DEVELOPMENT OF MICROEMULSION FOR TRANSDERMAL DRUG DELIVERY OF KETOPROFEN

By
Narumon Worachun

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree
MASTER OF PHARMACY
Program of Pharmaceutical Sciences
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การพัฒนาระบบไมโครอิมัลชันสำหรับนำส่งยาคีโตโพรเฟนทางผิวหนัง

โดย

นางสาวนฤมล วระชุน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต

สาขาวิชาวิทยาการทางเภสัชศาสตร์

บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร

ปีการศึกษา 2553

The Graduate School, Silpakorn University has approved and accredited the thesis title of "Development of Microemulsion for Transdermal Drug Delivery of Ketoprofen" submitted by Miss Narumon Worachun as a partial fulfillment of the requirements for the degree of Master of Pharmacy in Pharmaceutical Sciences.

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52353202 : MAJOR : PHARMACEUTICAL SCIENCES
KEY WORDS: KETOPROFEN/MICROEMULSION/SKIN PERMEATION
NARUMON WORACHUN: DEVELOPMENT OF MICROEMULSION FOR TRANSDERMAL DRUG DELIVERY OF KETOPROFEN. THESIS ADVISORS: ASSOC.PROF. TANASAIT NGAWHIRUNPAT, Ph.D., AND ASSOC.PROF. PRANEET OPANASOPIT, Ph.D.. 105 pp.

The aim of this study was prepared microemulsion for transdermal drug delivery of ketoprofen (KP). The physicochemical and chemical properties were evaluated. The microemulsion composed of isopropyl myristate (IPM) as oil phase, water, PEG40-hydrogenated castor oil (Cremophor® RH40) as surfactant with different co-surfactant (ethanol absolute, n-butanol and PEG400) were prepared. The formulations conposed of IPM, water, Cremophor® RH40:PEG400 (ratio 1:1) were selected to be loaded with 2.5% w/w of KP. The viscosity, droplet size, pH, conductivity, and their skin permeation of KP through shed snake skin were evaluated. The particle size, viscosity and conductivity of microemulsions were in the range of 80-470 nm, 81-1158 cP and 0.20-37.27 μS/cm, respectively. As 2.5% KP loaded in the formulations, the viscosity, droplet size and conductivity of microemulsion were similar to unloaded formulation, however, the pH of formulations decreased. The ratio of IPM, water and surfactant mixture played the important role on KP loading capacity of microemulsions formulation and skin permeation of KP. As the amount of water increased, the loading capacity of KP in the microemulsions decreased, however, the skin permeation of KP increased. While amount of surfactant and IPM increased, the loading capacity of KP increased, but the skin permeation of KP decreased. The highest KP loading capacity was formulation B1 (30% IPM, 60% surfactant and 10% water) whereas the highest skin permeation flux was formulation B4 (30% IPM, 45% surfactant and 25% water). The results suggested that the novel microemulsion system composed of IPM, water, Cremophor® RH40:PEG400 (ratio 1:1) can be applied for using as a transdermal drug delivery carrier.

Program of Pharmaceutical Science Graduate School, Silpakorn University Academic Year 2010
Student's signature ……..……..……..
Thesis Advisor's signature 1……..…...……..... 2……..……..…

52353202: สาขาวิชาวิทยาการทางเภสัชศาสตร์
คำสำคัญ : คีโตโพรเฟน/ไมโครอิมัลชัน/การซึมผ่านผิวหนัง

นฤมล วระชุน : การพัฒนาระบบไมโครอิมัลชันสำหรับนำส่งยาคีโตโพรเฟนทางผิวหนัง. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : ภก.รศ.ดร.ธนะเศรษฐ์ ง้าวหิรัญพัฒน์ และ ภญ.รศ.ดร.ปราณีต โอปณะโสภิต. 105 หน้า.

