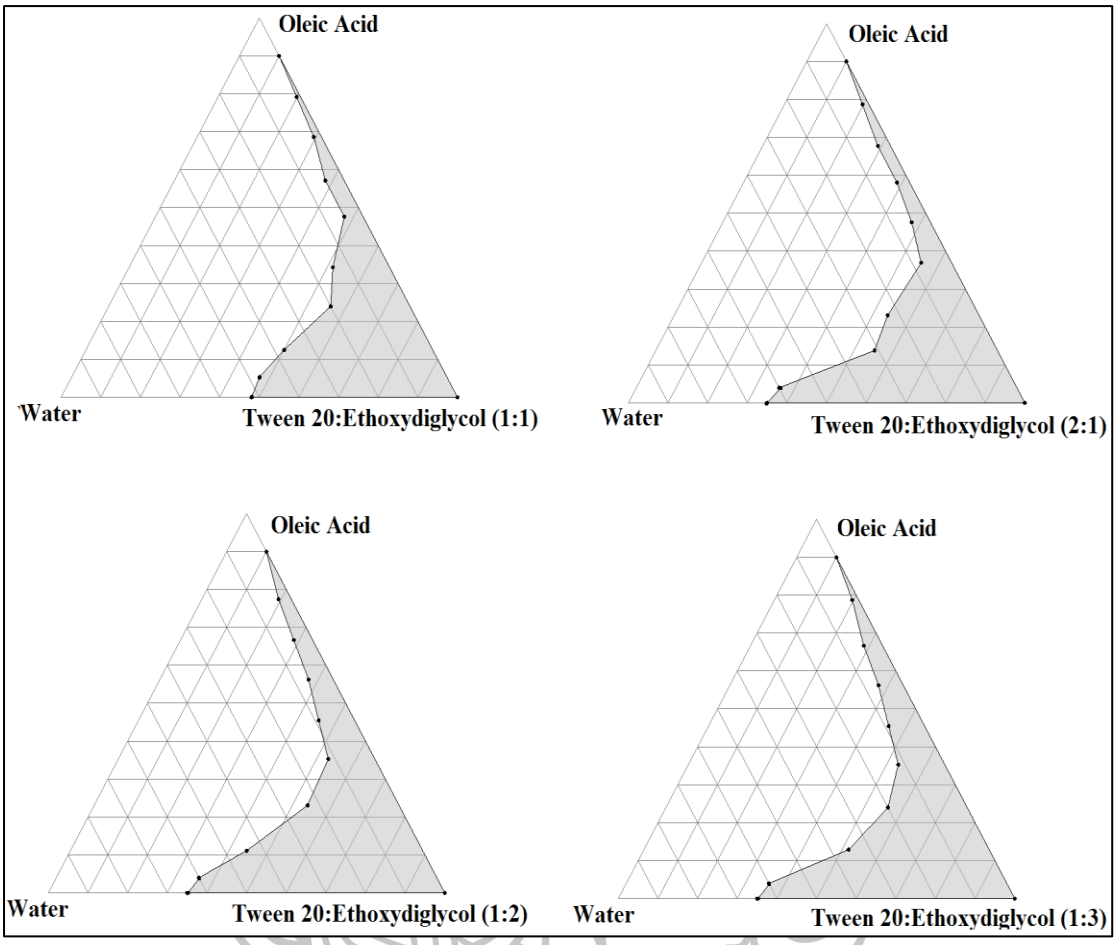


Figure 4.9 Pseudo-ternary phase diagrams containing oleic acid, S_{mix} (Tween 20: ethoxydiglycol) and water at various S_{mix} ratios.

Among the eight phase diagrams (Figure 4.8 and 4.9), the largest microemulsion region was observed in S_{mix} 1:2. As the ratio of co-surfactant in the S_{mix} was increased from 1:1, the microemulsion area expanded. However, microemulsion area of S_{mix} ratio 1:2 was larger than 1:3, indicating that co-surfactant in ratio 1:2 sufficiently reduced interfacial tension for microemulsion systems. Furthermore, the microemulsion area decreased when the concentration of Tween 20 in S_{mix} was increased.

In addition, various volume of water (20, 25, 30 and 35 % v/v.) was used to form microemulsions. Only formulation containing PG as co-surfactant with 35 % of water could form microemulgel with adding gelling agent. Therefore, volume of

water of microemulsions at 35 % v/v was selected to prepare micromulsions for further study. The microemulsions selected from the phase diagram were composed of oleic acid as oil phase (5%), Tween 20 as surfactant (20%), PG or ethoxydiglycol as co-surfactant (40%) and water phase (35%).

4.2.3 KP extract-loaded microemulsions

It has been reported that 10% w/w KP extract showed an anti-inflammatory effect [1]. Therefore, 10% w/w KP extract was used for this study. Microemulsions composed of oleic acid as oil phase, Tween 20 as surfactant, PG (ME1) or ethoxydiglycol (ME2) as co-surfactant (S_{mix} ratio 1:2) and water as aqueous phase were formulated. After that, 10% w/w KP extract was added in the ME1 and ME2 and coded as KPME1 and KPME2, respectively. The amount of total methoxyflavones content incorporated into KPME1 and KPME2 were 101.54±0.66 and 100.48±0.91 %, respectively.

4.2.3.1 Characterization of KP extract-loaded microemulsions

The pH values of blank ME and KP extract-loaded ME were 5.42-5.89 and 5.34-5.67, respectively (Figure 4.10a). After loaded KP extract, the pH of the KPME was slightly decreased.

The conductivity of the blank-ME was in the range of 24.9-28.7 μS/cm, and KP extract-loaded ME was in the range of 58.1-68.6 μS/cm (Figure 4.10b). The conductivity of KPME was significantly higher than that of blank-ME. This might be due to the high electrical conductivity of KP extract (69.2±1.8 μS/cm).In the previous study, the water in oil microemulsions represents very low specific conductivity (10^{-9}–10^{-7} $\Omega^{-1}cm^{-1}$ or 0.001-0.1 μS/cm) [70]. In our study, all the formulations were the oil in water microemulsions.

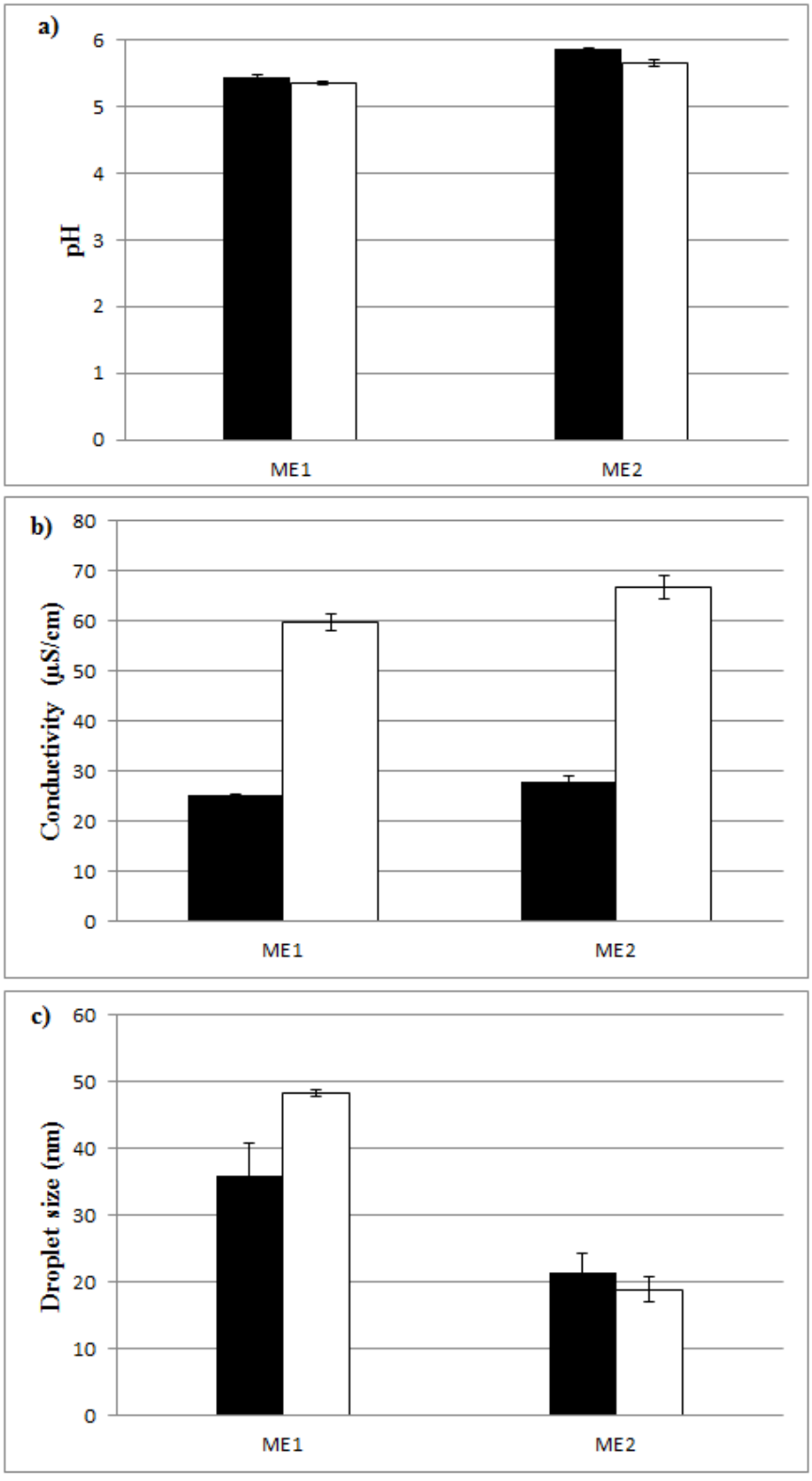


Figure 4.10 Characterization of (■) Blank-microemulsions and (□) KP extract-loaded microemulsions: a) pH b) Conductivity c) Droplet size.

The average droplet size and polydispersity index (PDI) of the microemulsions were characterized by DLS. The droplet sizes of the MEs, blank-ME (18.30-24.93 nm) and KP extract-loaded microemulsions (17.32-47.34 nm) were in the nano-size range (Figure 4.10c). The previous study showed that a small droplet sizes showed an increase in stability against sedimentation, flocculation and coalescence [71]. The PDI value exhibited the homogeneous droplet size and should be less than 0.5 [72]. The PDI values of microemulsion formulations, with and without KP extract were 0.183-0.368, indicating that the droplet size had high homogeneity.

4.2.3.2 Skin permeation of KP extract-loaded microemulsions

The skin permeation profiles of total methoxyflavones through abdominal porcine skin are shown in Figure 4.11. The skin permeation fluxes of methoxyflavones from KPME1 and KPME2 were 0.090±0.006 and 0.085±0.008 μg/cm^2/h, respectively. This result revealed that the type of co-surfactants (PG (KPME1) and ethoxydiglycol (KPME2)) did not significantly influence on skin permeation. The skin permeation fluxes of the KPME were significantly higher than the KP extract in water (0.013±0.001 μg/cm^2/h). There are several possible mechanisms of microemulsions in enhancing drug permeation for transdermal delivery. Firstly, it may be related to the high drug-loading capacity of microemulsions. Secondly, surfactant and co-surfactant in the microemulsion exhibit high penetration enhancing effect. Thirdly, the microemulsion components can enter the skin, thus, the solubility of the drug in the skin is increased. Fourth, the microstructure of the system provides large surface area for drug transfer and diffusion. Finally, supersaturation of micoremulsion system increases the thermodynamic activity and driving force for the transdermal drug delivery [52].

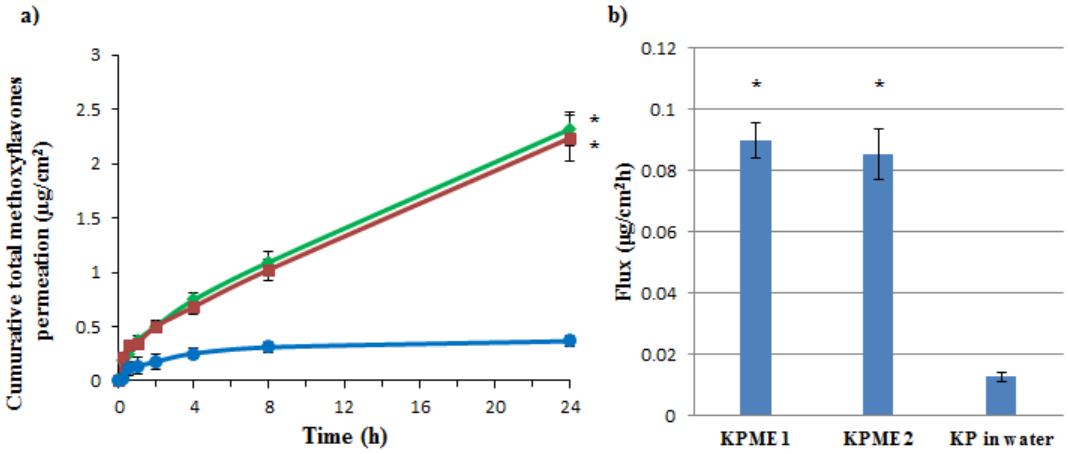


Figure 4.11(a) Skin permeation profiles; (♦) KPME1, (■) KPME2, (▲) KP extract in water and (b) flux value of KP extract-loaded microemulsions. Each value represents the mean ± standard deviation (n=3).* $p < 0.05$, compared with the KP extract in water.

4.2.4 KP extract-loaded microemulsions with limonene

Limonene is classified as terpene in chemical permeation enhancer that found to effectively improve skin permeation for transdermal delivery system. Therefore, in this study, various amount of limonene (1, 5 and 10 % w/w) was added in KPME1 and coded as KPME3, KPME4 and KPME5, respectively. The percent amount of total methoxyflavones was determined in the range of 99.60-102.12%**.** The physicochemical characteristics are shown in Figure 4.12**.** The pH value and conductivity of the blank microemulsion with limonene were in the range of 5.37-5.44 and 24.0-24.9 µS/cm, respectively (Figure 4.12a). The pH value and conductivity of the KP extract-loaded microemulsions with limonene were in the range of 5.28-5.38 and 52.0-58.1 µS/cm, respectively (Figure 4.12b). The conductivity of all formulations tended to decrease when the amount of limonene increased. The average droplet size of the blank microemulsion with limonene (20.45-49.26 nm) and KP extract-loaded microemulsion with limonene (42.99-110.65 nm) exhibited nano-size droplets (Figure 4.12c). The KP extract did not significantly influence on the droplet size of microemulsions. Nevertheless, the average droplet size of microemulsion depended on the amount of limonene. The droplet size tended to be larger, when the concentration of limonene increased.

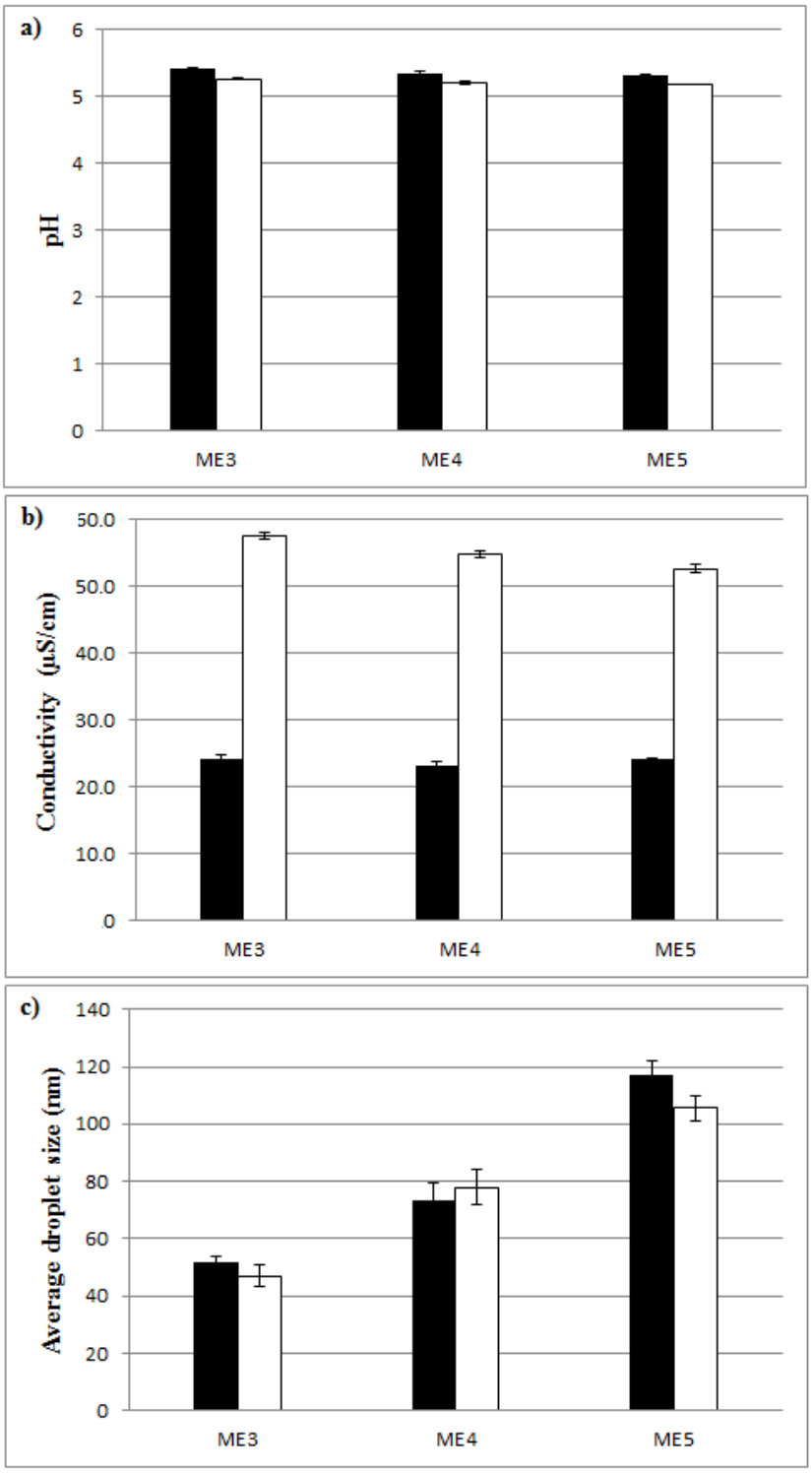


Figure 4.12 Characterization of (■) Blank-microemulsions with limonene and (□) KP extract-loaded microemulsion with limonene:

a) pH b) Conductivity c) Droplet size.

The *in vitro* skin permeation studies found that increasing the limonene content significantly increased permeation flux through porcine abdominal skin (Figure 4.13). This study revealed that increase amount of limonene in the microemulsion tended to increase the permeation flux. The highest skin permeation flux (0.132±0.006 µg/cm^2/h) was found in the KP extract-loaded microemulsion with 10 % limonene (KPME5) which was 10.4-fold and 1.5-fold higher than the control (KP extract in water) and microemulsions without limonene, respectively. This result demonstrated the potential use of limonene as an effective penetration enhancer for KP delivery through skin [73].

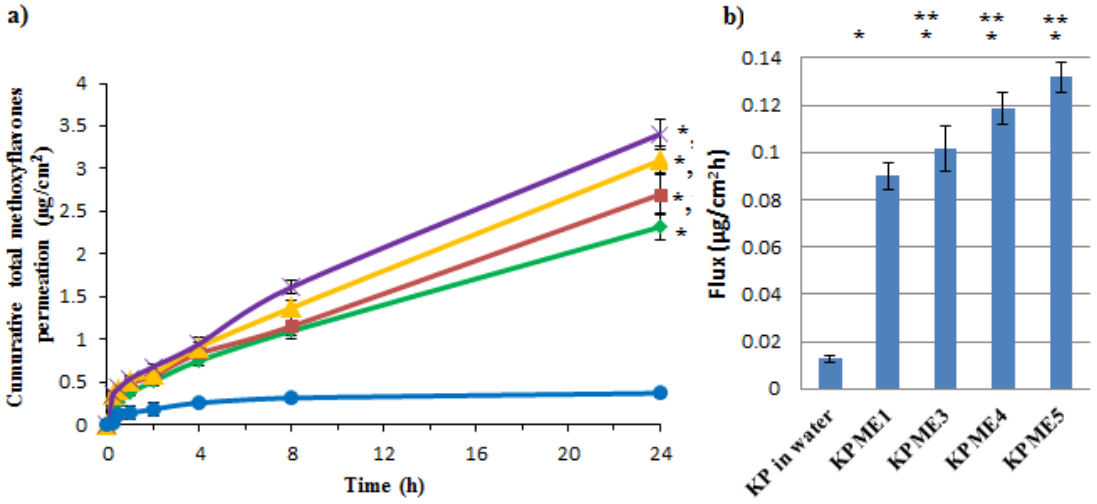


Figure 4.13(a) Skin permeation profiles of KP extract-loaded microemulsion with limonene; (♦) KPME1, (■) KPME3, (▲) KPME4 and (X) KPME5 and (●) KP extract in water and (b) flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with the KP extract in water. ** $p < 0.05$, compared with KPME1.

4.3 KP extract-loaded microemulgels

Various gelling agents such as hydroxyethyl cellulose (HEC), poloxamer 407, hydroxypropyl methyl cellulose (HPMC), carbopol 940 (with TEA) and xanthan gum were selected. Only ME1 could form microemulgel with gelling agents. Therefore, ME1 was selected and KP extract-loaded microemulgels were developed. Various concentrations of gelling agents were added to ME1, and microemulgels were inspected visually after 24 h for physical stability (Table 4.1). The results revealed

that only xanthan gum could form microemulgel whereas HEC, poloxamer 407, HPMC and carbopol 940 were unable to yield acceptable gels. These might be due to insufficient amount of water in microemulsions to produce microemulgel [56, 57]. Moreover, high surfactant of microemulsion disrupted to form the gel formations [74].

Table 4.1 Evaluation of various gelling agents in ME1.

Gelling agents	Concentrations (% w/w)	Observed state after 24 h
HEC	1.0, 3.0 and 5.0	Viscous liquid
Poloxamer 407	15.0, 20.0 and 25.0	Sedimentation
HPMC	1.0, 3.0 and 5.0	Viscous liquid
Carbopol 940	1.0, 1.5 and 2.0	Phase separation
Xanthan Gum	1.6, 1.8 and 2.0	Clear colorless gel

The microemulsion systems for KP extract-loaded microemulgels were selected from KP extract-loaded microemulsionswith 10 % w/w limonene (KPME5) resulting in the highest skin permeation. Various amounts of xanthan gum (1.6, 1.8 and 2 % w/w) with and without limonene were coded as KPGE4, KPGE5, KPGE6, KPGE1, KPGE2 and KPGE3, respectively (Table 4.2). The percent total methoxyflavones of KP extract-loaded microemulgels were in the range of 98.64-102.72%.

Table 4.2 Formulations of KP extract-loaded microemulgels with and without limonene.

Formulations	Percent (%)						
	KP Extract	Limonene	Xanthan gum	Oleic acid	Tween20	PG	Water
KPGE1	10.00	0	1.60	4.42	17.68	35.36	30.94
KPGE2	10.00	0	1.80	4.41	17.64	35.28	30.87
KPGE3	10.00	0	2.00	4.40	17.60	35.20	30.80
KPGE4	10.00	10.00	1.60	3.92	15.68	31.36	27.44
KPGE5	10.00	10.00	1.80	3.91	15.64	31.28	27.37
KPGE6	10.00	10.00	2.00	3.90	15.60	31.20	27.30

4.3.1 Characterization of KP extract-loaded microemulgels

The pH values of blank-microemulgels were 4.99-5.14. The pH values of 10% w/w KP extract loaded microemulgels were 4.86-4.98 (Figure 4.14a). After KP extract was loaded into microemulgels formulations, the pH of the microemulgels was decreased. This result was similar to KP-loaded microemulsions. In addition, the pH values of microemulgels were not significantly different when xanthan gum was added.

The viscosity in blank-microemulgels and KP extract-loaded microemulgels was in the range of 15,521.29-27,723.72 and 9,846.67-11,269.77cPs, respectively (Figure 4.14b). The viscosity of microemulgels significantly decreased when KP extract and limonene were incorporated. This might be caused from that amount of water in the formulation decreased, which affected the viscosity property of xanthan gum [59].

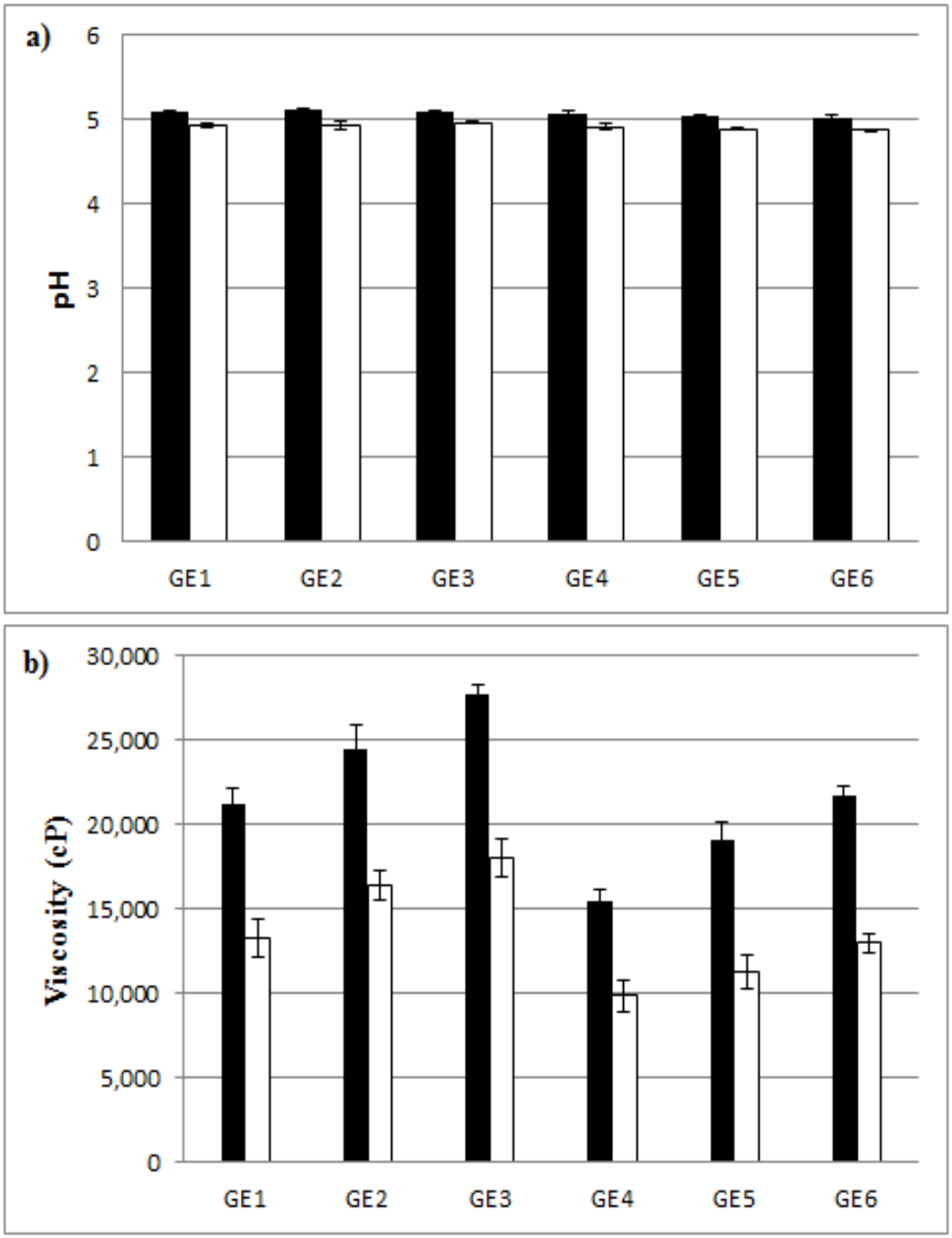


Figure 4.14 Characterization of (■) Blank-microemulgels with limonene and (□) KP extract-loaded microemulgels with and without limonene: a) pH b) Viscosity.

4.3.2 Skin permeation studies of KP extract-loaded microemulgels

The *in vitro* permeation profiles of KP extract-loaded microemulgels with and without limonene through the porcine abdominal skin are shown in Figure 4.15. 10 % w/w limonene in KP extract-loaded microemulgels significantly increased permeation flux. Nevertheless, amount of xanthan (1.6, 1.8 and 2 % w/w) did not significantly affect total methoxyflavones permeated through the skin.

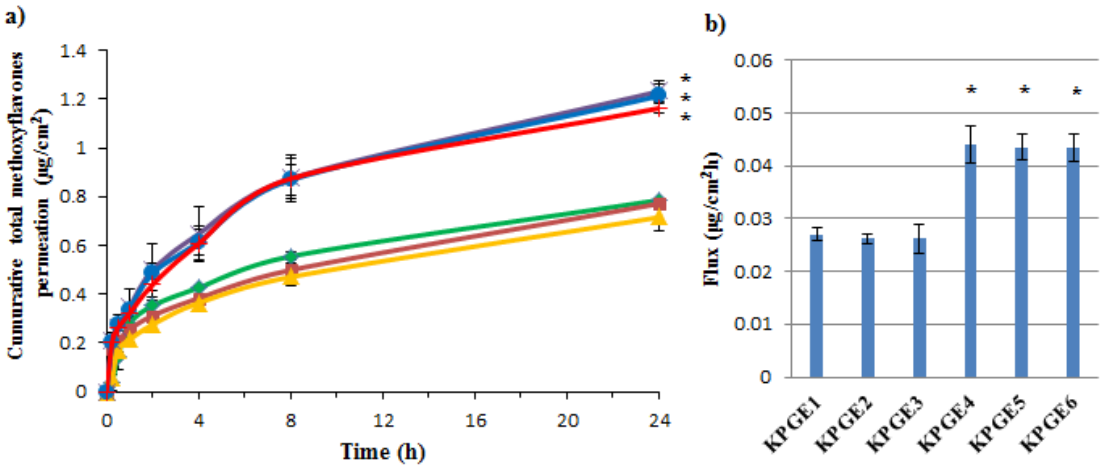


Figure 4.15 (a) Skin permeation profiles of KP extract-loaded microemulgel with and without limonene; (♦) KPGE1, (■) KPGE2, (▲) KPGE3, (X) KPGE4, (●) KPGE5 and (+) KPGE6 and (b) flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with KP extract-loaded microemulgels without limonene at same amount of xanthan gum.

The total methoxyflavones permeated across the skin of KP extract-loaded microemulsion (KPME5; without gelling agent) was significantly higher than KP extract-loaded microemulgel (KPGE5; with gelling agent) (Figure 4.16). This might be attributed to the gel formation in the microemulsion. Microemulgel increase its viscosity and decrease the permeation of methoxyflavones in the skin. The diffusion through the double layer microemulsion might be a rate-determining step, as the viscosity plays an important role in controlling the permeation of the drug into the skin [74].

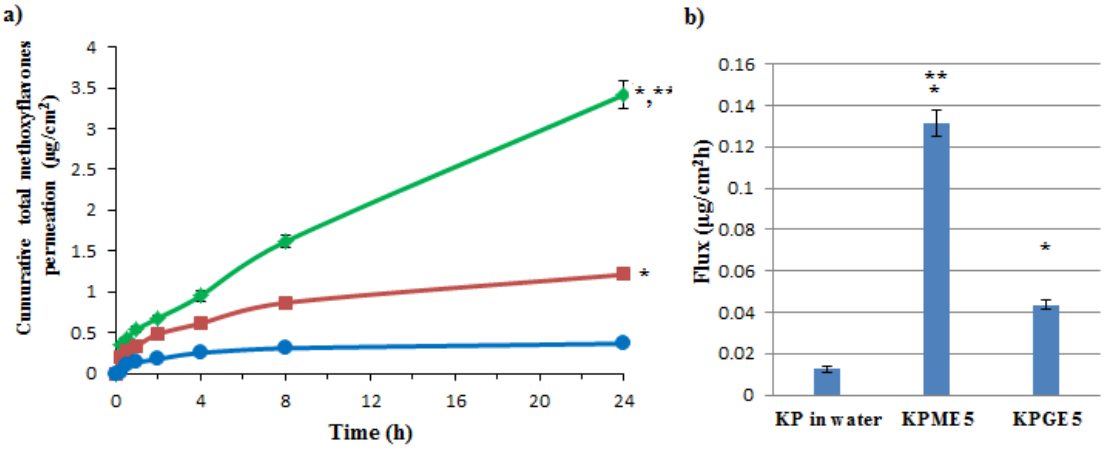


Figure 4.16 (a) Skin permeation profiles of (KPME5, ♦) KP extract-loaded microemulsion with 10 % limonene, (KPGE5, ■) KP extract-loaded microemulgels with 10 % limonene and (●) KP extract in water and (b) flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with the KP extract in water. ** $p < 0.05$, compared with KPGE5.

4.4 KP extract-loaded PLO gels

PLO gels were developed for transdermal delivery of KP extract. The effects of PLO gel components on the intrinsic properties and in vitro skin permeation of KP extract-loaded PLO gels were studied as follows;

4.4.1 Effect of types of oils

KP extract-loaded PLO gels containing 10 % KP extract, lecithin, poloxamer 407 (Poloxamer), water and various types of oil (IPM, IPP, IPM:oleic acid (1:1) and IPP:oleic acid (1:1) were formulated and coded as KPPLO1, KPPLO2, KPPLO3 and KPPLO4, respectively (Table 4.3). KPPLO1, KPPLO2 and KPPLO3 were successfully prepared, whereas KPPLO4 could not be prepared because lecithin was unable to dissolve in the mixture of IPP and oleic acid (1:1). The percent total methoxyflavones content of KPPLO1, KPPLO2 and KPPLO3 were 99.84±0.20, 98.36±0.82 and 99.44±1.28, respectively.

Table 4.3 Formulations of KP extract-loaded PLO gels with various types of oils.

Formulations	Percent (%)						
	KP Extract	Lecithin	IPM	IPP	Oleic acid	Poloxamer	Water
KPPLO1	10.00	10.00	10.00	0.00	0.00	20.00	50.00
KPPLO2	10.00	10.00	0.00	10.00	0.00	20.00	50.00
KPPLO3	10.00	10.00	5.00	0.00	5.00	20.00	50.00
KPPLO4	10.00	10.00	0.00	5.00	5.00	20.00	50.00

The characteristics of KP extract-loaded PLO gels (KPPLO1, KPPLO2 and KPPLO3) were brown opaque gel with a pH range of 4.59-4.73. The viscosity of KPPLO1, KPPLO2 and KPPLO3 were 34,405.52 ± 987.88, 37,114.82 ± 893.92 and 42,256.96 ± 771.47 cPs, respectively (Figure 4.17). The viscosity of the formulations tended to decrease when the KP extract was added. These phenomena were the same reason as described in microemulsion and microemulgel section.

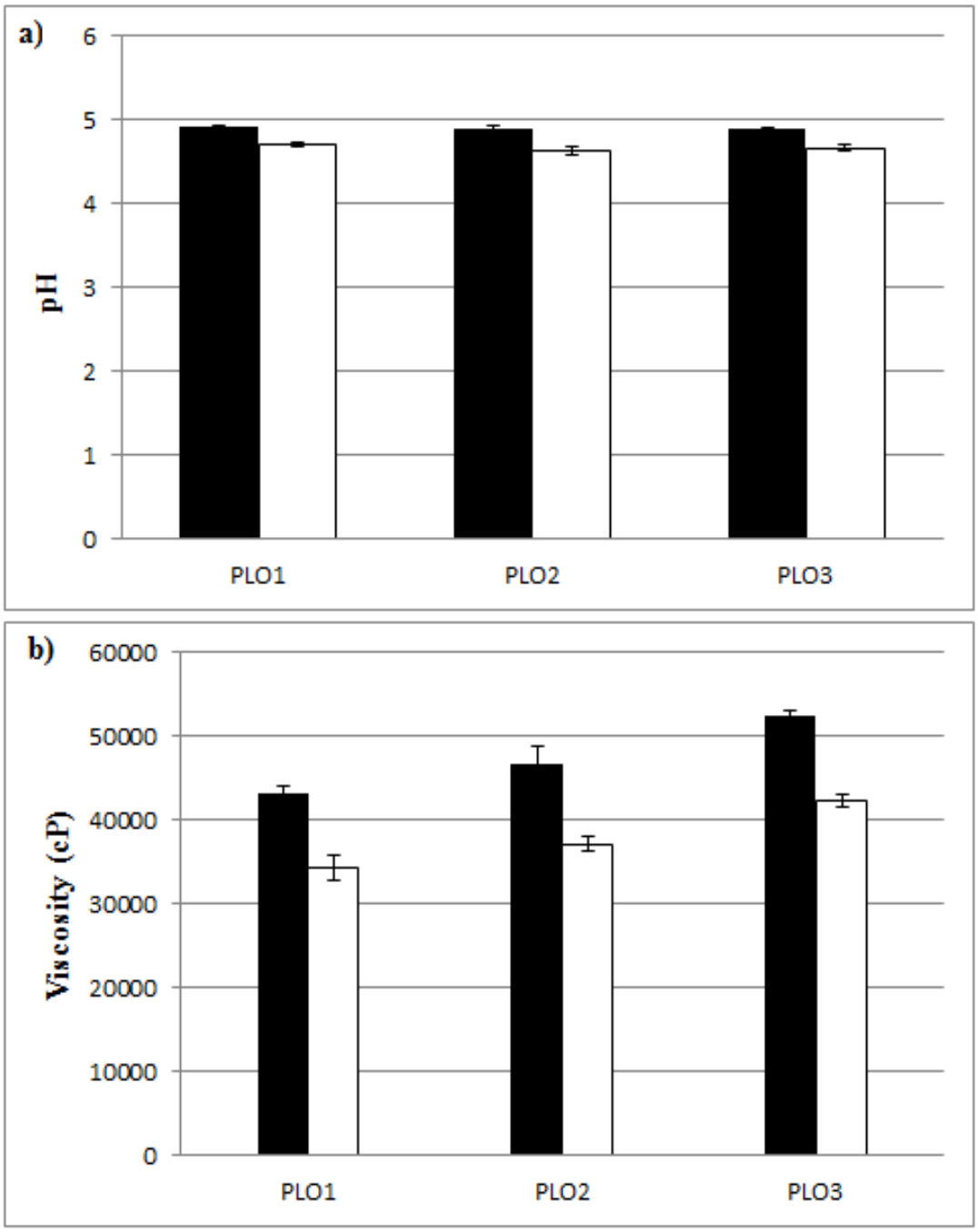


Figure 4.17 Characterization of (■) Blank- PLO gel and (□) KP extract-loaded PLO gels with various types of oils:
a) pH b) Viscosity.

The skin permeation profiles of the KP extract-loaded PLO gels containing different types of oils are shown in Figure 4.18. The cumulative total methoxyflavones permeations of KP-loaded PLO gels were significantly higher than the KP extract in water. The maximum cumulative total skin permeation of methoxyflavone was observed in KPPLO3. The skin permeation flux of KPPLO3 was 1.84 times compared with the KP extract in water. This result revealed that oleic acid strongly affected the KP permeation flux. Due to oleic acid was reported to increase the fluidization in the vesicle bilayer of the stratum corneum [46].

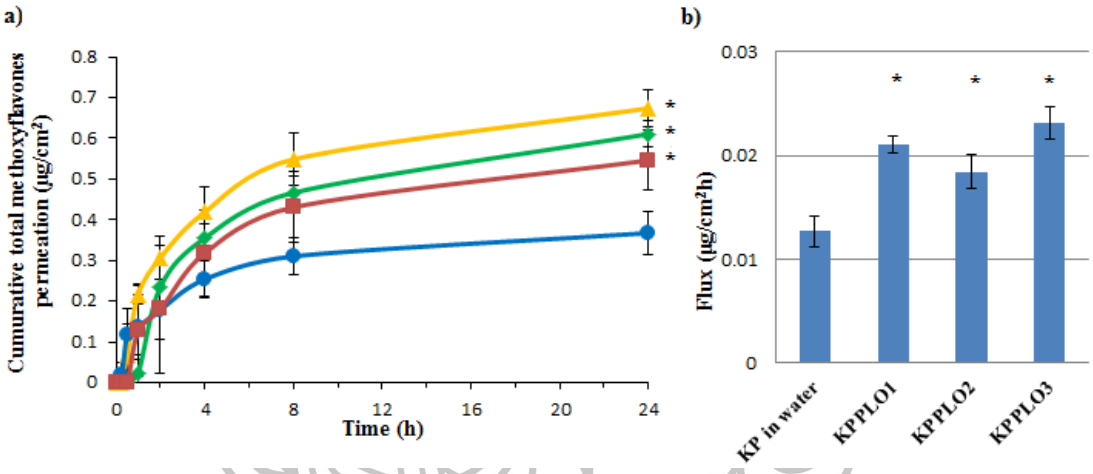


Figure 4.18 (a) Skin permeation profiles of KP extract-loaded PLO gel with various types of oils; (♦) KPPLO1, (■) KPPLO2, (▲) KPPLO3 and (●) KP in water and (b) flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with KP extract in water.

4.4.2 Effect of amount of poloxamer

The KP extract-loaded PLO gels (KPPLO3) containing different amount of poloxamer (15, 20 and 25 % w/w) were formulated and coded as KPPLO5, KPPLO3 and KPPLO6, respectively (Table 4.4). The percent content of KP in KPPLO5, KPPLO3 and KPPLO6 was 101.07±0.25, 99.44±1.28 and 98.37±1.03, respectively.

Table 4.4 Formulations of KP extract-loaded PLO gels with various amount of poloxamer.

Formulations	Percent (%)					
	KP Extract	Lecithin	IPM	Oleic acid	Poloxamer 407	Water
KPPLO3	10.00	10.00	5.00	5.00	20.00	50.00
KPPLO5	10.00	10.00	5.00	5.00	15.00	55.00
KPPLO6	10.00	10.00	5.00	5.00	25.00	45.00

The pH values of blank-PLO gels and KP extract-loaded PLO gels containing different amount of poloxamer (15, 20 and 25 % w/w) were 4.63-4.93. The amount of poloxamer did not significantly affect pH value of blank-PLO gels and KP extract-loaded PLO gels. The viscosity of blank-PLO gels and KP extract-loaded PLO gels containing different amount of poloxamer (15, 20 and 25 % w/w) was in the range of 38,730.81±1,007.86 to 49,458.24±1,459.63 cPs. An increase in viscosity when increasing the amount of poloxamer might be due to the formation of complex network of gel.

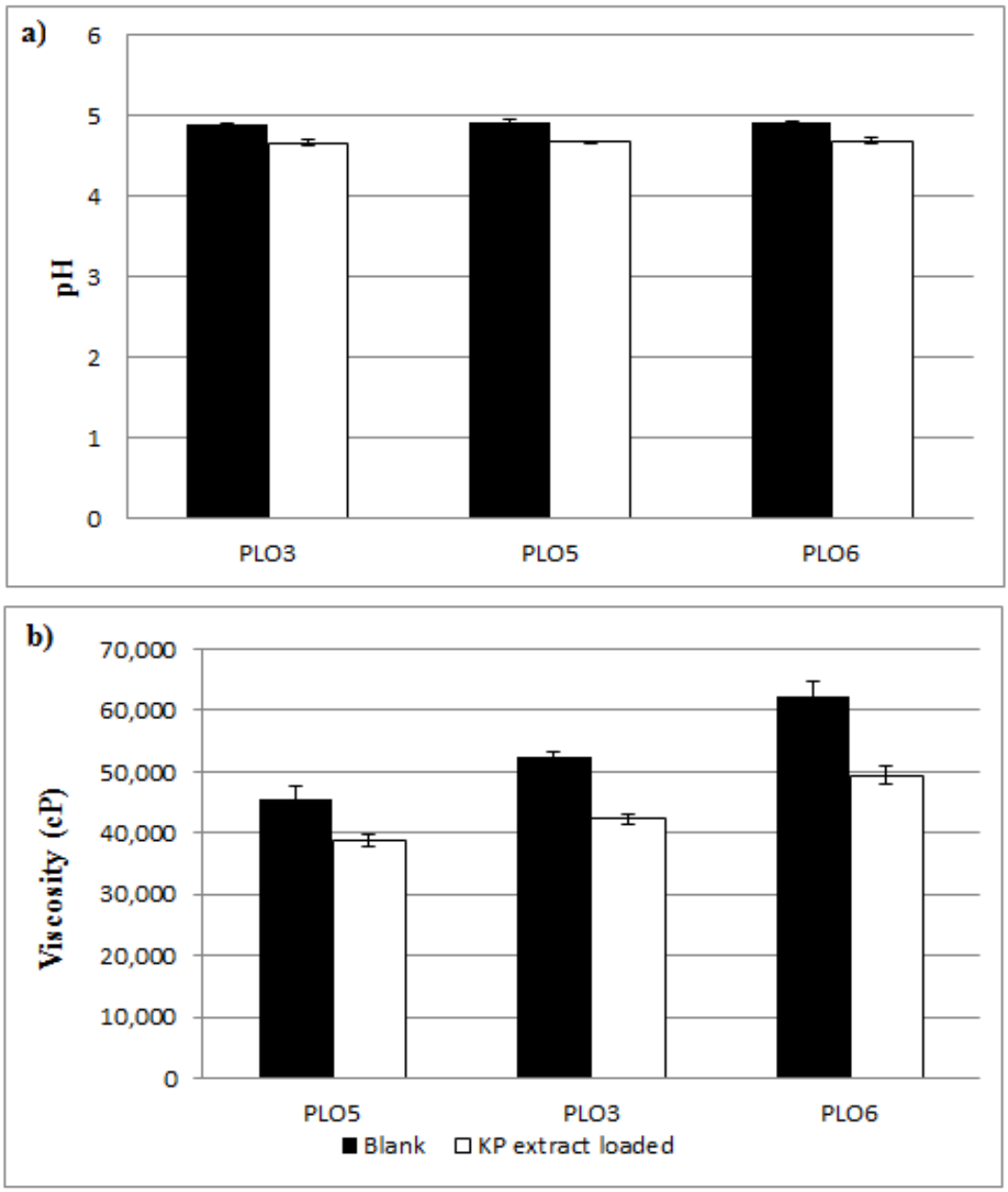


Figure 4.19 Characterization of (■) Blank- PLO gel and (□) KP extract-loaded PLO gels with various amount of poloxamer:
a) pH b) Viscosity.

The skin permeation profiles of the KP extract-loaded PLO gels containing different amount of poloxamers are shown in Figure 4.20. The cumulative total skin permeation of methoxyflavones of KP-loaded PLO gels was significantly higher than the KP extract in water. The cumulative total skin permeation of methoxyflavones of KPPLO3 was significantly higher than KPPLO6 and KPPLO7. The amount of poloxamer slightly affected the skin permeation. Poloxamer could hydrolyze the circulating triglyceride of skin, however it acts as the gelling agent as well [75]. Moreover, high viscosity of formulations decreased total skin permeation of methoxyflavones.

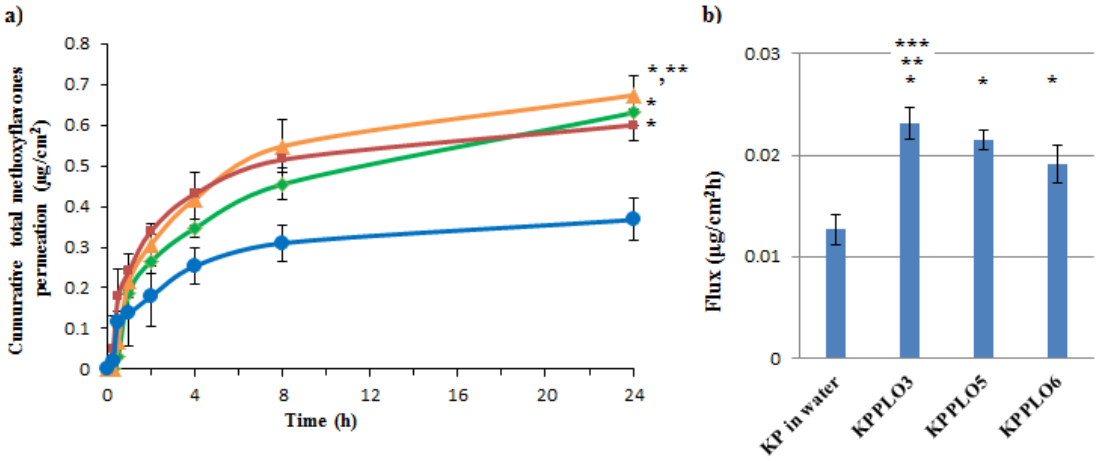


Figure 4.20 (a) Skin permeation profiles of KP extract-loaded PLO gel with various amount of poloxamer 407; (▲) KPPLO3, (♦) KPPLO5, (■) KPPLO6 and (●) KP in water and (b) flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with KP extract in water. ** $p < 0.05$, compared with KPPLO5. *** $p < 0.05$, compared with KPPLO6.

4.4.3 Effect of amount of limonene

The highest skin permeation of KP extract-loaded PLO gels with 20 % w/w poloxamer (KPPLO3) was selected, then various amount of limonene (1, 5 and 10 % w/w) were added in the gels and coded as KPPLO7, KPPLO8 and KPPLO9, respectively (Table 4.5). The percent of total methoxyflavones of KP extract-loaded PLO gels were of 99.86 and 99.37%.

Table 4.5 Formulations of KP extract-loaded PLO gels with various amounts of limonene.

Formulations	Percent (%)						
	KP Extract	Limonene	Lecithin	IPM	Oleic acid	Poloxamer	Water
KPPLO7	10.00	1.00	10.00	5.00	5.00	20.00	49.00
KPPLO8	10.00	5.00	10.00	5.00	5.00	20.00	45.00
KPPLO9	10.00	10.00	10.00	5.00	5.00	20.00	40.00

All formulations exhibited brown gel with the odor of limonene. Nevertheless, KPPLO9 was unstable gel after the 72-h storage, which the phase separation of limonene could be observed in PLO gel. The pH of Blank-PLO gels containing 1 and 5 % w/w limonene was 4.89±0.01 and 4.86±0.01, respectively. The pH of KP extract-loaded PLO gels containing 1 and 5 % w/w limonene was 4.70 ± 0.02 and 4.66 ± 0.2, respectively (Figure 4.21a). The viscosity of both Blank-PLO gels and KP extract-loaded PLO gels containing 1 and 5 % w/w limonene decreased when the concentration of limonene increased. The increasing concentration of limonene might decrease the amount of water in the formulation, thus reducing the gel formation [46].

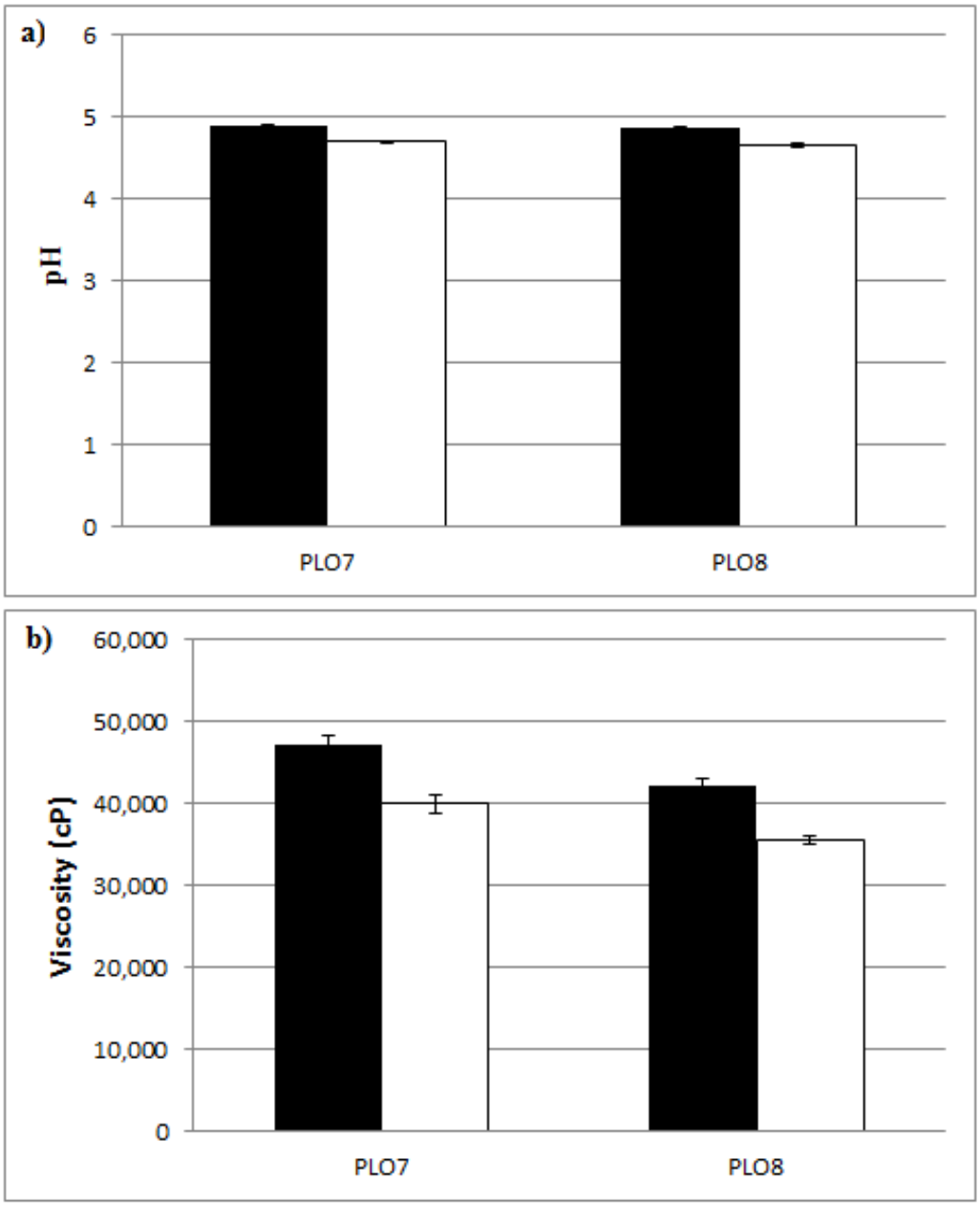


Figure 4.21 Characterization of (■) Blank- PLO gels and (□) KP extract-loaded PLO gels containing 1 and 5 % w/w limonene:
a) pH b) Viscosity.

The skin permeation profiles of KP extract-loaded PLO gels with and without limonene are shown as Figure 4.22. The skin permeation flux of KP extract-loaded PLO gels containing limonene (KPPLO7 and KPPLO8) was significantly higher than KP extract-loaded PLO gels without limonene (KPPLO3). KP extract-loaded PLO gels containing 5 % w/w limonene (KPPLO8) showed the highest skin permeation flux. Therefore, the best formula of KP extract-loaded PLO gels was composed of 10% KP extract, 10% lecithin, 5% oleic acid, 5% IPM, 5% limonene, 20% poloxamer and 45% water.

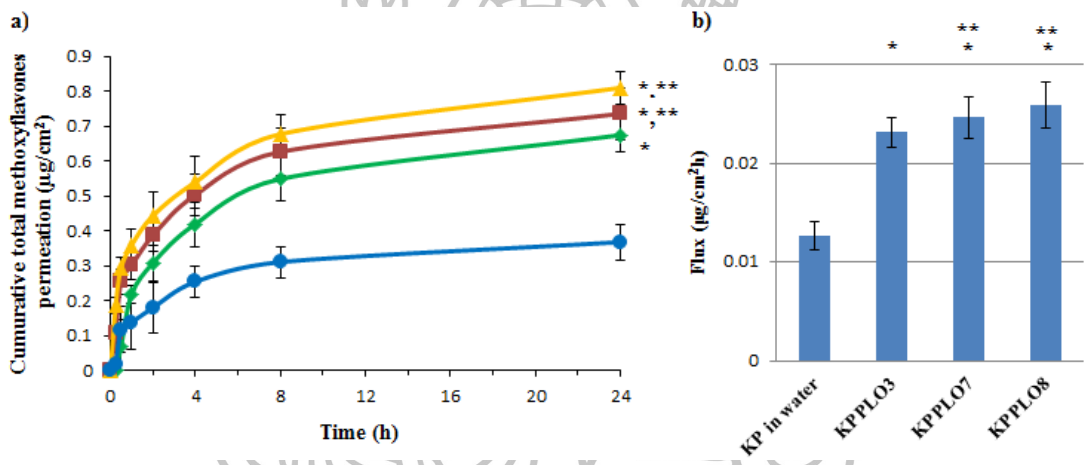


Figure 4.22 (a) Skin permeation profiles of KP extract-loaded PLO gel with various amount of limonene; (♦) KPPLO3, (■) KPPLO7, (▲) KPPLO8 and (●) KP in water and (b) flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with KP extract in water. ** $p < 0.05$, compared with KP extract-loaded PLO gel without limonene.

The highest skin permeation of KP extract-loaded microemulsion (KPME5), KP extract-loaded microemulgel (KPGE5) and KP extract-loaded PLO gel (KPPLO8) were selected and their skin permeation profiles and the flux were compared (Figure 4.23). The flux values of KPME5 (0.132±0.006 µg/cm^2/h) KPGE5 (0.044±0.002 µg/cm^2/h), KPPLO8 (0.026±0.002 µg/cm^2/h) were 10.4-, 3.4- and 2.0-fold higher than KP extract in water (0.013±0.002 µg/cm^2/h), respectively. These results indicated that the formulations affected the skin penetration of total methoxyflavones of KP extract. KPGE5 and KPPLO8 were developed in order to optimize their viscosity and appearance for topical application. Fouad et al. reported that microemulgel penetrated through stratum corneum and acted as drug sustained delivery [13]. Boddu et al. showed the efficiency of PLO gel for sustained drug delivery [67]. Nevertheless, this study showed that the skin permeation flux of KPME5 was significantly higher than KPGE5 and KPPLO8. From the *in vitro* skin permeation results, KP extract formulations that could be developed as product was KPME5. However, KPGE5 and KPPLO8 were appropriate to prepare for sustained transdermal delivery.

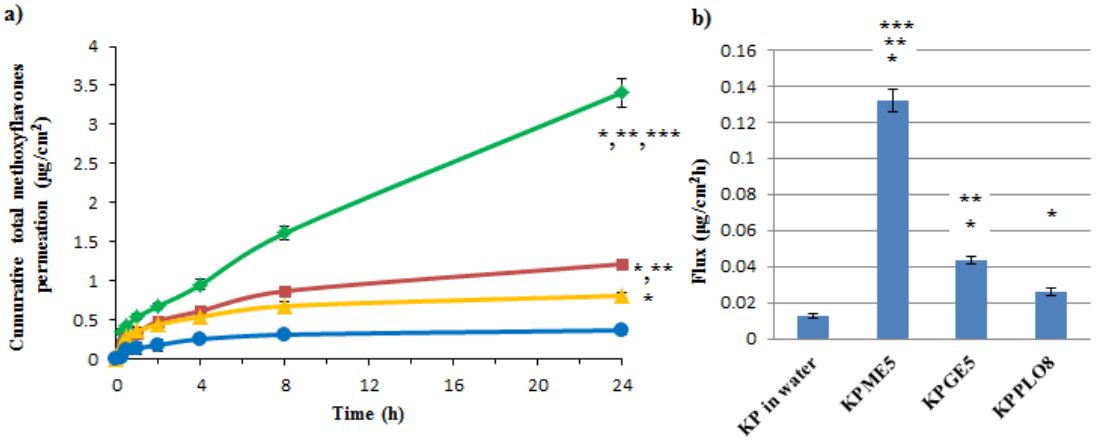


Figure 4.23 (a) Skin permeation profiles of (♦) KPME5, (■) KPGE5, (▲) KPPLO8 and (●) KP extract in water (b) and flux value. Each value represents the mean ± standard deviation (n=3). * $p < 0.05$, compared with KP extract in water. ** $p < 0.05$, compared with KPPLO8. *** $p < 0.05$, compared with KPGE5.

4.5 Stability evaluation

KP extract-loaded microemulsion (KPME5), KP extract-loaded microemulgel (KPGE5) and KP extract-loaded PLO gel (KPPLO8) were stored under accelerated condition (40 ± 2^{o} C, 75 ± 5 % RH) comparing with long term condition (room temperature) for 3 months. Both chemical and physical stability of the methoxyflavones were evaluated and the data are shown in Table 4.6 and 4.7. There was no significant change in the appearance over a period of 3 months. The pH of all the formulations slightly changed from the initial period but the pH of the KPPLO8 was tended to decrease under accelerated condition. The conductivity of KPME5 slightly changed from the initial period. The droplet sizes of microemulsion formulations slightly changed from the initial period, however, they were within the nano-size range (100.59-111.57 nm). The PDI values of microemulsion formulations were less than 0.500 (0.167-0.486) under both condition, indicating that the droplet size had high homogeneity [71]. The viscosity of KPGE5 and KPPLO8 was slightly decreased at accelerated condition. The percent of total methoxyflavone contents also slightly decreased under both condition. The results indicated that the KP extract loaded microemulsion, microemulgel and PLO gel were stable for 3 months under long term and accelerated condition.

Table 4.6 Percent of total methoxyflavones remaining and characterization of KP extract-loaded microemulsion (KPME5), KP extract-loaded microemulgel (KPGE5) and KP extract-loaded PLO gel (KPPLO8) under long term condition (room temperature). Each value represents the mean ± standard deviation (n=3).

Parameters	Initial	1 month	2 months	3 months
Appearance				
- KPME5	- Clear brown liquid	- Clear brown liquid	- Clear brown liquid	- Clear brown liquid
- KPGE5	- Clear brown gel	- Clear brown gel	- Clear brown gel	- Clear brown gel
- KPPLO8	- Opaque brown gel	- Opaque brown gel	- Opaque brown gel	- Opaque brown gel
% Methoxyflavones remaining				
- KPME5	100.00±0.00	98.26±1.46	101.44±0.70	98.52±1.02
- KPGE5	100.00±0.00	100.23±1.79	99.10±0.90	99.12±1.44
- KPPLO8	100.00±0.00	99.11±2.34	98.35±1.14	98.60±1.25
pH				
- KPME5	5.32±0.04	5.33±0.03	5.26±0.01	5.24±0.03
- KPGE5	4.89±0.02	4.86±0.03	4.84±0.02	4.79±0.01
- KPPLO8	4.66±0.02	4.63±0.03	4.58±0.02	4.51±0.06
Conductivity (µS/cm)				
- KPME5	52.6±0.6	53.4±0.2	56.5±0.2	56.6±0.7
Droplet size (nm)				
- KPME5	106.60±4.48	100.59±2.21	106.27±2.65	108.88±2.90
PDI				
- KPME5	0.196±0.065	0.326±0.095	0.351±0.102	0.309±0.124
Viscosity (cP)				
- KPGE5	11270±959	12129±769	12008±37	11156±881
- KPPLO8	35513±561	36090±902	36,965±552	35345±444

Table 4.6 Percent of total methoxyflavones remaining and characterization of KP extract-loaded microemulsion (KPME5), KP extract-loaded microemulgel (KPGE5) and KP extract-loaded PLO gel (KPPLO8) under accelerated condition (40 ± 2^{o} C, 75 ± 5 %RH). Each value represents the mean ± standard deviation (n=3).

Parameters	Initial	1 month	2 months	3 months
Appearance				
- KPME5	- Clear brown liquid	- Clear brown liquid	- Clear brown liquid	- Clear brown liquid
- KPGE5	- Clear brown gel	- Clear brown gel	- Clear brown gel	- Clear brown gel
- KPPLO8	- Opaque brown gel	- Opaque brown gel	- Opaque brown gel	- Opaque brown gel
% Methoxyflavones remaining				
- KPME5	100.00±0.00	98.76±0.34	99.20±1.95	98.29±0.89
- KPGE5	100.00±0.00	98.56±0.84	99.11±0.25	97.81±0.81
- KPPLO8	100.00±0.00	99.81±1.83	98.45±1.65	97.41±1.99
pH				
- KPME5	5.32±0.04	5.30±0.03	5.26± 0.01	5.24±0.03
- KPGE5	4.89±0.02	4.83±0.02	4.79±0.02	4.77±0.02
- KPPLO8	4.66±0.02	4.60±0.02	4.54±0.03	4.47± 0.02
Conductivity (µS/cm)				
- KPME5	52.6±0.6	55.5±0.3	58.2±0.3	59.1±0.2
Droplet size (nm)				
- KPME5	106.60±4.48	105.90±5.76	109.73±2.09	111.57±3.62
PDI				
- KPME5	0.196±0.065	0.272±0.109	0.432±0.165	0.486±0.097
Viscosity (cP)				
- KPGE5	11270±959	12371±609	11153±587	10201±239
- KPPLO8	35513±561	34950±552	33597±472	34508±2438

CHAPTER 5
CONCLUSIONS

In the present study, three types of transdermal delivery systems namely microemulsions (ME), microemulgels and PLO gels were successfully prepared for skin permeation enhancement of KP extract. The effect of the composition on the physicochemical characteristics and *in vitro* skin permeation were evaluated. The results of this study could be concluded as follow:

The ME systems composed of oleic acid as oil phase, Tween 20 as surfactant, propylene glycol as co-surfactant and water as aqueous phase were formulated. The surfactant mixture consisting of the Tween 20/propylene glycol was found to be better than another investigated surfactant systems as it could construct the largest ME area of pseudo-ternary phase diagram. ME consisted of the mixture of surfactant/co-surfactant at the ratio of 1:2 was selected for incorporating 10% KP extract. Limonene was used as the skin enhancer for enhancing the skin permeation of KP extract. 10% limonene incorporating into ME showed the highest total methoxyflavones flux.

KP extract-loaded ME with 10 % limonene were selected for incorporating gelling agent to form microemulgels. Among gelling agents, only xanthan gum could form microemulgels. 1.8% xanthan gum had a suitable viscosity for transdermal application. In addition, the amount of xanthan gum (1.6, 1.8 and 2.0 % w/w) did not significantly affect the total methoxyflavones flux. In comparison, the total methoxyflavones flux of KP extract-loaded ME (KPME5; without gelling agent) was significantly 3.0-fold greater than KP extract-loaded microemulgel (KPGE5; with 1.8% xanthan gum). Therefore, it was suggested that the type of gelling agents affected the forming of microemulgels and the skin permeation of KP extract.

In case of KP extract-loaded PLO gels, the factors affecting the skin permeation were types of oils, amount of poloxamer 407 and amount of limonene. KP extract-loaded PLO gels containing 10 % KP extract, 10% lecithin, 5% oleic acid, 5%

IPM, 5% limonene, 20% poloxamer 407 and 45% water showed the greatest skin permeation flux.

In comparison, the skin permeation flux of methoxyflavones from the investigate formulations was the increasing order of KPME5 (0.132±0.006 μg/cm^2/h) > KPGE5 (0.044±0.002 μg/cm^2/h) > KPPLO8 (0.026±0.002 μg/cm^2/h). However, microemulgels and PLO gels provided sustained skin permeation profiles and showed good appearance for transdermal delivery. The selected ME (KPME5), microemulgels (KPGE5) and PLO gels (KPPLO8) formulation showed good physical and chemical stability under storage condition. The results indicated that the KP-loaded ME, microemulgels and PLO gels were stable for storage at least 3 months.

REFERENCES

[1] Sripanidkulchai B. (2014). ***Kaempferia parviflora*: research and product development**. 2nd ed. Khonkaen: Klungnana inc.

[2] Chuthaputti A. (2013). "Krachai Dam (*Kaemferia parviflora* Wall. ex Baker): A Champion Herbal Product." **Journal Thai traditional & Alternative Medicine** 11, 1 (January-April): 4-16.

[3] Tewtrakul S, Subhadhirasakul S. (2008). "Effects of compounds from *Kaempferia parviflora* on nitric oxide, prostaglandin E_2 and tumor necrosis factor-alpha productions in RAW264.7 macrophage cells." **Journal of Ethnopharmacology** 120. 1 (October 30): 81-84.

[4] Sae-wong C, Tansakul P, Tewtrakul S. (2009). "Anti-inflammatory mechanism of *Kaempferia parviflora* in murine macrophage cells (RAW264.7) and in experimental animals." **Journal of Ethnopharmacology** 124, 3 (July 30): 576-580.

[5] Sae-wong C, Matsuda H, Tewtrakul S, Tansakul P, Nakamura S, Nomura Y, Yoshikawa M. (2011) "Suppressive effects of methoxyflavonoids isolated from *Kaempferia parviflora* on inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells." **Journal of Ethnopharmacology** 136, 3 (July 14): 488-495.

[6] Sutthanut K, Sripanidkulchai B, Yenjai C, Jay M. (2007). "Simultaneous identification and quantitation of 11 flavonoids constituents in *Kaempferia parviflora* by gas chromatography." **Journal of Chromatography A** 1143, 1-2 (March 2): 227-233.

[7] Sutthanut K, Lu X, Sripanidkulchai B, Yenjai C, Jay M. (2009). "Solid liquid nanoparticles for transdermal delivery of *Kaempferia parviflora* extracts." **Journal of Biomedical Nanotechnology** 5, 2 (April): 224-232.

[8] Mekjaruskul C, Jay M, Sripanidkulchai B. (2012). “Pharmacokinetics, tissue distribution, excretion, and metabolite identification of methoxyflavones in *Kaempferia parviflora* extract in rats.” **Drug Metabolism Disposition** 40, 12 (December): 2342-2353.

[9] Tuntiyasawasdikul S, Limpongsa E, Jaipakdee N, Sripanidkulchai B. (2014). “Transdermal permeation of *Kaempferia parviflora* methoxyflavones from isopropyl myristate-based vehicles.” **American Association of Pharmaceutical Scientists** 15, 4 (August): 947-955.

[10] Lawrence MJ, Rees GD. (2012). “Microemulsion-based media as novel drug delivery systems.” **Advanced Drug Delivery Reviews** 64, Supplement (December): 175-193.

[11] Patel HK, Barot BS, Parejiya PB, Shelat PK, Shukla A. (2013). “Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: Ex vivo permeation and skin irritation studies.” **Colloids and Surfaces B: Biointerfaces** 102, (February 1): 86-94.

[12] Tavano L, Alfano P, Muzzalupo R, Cindio BD. (2011). “Niosomes vs microemulsions: New carriers for topical delivery of Capsaicin.” **Colloids and Surfaces B: Biointerfaces** 87, 2 (October 15): 333-339.

[13] Fouad SA, Basalious EB, EL-Nabarawi MA, Tayel SA. (2013). “Microemulsion and poloxamer microemulsion-based gel for sustained transdermal delivery of diclofenac epolamine using in-skin drug depot: In vitro/in vivo evaluation**.**” **International Journal of Pharmaceutics** 453: 2 (September 10): 569-578.

[14] Vintiloiu A, Leroux JC. (2008). “Organogels and their use in drug delivery.” **Journal of Controlled Release** 125, 3 (February 11): 179-192.

[15] Kumar R, Katare OP. (2005). “Lecithin Organogels as a potential phospholipid-structured system for topical drug delivery: A review.” **American Association of Pharmaceutical Scientists** 6, 2 (October 6): 298-309.

[16] Bramwell BL & Williams LVA. (2012). “The use of Pluronic lecithin organogels in the transdermal delivery of drugs.” **International Journal of Pharmaceutics Compounding** 16, 1 (January/February): 62-63.

[17] Almeida H, Amaral MH, Lobão P, Lobo JMS. (2012). "Pluronic F-127 and pluronic lecithin organogel (PLO): Main features and their applications in topical and transdermal administration of drugs." **Journal of Pharmacy & Pharmaceutical Sciences** 15, 4 (October 25): 592–605.

[18] Department of Medical Sciences (2009). **Thai Herbal Pharmacopoeia 2009 volume III.** Bangkok : Prachachon Co.,Ltd.

[19] Picheansoonthon C & Koonterm S. (2008). "Notes on the genus Kaempferia L. (Zingiberaceae) in Thailand" **Journal Thai traditional & Alternative Medicine** 6: 73-93.

[20] Chaipech,S, Morikawa T, Ninomiya K, Yoshikawa M, Pongpiriyadacha Y, Hayakawa T, Muraoka O. (2012). "Structures of Two New Phenolic Glycosides, Kaempferiaosides A and B, and Hepatoprotective Constituents from the Rhizomes of *Kaempferia parviflora*" **Chem Pharm Bull** 60, 1 (October): 62-69.

[21] Chaipech,S, Morikawa T, Ninomiya K, Yoshikawa M, Pongpiriyadacha Y, Hayakawa T, Muraoka O. (2012). "New flav-3-en-3-ol glycosides, kaempferiaosides C and D, and acetophenone glycosides, kaempferiaosides E and F, from the rhizomes of *Kaempferia parviflora*" **Journal of Natural Medicines** 66, 3 (July): 486-492.

[22] Mekjaruskul C, Jay M, Sripanidkulchai B. (2012). "Modulatory effects of *Kaempferia parviflora* extract on mouse hepatic cytochrome P450 enzymes" **Journal of Ethnopharmacology** 141, 3 (June 14): 831-839.

[23] Horigome S, Yoshida I, Tsuda A, Harada T, Yamaguchi A, Yamazaki K, Inohana S, Isagawa S, Kibune N, Satoyama T, Katsuda S, Suzuki S, Watai M, Hirose N, Mitsue T, Shirakawa H, Komai M. (2014). "Identification and evaluation of anti-inflammatory compounds from *Kaempferia parviflora*" **Bioscience, Biotechnology, and Biochemistry** 78, 5 (May 15): 851-860.

[24] Sudwan P, Saenphet K, Saenphet S, Suwansirikul S. (2006). "Effect of *Kaempferia parviflora* Wall. Ex. Baker on sexual activity of male rats and its toxicity" **Southeast Asian Journal Tropic Medicine Pubic Heath** 37, 3: 210-215.

[25] Chaturapanich G, Chaiyakul S, Verawatnapakul V, Pholpramool C. (2008). "Effects of *Kaempferia parviflora* extracts on reproductive parameters and spermatic blood flow in male rats" **Society for Reproduction and Fertility** 136, 4 (October): 515-522.

[26] Temkitthawon P, Hinds TR, Beavo JA, Viyoch J, Suwanborirux K, Pongamornkul W, Sawasdee P, Ingkaninan K. 2011. "*Kaempferia parviflora*, a plant used in traditional medicine to enhance sexual performance contains large amounts of low affinity PDE5 inhibitors" **Journal of Ethnopharmacology** 137, 3 (October 11): 1437-1441.

[27] Wattanathorn J, Pangphukiew P, Muchimapura S, Sripanidkulchai K, Sripanidkulchai B. 2012. "Aphrodisiac activity of *Kaempferia parviflora*" **American Journal of Agricultural and Biological Sciences** 7, 2 (January): 114-120.

[28] Horigome S, Maeda M, Ho HJ, Shirakawa H, Komai M. 2016. "Effect of *Kaempferia parviflora* extract and its polymethoxyflavonoid components on testosterone production in mouse testis-derived tumour cells" **Journal of Functional Foods** 26, (October): 529-538.

[29] Akase T, Shimada T, Terabayashi S, Ikeya Y, Sanada H, Aburada M. 2011. "Antiobesity effects of *Kaempferia parviflora* in spontaneously obese type II diabetic mice" **Journal of Natural Medicines** 65, 1 (January) 65: 73-80.

[30] Shimada T, Horikawa T, Ikeya Y, Matsuo H, Kinoshita K, Taguchi T, Ichinose K, Takahashi K, Aburada M. 2011. "Preventive effect of *Kaempferia parviflora* ethyl acetate extract and its major components Polymethoxyflavonoid on metabolic diseases" **Fitoterapia** 82, 8 (September 1): 1272-1278.

[31] Horikawa T, Shimada T, Okabe Y, Kinoshita K, Koyama K, Miyamoto K, Ichinose K, Takahashi K, Aburada M. 2012. "Polymethoxyflavonoids from *Kaempferia parviflora* induce adipogenesis on 3T3-L1 preadipocytes by regulating transcription factors at an early stage of differentiation" **Biological & Pharmaceutical Bulletin** 35, 5 (Feburary 29): 686-692.

[32] Yenjai C, Prasanphen K, Daodee S, Wongpanich V, Kittakoop P. 2004. "Bioactive flavonoids from *Kaempferia parviflora*" **Fitoterapia** 75, 1 (January): 89-92.

[33] Kummee S, Tewtrakul S, Subhadhirasakul S. 2008. "Antimicrobial activity of the ethanol extract and compounds from the rhizomes of *Kaempferia parviflora*" **Songklanakarin Journal Science Technology** 30, 4 (July-August): 463-466.

[34] Chaichanawongsaroj N, Amonyingcharoen S, Saifah E, Poovorawan Y. 2010. "The effects of *Kaempferia parviflora* on anti-internalization activity of *Helicobacter pylori* to HEp-2 cells" **African Journal of Biotechnology** 30, 9 (July): 4796-4801.

[35] Tewtrakul S, Subhadhirasakul S, Kummee S. 2008. "Anti-allergic activity of compounds from *Kaempferia parviflora*" **Journal of Ethnopharmacology** 116, 1 (February 28): 191-193.

[36] Kobayashi S, Kato T, Azuma T, Kikuzaki H, Abe K. 2015. "Anti-allergenic activity of polymethoxyflavones from *Kaempferia parviflora*" **Journal of functional foods** 13, (March) 100-107.

[37] Rujjanawate C, Kanjanapothi D, Amornlerdpison D, Pojanagaroon S. 2005. "Anti-gastric ulcer effect of *Kaempferia parviflora*" **Journal of Ethnopharmacology** 102, 1 (October 31): 120-122.

[38] Wattanapitayakul SK, Suwatronnakorn M, Chularojmontri L, Herunsalee A, Niumsakul S, Charuchongkolwongse S, Chansuvanich N. 2007. "*Kaempferia parviflora* ethanolic extract promoted nitric oxide production in human umbilical vein endothelial cells" **Journal of Ethnopharmacology** 110, 3 (April 4): 559-562.

[39] Nakao K, Murata K, Deguchi T, Itoh K, Fujita T, Higashino M, Yoshioka Y, Matsumura S, Tanaka R, Shibada T, Ohfune Y, Matsuda H. 2012. "Xanthine oxidase inhibitory activities and crystal structures of methoxyflavones from Kaempferia parviflora rhizome" **Biological & Pharmaceutical Bulletin** 34, 7 (April 12): 1143-1146.

[40] Chivapat S, Chansuvanich S, Chavalittumrog P, Attawish A, Phadungpat S, Punyamong S, Pama K. 2004. "Acute and chronic toxicity study of *Kaempferia parviflora* Wall ex. Baker powder" **Journal Thai traditional & Alternative Medicine** 2, 2 (February-May): 3-16.

[41] Chivapat S, Chavalittumrong P, Attawish1 A. Rungsipipat A. 2010. "Chronic toxicity study of *Kaempferia parviflora* Wall ex. extract" **The Thai Journal of Veterinary Medicine** 40, 4: 377-383.

[42] Yorsin S, Kanokwiroon K, Radenahmad N, Jansakul C. 2014. "Effects of *Kaempferia parviflora* rhizomes dichloromethane extract on vascular functions in middle-aged male rat" Journal of **Ethnopharmacology**156, (October 28):162-174.

[43] Mekjaruskula C, Yang YT, Leed MGD, Sadgrove MP, Jay M, Sripanidkulchai B. 2013. "Novel formulation strategies for enhancing oral delivery of methoxyflavones in *Kaempferia parviflora* by SMEDDS or complexation with 2-hydroxypropyl-β-cyclodextrin" **International Journal of Pharmaceutics** 445, 1-2 (March 10): 1-11.

[44] Tuntiyasawasdikul S, Limpongsa E, Jaipakdee N, Sripanidkulchai B. 2015. "A monolithic drug-in-adhesive patch of methoxyflavones from *Kaempferia parviflora*: In vitro and in vivo evaluation" **International Journal of Pharmaceutics** 478, 2 (January 30): 486-495.

[45] Burguera JL, Burguera M. 2012. "Analytical applications of emulsions and microemulsions." **Talanta** 96, (July 15): 11-20.

[46] Kogan A, Garti N. 2006. "Microemulsions as transdermal drug delivery vehicles." **Advances in Colloid and Interface Science** 123-126, (November 16): 369-385.

[47] Talegaonkar S, Azeem A, Ahmad FJ, Khar RK, Pathan SA, Khan ZI. 2008. "Microemulsions: A Novel Approach to Enhanced Drug Delivery" **Recent Patents on Drug Delivery & Formulation** 2, 3: 238-257.

[48] Jadhav KR, Shaikh IM, Ambade KW, Kadam VJ. 2006. "Applications of microemulsion based drug delivery system." **Current Drug Delivery** 3, 3 (July): 267-273.

[49] Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, Ali M. 2007. "Development and bioavailability assessment of ramipril nanoemulsion formulation." **European Journal of Pharmaceutical and Biopharmaceutics** 66, 2 (May): 227-243.

[50] Amnon C. Sintov. 2015. "Transdermal delivery of curcumin *via* microemulsion" **International Journal of Pharmaceutics** 481, 1–2 (March 15): 97-103.

[51] Hathout RM. Mansour S, Geneidi AS, Mortada ND. 2011."Visualization, dermatopharmacokinetic analysis and monitoring the conformational effects of a microemulsion formulation in the skin stratum corneum." **Journal of Colloid and Interface Science** 354, 1 (February 1) 124–130.

[52] Maghraby GE. 2012. "Microemulsions as transdermal drug delivery systems." **Current Nanoscience** 8, 4 (September): 504-511.

[53] Che J, Wu Z, Shao W, Guo P, Lin Y, Pan W, Zeng W, Zhang G, Wu C, Xu Y. 2015. "Synergetic skin targeting effect of hydroxypropyl-β-cyclodextrin combined with microemulsion for ketoconazole" European **Journal of Pharmaceutics and Biopharmaceutics** 93, (June): 136-148**.**

[54] Kalpesh C. Ashara KC, Paun JS, Soniwala MM, Chavda JR, Mendapara VP, Mori NM. 2016. "Microemulgel: an overwhelming approach to improve therapeutic action of drug moiety" **Saudi Pharmaceutical Journal** 24, 4 (July): 452-457.

[55] Spiclin P, Homar M, Valant AZ, Gasperlin M. 2003. "Sodium ascorbyl phosphate in topical microemulsions". **International Journal of Pharmaceutics** 256, 1-2 (April): 65-73.

[56] Bachhav YG, Patravale VB. 2009. "Microemulsion based vaginal gel of fluconazole: Formulation, *in vitro* and *in vivo* evaluation". **International Journal of Pharmaceutics** 365, 1-2 (January 5): 175-179.

[57] Patel HK, Barot BS, Parejiya PB, Shelat PK, Shukla A. 2014. "Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo – Part II: Rheological characterization and in vivo assessment through dermatopharmacokinetic and pilot clinical studies" **Colloids and Surfaces B: Biointerfaces** 119, (July 1): 145-153.

[58] Wan T, Xu T, Pan J, Qin M, Pan W, Zhang G, Wu Z, Wu C, Xu Y. 2015. "Microemulsion based gel for topical dermal delivery of pseudolaric acid B: In vitro and in vivo evaluation". **International Journal of Pharmaceutics** 493, 1-2 (September 30): 111-120.

[59] Hoffman AS. 2012. "Hydrogels for biomedical applications" **Advanced Drug Delivery Reviews** 64, 1 (January 17) 18-23.

[60] Anand B, Pisal SS, Paradkar AR, Mahadik, K.R. 2001. "Applications of organogels in pharmaceuticals". **Journal of scientific and industrial research** 60, 4(April): 311-318.

[61] Kirilov P, Tran VH, Tassela AD, Salvia JP, Perrot S, Haftek M, Boulieu R, Pirot F. 2016. "Ex-Vivo percutaneous absorption of enrofloxacin: Comparison of LMOG organogel vs. pentravan cream". **International Journal of Pharmaceutics** 498, 1-2 (Februray 10) 170-177.

[62] Trickett K, Eastoe J. 2008. "Surfactant-based gels." **Advances in Colloid and Interface Science** 144, 1-2 (December 2) 66-74.

[63] Raut S, Bhadoriya SS, Uplanchiwar V, Mishra V, Gahane A, Jain SK. 2012. "Lecithin organogel: A unique micellar system for the delivery of bioactive agents in the treatment of skin aging." **Acta Pharmaceutica Sinica B** 2. 1 (February 10): 8-15.

[64] Jones M. 2005. "The history of pluronic lecithin organogel." **International \ Journal of Pharmaceutical Compounding** 7, 3 (May-June): 180-182.

[65] Dumortier G, Grossiord JL, Agnely F, Chaumeil JC. 2006. "A Review of Poloxamer 407 Pharmaceutical and Pharmacological Characteristics." **Pharmaceutical Research** 23, 12 (December): 2709-2728.

[66] Agrawal V, Gupta V, Ramteke S, Trivedi P. 2010. “Preparation and evaluation of tubular micelles of pluronic lecithin organogel for transdermal delivery of sumatriptan.” **American Association of Pharmaceutical Scientists** 11, 4 (December): 1718-1725.

[67] Boddu SHS, Gupta H, Bonam SP. 2014. “Preclinical evaluation of a ricinoleic acid poloxamer gel system for transdermal eyelid delivery.” **International Journal of Pharmaceutics** 470, 1-2 (August 15): 158-161.

[68] Santoyo, S, Ygartua P. (2000). "Effect of skin pretreatment with fatty acids on percutaneous absorption and skin retention of piroxicam after its topical application." **European Journal of Pharmaceutics and Biopharmaceutics** 50, 2 (September): 245-250.

[69] Engelbrecht TN, Schroeter A, Hauss T, Neubert RH. (2011). “Lipophilic penetration enhancers and their impact to the bilayer structure of stratum corneum lipid model membranes: Neutron diffraction studies based on the example oleic acid. **Biochimica et Biophysica Acta (BBA) –Biomembranes** 1808, 2 (December): 2798-2806.

[70] Mehta SK and Kaur G. (2011). “Microemulsions: Thermodynamic and Dynamic Properties.” **Thermodynamics**. Accessed January 10. Available from:http://www.intechopen.com/books/thermodynamics/microemulsions-thermodynamic-and-dynamic-properties.

[71] Hathout RM, Woodman TJ, Mansour S, Mortada ND, Geneidi AS, Guy RH. (2010). "Microemulsion formulations for the transdermal delivery of testosterone." **European Journal of Pharmaceutical Sciences** 40, 3 (June 4): 188-196.

[72] Okur NU, Apaydın S, Yavaşoğlu NUK, Yavaşoğlu A, Karasulu HY. (2011). "Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations." **International Journal of Pharmaceutics** 416, 1 (September 5): 136-144.

[73] Liu CH, Chang FY, Hung DK**. (2011). “**Terpene microemulsions for transdermal curcumin delivery: Effects of terpenes and cosurfactants.” **Colloids and Surfaces B: Biointerfaces** 82, 1 (January 1): 63-70.

[74] Gannu R, Palem CR, Yamsani VV, Yamsani SK, Yamsani MR. (2011). Enhanced bioavailability of lacidipine via microemulsion based transdermal gels: Formulation optimization, ex vivo and in vivo characterization. **International Journal of Pharmaceutics** 388, 1-2 (March 30): 231–241.

[75] Devi DR, Sandhya P, Hari BV. (2013). Poloxamer: A novel functional molecule for drug delivery and gene therapy. **Journal of Pharmaceutical Sciences and Research** 5, 8: 159-165.

APPENDIX

APPENDIX A

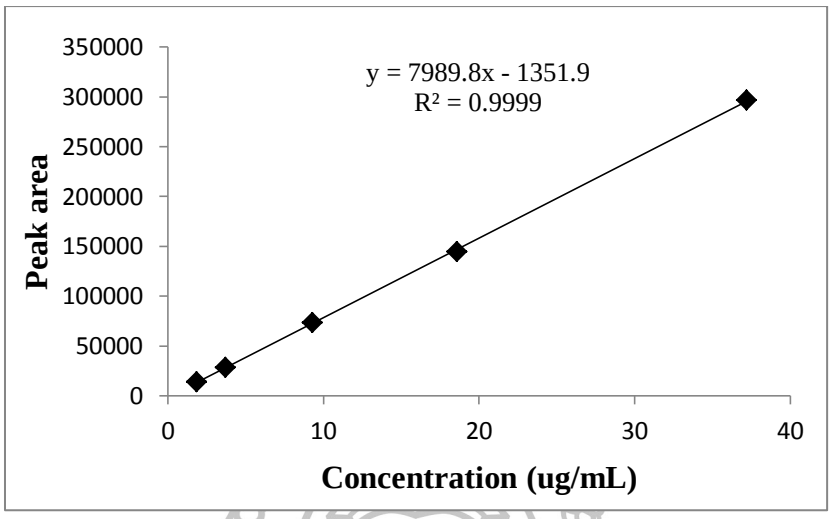


Figure A.1 Standard curve of DMF for analysis solubility.

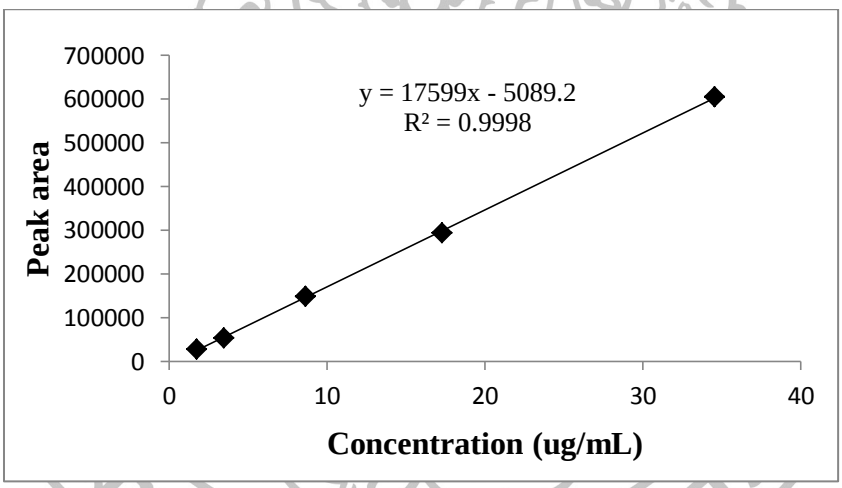


Figure A.2 Standard curve of TMF for analysis solubility.

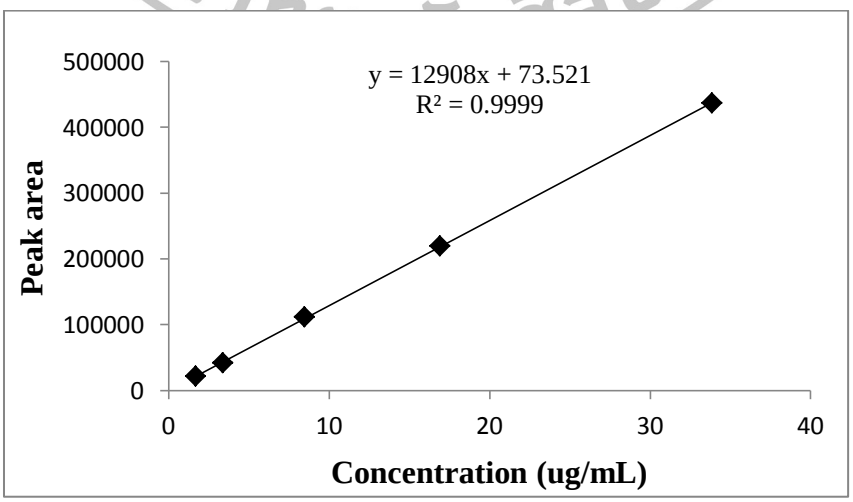


Figure A.3 Standard curve of PMF for analysis solubility.

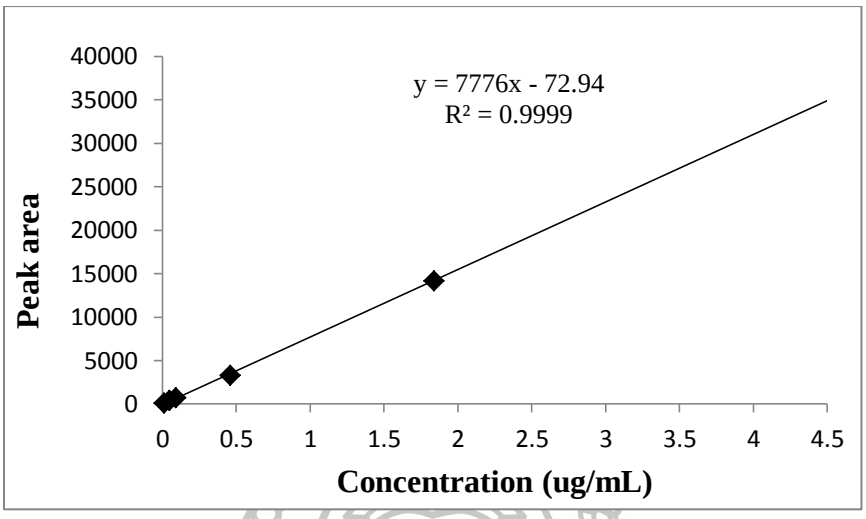


Figure A.4 Standard curve of DMF for percent content and skin permeation studies.

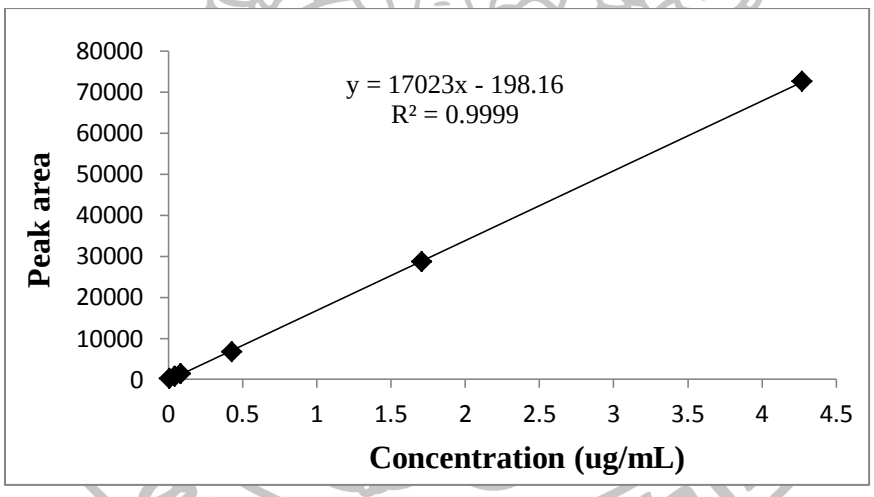


Figure A.5 Standard curve of TMF for percent content and skin permeation studies.

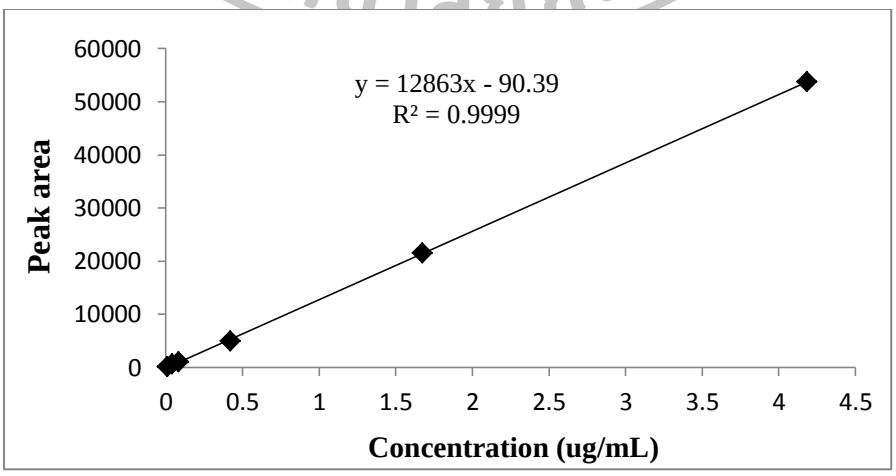


Figure A.6 Standard curve of PMF for percent content and skin permeation studies.

APPENDIX B

Table B.1 The solubility of DMF, TMF, PMF and total methoxyflavone in water, oils, surfactants and co-surfactants. Each value represents the mean ± standard deviation (n=3).

Ingredients	Solubility (mg/mL)			
	DMF	TMF	PMF	Total methoxyflavones
Water	0.392 ± 0.015	0.315 ± 0.012	0.408 ± 0.014	1.115 ± 0.050
Isopropyl palmitate	5.520 ± 0.487	3.417 ± 0.279	3.263 ± 0.062	12.201 ± 1.261
Isopropyl myristate	10.097 ± 0.123	3.324 ± 0.048	4.544 ± 0.083	17.965 ± 3.610
Oleic Acid	73.147 ± 0.953	72.262 ± 1.003	73.533 ± 1.348	218.942 ± 0.651
Caprylic/capric triglyceride	14.620 ± 0.440	11.114 ± 0.319	12.232 ± 0.427	37.965 ± 1.791
Cremophor® RH40	19.857 ± 1.061	19.396 ± 1.365	20.792 ± 1.415	60.045 ± 0.711
Cremophor® EL	22.934 ± 1.255	22.557 ± 1.294	24.370 ± 1.480	69.860 ± 0.957
Tween 20	39.109 ± 2.727	38.171 ± 2.666	38.580 ± 2.715	115.859 ± 0.470
Propylene glycol	67.562 ± 4.639	67.076 ± 4.536	68.318 ± 4.677	202.956 ± 0.626
Butylene glycol	45.313 ± 1.755	45.322 ± 2.240	46.503 ± 2.949	137.139 ± 0.684
Ethoxydiglycol	69.083 ± 1.134	68.849 ± 1.159	70.119 ± 1.117	208.051 ± 0.676

Table B.2 Percent total methoxyflavone content and physicochemical properties of Blank-microemulsion and KP extract-loaded microemulsion formulations. Each value represents the mean ± standard deviation (n=3).

Formulations	Percent content	pH	Conductivity (μS/cm)	Size (nm)	PDI
KPME1	101.54 ± 0.66	5.36 ± 0.02	59.73 ± 1.60	48.27 ± 0.54	0.311 ± 0.057
ME1	-	5.45 ± 0.03	25.17 ± 0.38	35.82 ± 5.02	0.217 ± 0.015
KPME2	100.48 ± 0.91	5.65 ± 0.02	66.87 ± 2.34	18.91 ± 1.84	0.289 ± 0.027
ME2	-	5.86 ± 0.04	28.03 ± 0.99	21.44 ± 2.87	0.202 ± 0.024
KPME3	100.08 ± 0.43	5.35 ± 0.01	57.53 ± 0.51	46.87 ± 3.76	0.261 ± 0.017
ME3	-	5.42 ± 0.02	24.20 ± 0.70	51.66 ± 2.20	0.226 ± 0.046
KPME4	99.88 ± 0.50	5.34 ± 0.04	54.83 ± 0.55	77.98 ± 6.00	0.306 ± 0.048
ME4	-	5.40 ± 0.02	24.10 ± 0.17	73.20 ± 6.23	0.236 ± 0.052
KPME5	102.12 ± 1.60	5.32 ± 0.04	52.63 ± 0.57	105.60 ± 4.48	0.289 ± 0.075
ME5	-	5.39 ± 0.02	26.60 ± 0.20	116.96 ± 5.04	0.196 ± 0.059

Table B.3 Percent total methoxyflavone content and physicochemical properties of Blank-microemulgel and KP extract-loaded microemulgel formulations. Each value represents the mean ± standard deviation (n=3).

Formulations	Percent content	pH	Viscosity (cP)
KPGE1	101.12 ± 0.16	4.94 ± 0.03	13285.23 ± 1173.07
GE1	-	5.09 ± 0.02	21234.49 ± 894.53
KPGE2	101.02 ± 1.53	4.92 ± 0.05	16402.66 ± 915.95
GE2	-	5.13 ± 0.01	24530.40 ± 1357.75
KPGE3	99.87 ± 1.06	4.96 ± 0.02	18075.74 ± 1125.66
GE3	-	5.10 ± 0.02	27723.72 ± 606.82
KPGE4	100.27 ± 1.10	4.92 ± 0.04	9846.67 ± 921.66
GE4	-	5.07 ± 0.03	15521.79 ± 617.41
KPGE5	100.33 ± 1.92	4.89 ± 0.02	11269.77 ± 959.26
GE5	-	5.04 ± 0.03	19137.24 ± 987.96
KPGE6	100.51 ± 0.22	4.87 ± 0.02	12961.74 ± 533.74
GE6	-	5.02 ± 0.03	21752.40 ± 579.89

Table B.4 Percent total methoxyflavone content and physicochemical properties of Blank-PLO gel and KP extract-loaded PLO gel formulations. Each value represents the mean ± standard deviation (n=3).

Formulations	Percent content	pH	Viscosity (cP)
KPPLO1	99.84 ± 0.20	4.70 ± 0.03	34405.52 ± 1494.80
PLO1	-	4.91 ± 0.02	43162.05 ± 987.88
KPPLO2	98.36 ± 0.82	4.63 ± 0.05	37114.82 ± 893.92
PLO2	-	4.90 ± 0.03	46729.15 ± 1969.79
KPPLO3	99.44 ± 1.28	4.66 ± 0.04	42256.96 ± 771.47
PLO3	-	4.88 ± 0.03	52453.06 ± 651.20
KPPLO5	101.07 ± 0.25	4.67 ± 0.02	38730.81 ± 1007.86
PLO5	-	4.91 ± 0.03	45368.75 ± 2350.92
KPPLO6	98.37 ± 1.03	4.69 ± 0.04	49458.24 ± 1459.63
PLO6	-	4.92 ± 0.02	62266.03 ± 2507.50
KPPLO7	99.86 ± 1.01	4.70 ± 0.02	39933.79 ± 1158.45
PLO7	-	4.89 ± 0.01	47250.00 ± 977.04
KPPLO8	99.37 ± 2.07	4.66 ± 0.02	35513.29 ± 561.49
PLO8	-	4.86 ± 0.01	42137.04 ± 998.88

Table B.5 Percent total methoxyflavone content of extract-loaded microemulsion (KPME5), KP extract-loaded microemulgel (KPGE5) and KP extract-loaded PLO gel (KPPLO8) under long term condition (room temperature). Each value represents the mean ± standard deviation (n=3).

Formulations	Percent content			
	Initial	1 month	2 months	3 months
KPME5	102.12 ± 1.60	100.33 ± 0.52	103.59 ± 1.32	100.62 ± 1.97
KPGE5	100.33 ± 1.92	100.57 ± 3.23	99.43 ± 2.16	99.47 ± 2.91
KPPLO8	99.37 ± 2.07	98.46 ± 0.86	97.74 ± 3.02	97.97 ± 2.02

Table B.6 Percent total methoxyflavone content of extract-loaded microemulsion (KPME5), KP extract-loaded microemulgel (KPGE5) and KP extract-loaded PLO gel (KPPLO8) under accelerated (40 ± 2^{o} C, 75 ± 5 %RH). Each value represents the mean ± standard deviation (n=3).

Formulations	Percent content			
	Initial	1 month	2 months	3 months
KPME5	102.12 ± 1.60	100.86 ± 1.66	101.30 ± 2.05	100.38 ± 1.94
KPGE5	100.33 ± 1.92	98.90 ± 2.21	99.45 ± 1.99	98.13 ± 1.18
KPPLO8	99.37 ± 2.07	99.16 ± 1.95	97.85 ± 3.49	96.73 ± 1.41

APPENDIX C

Table C.1 The cumulative permeation of KPME1.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0623±0.0077	0.0630±0.0087	0.0617±0.0067	0.1870±0.0231
0.5	0.0828±0.0097	0.0833±0.0106	0.0854±0.0087	0.2516±0.0290
1	0.1289±0.0129	0.1168±0.0135	0.1270±0.0117	0.3726±0.0382
2	0.1746±0.0150	0.1591±0.0165	0.1735±0.0155	0.5072±0.0465
4	0.2538±0.0216	0.2371±0.0223	0.2573±0.0212	0.7483±0.0647
8	0.3796±0.0314	0.3468±0.0305	0.3689±0.0285	1.0952±0.0905
24	0.7906±0.0564	0.7243±0.0537	0.8019±0.0490	2.3167±0.1573

Table C.2 The cumulative permeation of KPME2.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0763±0.0051	0.0753±0.0043	0.0694±0.0045	0.2209±0.0139
0.5	0.1125±0.0084	0.1069±0.0067	0.1050±0.0073	0.3244±0.0223
1	0.1212±0.0090	0.1172±0.0074	0.1124±0.0082	0.3508±0.0245
2	0.1743±0.0138	0.1633±0.0110	0.1663±0.0129	0.5038±0.0376
4	0.2271±0.0243	0.2268±0.0162	0.2302±0.0255	0.6841±0.0658
8	0.3567±0.0292	0.3212±0.0241	0.3479±0.0426	1.0259±0.0956
24	0.7540±0.0689	0.7088±0.0571	0.7720±0.0835	2.2348±0.2093

Table C.3 The cumulative permeation of KPME3.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.1083±0.0069	0.1002±0.0055	0.1084±0.0077	0.3169±0.0201
0.5	0.1333±0.0089	0.1300±0.0092	0.1324±0.0108	0.3957±0.0265
1	0.1582±0.0113	0.1614±0.0152	0.1689±0.0132	0.4886±0.0393
2	0.1866±0.0137	0.1892±0.0177	0.1948±0.0229	0.5706±0.0538
4	0.2724±0.0209	0.2766±0.0211	0.2870±0.0275	0.8360±0.0694
8	0.3728±0.0352	0.3761±0.0348	0.4043±0.0407	1.1532±0.1106
24	0.9029±0.0802	0.8614±0.0760	0.9327±0.0856	2.6969±0.2418

Table C.4 The cumulative permeation of KPME4.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.1179±0.0061	0.1082±0.0053	0.1182±0.0068	0.3443±0.0182
0.5	0.1438±0.0071	0.1301±0.0069	0.1328±0.0077	0.4067±0.0217
1	0.1693±0.0084	0.1685±0.0081	0.1810±0.0096	0.5188±0.0260
2	0.1993±0.0096	0.1977±0.0092	0.2024±0.0104	0.5993±0.0292
4	0.2985±0.0155	0.3012±0.0154	0.3108±0.0171	0.9106±0.0479
8	0.4426±0.0248	0.4447±0.0238	0.4816±0.0276	1.3689±0.0761
24	1.0393±0.0568	0.9833±0.0572	1.0739±0.0601	3.0965±0.1741

Table C.5 The cumulative permeation of KPME5.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.1183±0.0169	0.1118±0.0071	0.1206±0.0189	0.3507±0.0428
0.5	0.1427±0.0054	0.1393±0.0.0099	0.1441±0.0099	0.4261±0.0230
1	0.1792±0.0076	0.1715±0.0132	0.1887±0.0181	0.5393±0.0386
2	0.2264±0.0101	0.2148±0.0116	0.2365±0.0112	0.6777±0.0325
4	0.3158±0.0167	0.2988±0.0234	0.3326±0.0296	0.9472±0.0687
8	0.5342±0.0235	0.5076±0.0281	0.5690±0.0333	1.6108±0.0847
24	1.1190±0.0638	1.0782±0.0560	1.2095±0.0617	3.4067±0.1788

Table C.6 The cumulative permeation of KPGE1.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0000±0.0000	0.0000±0.0000	0.0383±0.0332	0.0383±0.0332
0.5	0.0454±0.0403	0.0186±0.0322	0.0806±0.0021	0.1446±0.0565
1	0.0911±0.0097	0.0837±0.0121	0.1035±0.0028	0.2782±0.0216
2	0.1191±0.0101	0.1048±0.0084	0.1286±0.0049	0.3525±0.0208
4	0.1418±0.0020	0.1299±0.0055	0.1552±0.0027	0.4269±0.0085
8	0.1843±0.0076	0.1691±0.0057	0.2025±0.0041	0.5559±0.0153
24	0.2621±0.0051	0.2417±0.0042	0.2828±0.0087	0.7866±0.0032

Table C.7 The cumulative permeation of KPGE2.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0611±0.0017	0.0580±0.0017	0.0569±0.0016	0.1760±0.0050
0.5	0.0720±0.0022	0.0679±0.0021	0.0688±0.0021	0.2086±0.0064
1	0.0869±0.0027	0.844±0.0027	0.0840±0.0026	0.2554±0.0079
2	0.1080±0.0032	0.0991±0.0037	0.1050±0.0041	0.3121±0.0109
4	0.1296±0.0045	0.1228±0.0048	0.1311±0.0054	0.3835±0.0146
8	0.1691±0.0088	0.1574±0.0083	0.1729±0.0095	0.4994±0.0263
24	0.2611±0.0075	0.2468±0.0070	0.2641±0.0101	0.7721±0.0227

Table C.8 The cumulative permeation of KPGE3.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0151±0.0262	0.0000±0.0000	0.0448±0.0029	0.0599±0.0233
0.5	0.0586±0.0008	0.0545±0.0035	0.0583±0.0072	0.1715±0.0111
1	0.0708±0.0018	0.692±0.0049	0.0735±0.0056	0.2135±0.0123
2	0.929±0.0038	0.0859±0.0066	0.0968±0.0077	0.2755±0.0180
4	0.1185±0.0061	0.1141±0.0090	0.1318±0.0106	0.3644±0.0257
8	0.1539±0.0092	0.1455±0.0119	0.1719±0.0141	0.4713±0.0351
24	0.2384±0.0156	0.2237±0.0179	0.2540±0.0205	0.7162±0.0540

Table C.9 The cumulative permeation of KPGE4.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0732±0.0133	0.0671±0.0114	0.0679±0.0122	0.2083±0.0367
0.5	0.100±0.0204	0.0792±0.0159	0.0857±0.0170	0.2650±0.0532
1	0.1233±0.0264	0.1067±0.0229	0.1178±0.0243	0.3478±0.0735
2	0.1686±0.0373	0.1582±0.0339	0.1718±0.0370	0.4985±0.1078
4	0.2191±0.0423	0.2039±0.0300	0.2243±0.0378	0.6473±0.1096
8	0.2968±0.0280	0.2736±0.0238	0.3048±0.0345	0.8751±0.0841
24	0.4096±0.0202	0.3918±0.0094	0.4311±0.0132	1.2325±0.0416

Table C.10 The cumulative permeation of KPGE5.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0696±0.0037	0.0649±0.0019	0.0658±0.0012	0.2003±0.0055
0.5	0.0960±0.0035	0.0857±0.0012	0.0920±0.0015	0.2738±0.0041
1	0.1188±0.0035	0.1040±0.0009	0.1145±0.0022	0.3374±0.0038
2	0.1640±0.0175	0.1549±0.0129	0.1662±0.0155	0.4851±0.0455
4	0.2086±0.0155	0.1938±0.0173	0.2114±0.0210	0.6138±0.0529
8	0.2969±0.0239	0.2704±0.0180	0.3011±0.0236	0.8684±0.0643
24	0.3991±0.0172	0.3910±0.0220	0.4240±0.0115	1.2141±0.0504

Table C.11 The cumulative permeation of KPGE6.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0696±0.0012	0.0677±0.0062	0.0687±0.0029	0.2061±0.0064
0.5	0.0885±0.0014	0.0845±0.0087	0.0889±0.0041	0.2619±0.0095
1	0.1086±0.0018	0.1021±0.0114	0.1104±0.0055	0.3210±0.0130
2	0.1456±0.0070	0.1404±0.0126	0.1530±0.0127	0.4390±0.0237
4	0.2066±0.231	0.1879±0.0220	0.2123±0.0304	0.6069±0.0736
8	0.2911±0.0325	0.2786±0.0392	0.3052±0.0292	0.8749±0.0988
24	0.3929±0.0171	0.3775±0.0321	0.4278±0.0175	1.1633±0.0190

Table C.12 The cumulative permeation of KPPLO1.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
0.5	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
1	0.0000±0.0000	0.0000±0.0000	0.0244±0.0000	0.0244±0.0422
2	0.0765±0.0189	0.0772±0.0178	0.0821±0.0204	0.2358±0.0568
4	0.1210±0.0122	0.1114±0.0130	0.1227±0.0110	0.3551±0.0329
8	0.1549±0.0143	0.1504±0.0117	0.1612±0.0136	0.4665±0.0385
24	0.2026±0.0115	0.1981±0.0118	0.2100±0.0109	0.6106±0.0324

Table C.13 The cumulative permeation of KPPLO2.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
0.5	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
1	0.0428±0.0374	0.0416±0.0362	0.0434±0.0382	0.1278±0.1117
2	0.0610±0.0530	0.0592±0.0514	0.0606±0.0532	0.1808±0.1574
4	0.1082±0.0364	0.1039±0.0324	0.1059±0.0365	0.3180±0.1051
8	0.1477±0.0332	0.1359±0.0287	0.1471±0.0247	0.4307±0.0865
24	0.1831±0.0260	0.1705±0.0261	0.1922±0.0217	0.5458±0.0737

Table C.14 The cumulative permeation of KPPLO3.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
0.5	0.0244±0.0423	0.0000±0.0000	0.0450±0.0395	0.0694±0.0741
1	0.0730±0.0108	0.0696±0.0060	0.0747±0.0082	0.2173±0.0246
2	0.1030±0.0208	0.0988±0.0117	0.1054±0.0201	0.3073±0.0521
4	0.1409±0.0212	0.1309±0.0141	0.1470±0.0285	0.4187±0.0635
8	0.1870±0.0183	0.1670±0.0183	0.1944±0.0274	0.5484±0.0639
24	0.2215±0.0194	0.2084±0.0132	0.2440±0.0141	0.6740±0.0464

Table C.15 The cumulative permeation of KPPLO5.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000	0.0000±0.0000
0.5	0.0161±0.0279	0.0000±0.0000	0.0156±0.0269	0.0317±0.0548
1	0.0634±0.0038	0.0584±0.0042	0.0666±0.0054	0.1884±0.0124
2	0.0911±0.0094	0.0812±0.0073	0.0929±0.0141	0.2652±0.0306
4	0.1176±0.0055	0.1071±0.0042	0.1219±0.0126	0.3467±0.0216
8	0.1544±0.0107	0.1390±0.0078	0.1617±0.0202	0.4551±0.0385
24	0.2132±0.0088	0.1878±0.0034	0.2298±0.0238	0.6308±0.0330

Table C.16 The cumulative permeation of KPPLO6.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0160±0.0278	0.0159±0.0276	0.0158±0.0273	0.0477±0.0827
0.5	0.0648±0.0142	0.0452±0.0394	0.0680±0.0181	0.1780±0.0696
1	0.0826±0.0141	0.0757±0.0102	0.0847±0.0157	0.2430±0.0399
2	0.1111±0.0059	0.1013±0.0054	0.1276±0.0162	0.3400±0.0096
4	0.1382±0.0133	0.1269±0.0141	0.1661±0.0293	0.4313±0.0122
8	0.1710±0.0102	0.1511±0.0052	0.1936±0.0286	0.5156±0.0304
24	0.1987±0.0071	0.1776±0.0041	0.2242±0.0368	0.6005±0.0391

Table C.17 The cumulative permeation of KPPLO7.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0404±0.0351	0.0205±0.0356	0.0464±0.0414	0.1074±0.1024
0.5	0.0877±0.0123	0.0770±0.0109	0.0895±0.0144	0.2542±0.0361
1	0.1019±0.0142	0.0913±0.0121	0.1079±0.0170	0.3010±0.0417
2	0.1311±0.0169	0.1197±0.0124	0.1354±0.0186	0.3861±0.0476
4	0.1687±0.0224	0.1540±0.0166	0.1797±0.0216	0.5023±0.0604
8	0.2144±0.0252	0.1885±0.0188	0.2230±0.250	0.6260±0.0688
24	0.2502±0.0296	0.2237±0.0197	0.2611±0.0268	0.7351±0.0754

Table C.18 The cumulative permeation of KPPLO8.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0614±0.0090	0.0614±0.0062	0.0627±0.0095	0.1855±0.0247
0.5	0.0993±0.0132	0.0906±0.0078	0.1028±0.0106	0.2927±0.0317
1	0.1169±0.0123	0.1135±0.0132	0.1283±0.0183	0.3587±0.0438
2	0.1450±0.0235	0.1368±0.0192	0.1611±0.0250	0.4428±0.0674
4	0.1761±0.0242	0.1691±0.0225	0.1937±0.0296	0.5389±0.0760
8	0.2273±0.0204	0.2061±0.0164	0.2435±0.0209	0.6768±0.0567
24	0.2680±0.0124	0.2477±0.0140	0.2936±0.0212	0.8094±0.0474

Table C.19 The cumulative permeation of KP extract in water.

Times (h)	Cumulative permeated (μg/cm^2)			
	DMF	TMF	PMF	Total methoxyflavones
0.25	0.0000±0.0000	0.0000±0.0000	0.0180±0.0312	0.0180±0.0312
0.5	0.0395±0.0342	0.0187±0.0324	0.0585±0.0092	0.1166±0.0665
1	0.0463±0.0401	0.0223±0.0387	0.0688±0.0109	0.1374±0.0788
2	0.0735±0.0191	0.0257±0.0446	0.0798±0.0159	0.1791±0.0735
4	0.0888±0.0155	0.0678±0.0177	0.0971±0.0135	0.2537±0.0437
8	0.1102±0.0152	0.0815±0.0206	0.1185±0.0135	0.3102±0.0449
24	0.1299±0.0225	0.0981±0.0186	0.1395±0.0162	0.3674±0.0516

Table C.20 The skin permeation flux of metroxyflavones from the KP extract-loaded microemulsion, KP extract-loaded microemulgel and KP extract-loaded PLO gel formulations.

Formulations	Flux ($\mu g/cm^2/h$)
KPME1	0.090 ± 0.006
KPME2	0.085 ± 0.008
KPME3	0.102 ± 0.009
KPME4	0.118 ± 0.007
KPME5	0.132 ± 0.006
KPGE1	0.027 ± 0.001
KPGE2	0.026 ± 0.001
KPGE3	0.026 ± 0.003
KPGE4	0.044 ± 0.004
KPGE5	0.044 ± 0.002
KPGE6	0.043 ± 0.003
KPPLO1	0.021 ± 0.001
KPPLO2	0.018 ± 0.002
KPPLO3	0.023 ± 0.002
KPPLO5	0.022 ± 0.001
KPPLO6	0.019 ± 0.002
KPPLO7	0.025 ± 0.002
KPPLO8	0.026 ± 0.002
KP extract in water	0.013 ± 0.001

BIOGRAPHY

Name	Paisit Wattanasri, Mr.
Date of Birth	October 7, 1987
Place of Birth	Bangkok, Thailand
Nationality/Religion	Thai/Buddhism
E-mail address	watpaisit@gmail.com

Workplace

2011 – Present	Product Development Departments, Bangkok Lab and Cosmetic Co., Ltd.

Education

2014 – Present	Master of Pharmacy Program in Pharmaceutical Sciences Silpakorn University, Thailand
2006 – 2011	Bachelor of Pharmacy Silpakorn University, Thailand

Poster Presentation

1. **Paisit Wattanasri**, Tanasait Ngawhirunpat, Theerasak Rojanarata, Nattawat Nattapulwat and Praneet Opanasopit. "Development of *Kaempferia parviflora* extract-loaded microemulsions for skin permeation enhancement", 32nd International Annual Meeting in Pharmaceutical Sciences. 10-11 March 2016, Chulalongkorn University, Bangkok, Thailand.
2. **Paisit Wattanasri**, Tanasait Ngawhirunpat, Theerasak Rojanarata, Prasert Akkaramongkolporn and Praneet Opanasopit. "Development of Pluronic Lecithin Organogel loaded *Kaempferia parviflora* Extract for Transdermal Delivery", The 4th Current Drug Development International Conference (CDD 2016). 1-3 May 2016, Phuket, Thailand.
3. **Paisit Wattanasri**, Tanasait Ngawhirunpat, Theerasak Rojanarata Praneet Opanasopit. "Effect of d-limonene on skin permeation enhancer of *Kaempferia parviflora* extract-loaded microemulsions", 26th Federation of Asian Pharmaceutical Associations (FAPA). 9-13 November 2016, Bangkok, Thailand.