ไมโครอิมัลชันที่ประกอบด้วยไอโซโพรพิลไมริสเทต (ไอพีเอ็ม) เป็นวัตภาคน้ำมัน น้ำ พีอีจี 40 ไฮโดรจิเนท คาสเตอร์ ออยล์ (ครีโมฟอร์ อาร์เอช40) เป็นสารลดแรงตึงผิว โดยมีสารลดแรงตึงผิวร่วมชนิดต่างๆ คือ เอทานอล เอ็น-บิวทานอล และพอลีเอทิลีนไกลคอล 400 (พีอีจี 400) ถูกเตรียมขึ้น และทำการประเมินความคงตัวทางกายภาพ โดยเลือกตำรับที่ประกอบด้วย ไอพีเอ็ม น้ำ ครีโมฟอร์ อาร์เอช40 และพีอีจี 400 โดยมีอัตราส่วนของ ครีโมฟอร์ อาร์เอช 40ต่อพีอีจี 400 เท่ากับ 1 ต่อ 1 เพื่อบรรจุยาคีโตโพรเฟนความเข้มข้น 2.5 เปอร์เซนต์โดยน้ำหนัก เพื่อประเมินความหนืด ขนาดอนุภาค ความเป็นกรดด่าง การนำไฟฟ้า และการซึมผ่านคราบงูของยาคีโตโพรเฟน ผลการศึกษาพบว่า ขนาดของไมโครอิมัลชันอยู่ระหว่าง 80 ถึง 470 นาโนเมตร ความหนืดมีค่าระหว่าง 81 ถึง 1158 เซนติพอยส์ และการนำไฟฟ้ามีค่าระหว่าง 0.2 ถึง 37.2 ไมโครซีเมนต่อเซนติเมตร การบรรจุคีโตโพรเฟน 2.5 เปอร์เซนต์โดยน้ำหนักในไมโครอิมัลชันไม่มีผลต่อความหนืด ขนาดอนุภาค และการนำไฟฟ้า แต่มีผลลดความเป็นกรดด่างของตำรับ อัตราส่วนของไอพีเอ็ม น้ำ และของผสมของสารลดแรงตึงผิว และสารลดแรงตึงผิวร่วมมีผลต่อความสามารถในการบรรจุยา คุณลักษณะของตำรับไมโครอิมัลชัน และการซึมผ่านผิวหนังของยาคีโตโพรเฟน ปริมาณน้ำที่เพิ่มขึ้นส่งผลให้ความหนืด การนำไฟฟ้าของตำรับไมโครอิมัลชัน และการซึมผ่านผิวหนังของยาคีโตโพรเฟนเพิ่มขึ้นแต่ทำให้ความสามารถในการบรรจุยา และค่าความเป็นกรดด่างของตำรับลดลง เมื่อปริมาณสารลดแรงตึงผิวเพิ่มขึ้นทำให้ความสามารถในการบรรจุยา และค่าความเป็นกรดด่างของตำรับเพิ่มขึ้น แต่ทำให้ความหนืด และการซึมผ่านผิวหนังของยาคีโตโพรเฟนลดลง ปริมาณของไอพีเอ็มที่เพิ่มขึ้นส่งผลให้ค่าการนำไฟฟ้า และการซึมผ่านผิวหนังของยาคีโตโพรเฟนลดลง ความเข้มข้นของผสมของสารลดแรงตึงผิวและสารลดแรงตึงผิวร่วมและอัตราส่วนของน้ำมันต่อน้ำ ที่ทำให้ตำรับมีคุณ-ลักษณะและการซึมผ่านผิวหนังของยาที่เหมาะสม คือ 50% และ อัตราส่วน 1 ต่อ 1 ตามลำดับ ผลการศึกษาแสดงให้เห็นว่า ระบบไมโครอิมัลชันระบบใหม่ที่ประกอบด้วย ไอพีเอ็ม น้ำ ครีโมฟอร์ อาร์เอช40 และพีอีจี 400 ในอัตราส่วน 1 ต่อ 1 สามารถประยุกต์ใช้ในการเป็นตัวพาสำหรับนำส่งยาผ่านทางผิวหนังได้

สาขาวิทยาการทางเภสัชศาสตร์ บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร ปีการศึกษา 2553
ลายมือชื่อนักศึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ 1..... 2.....

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and appreciation to my thesis advisor, Associate Professor Dr. Tanasait Ngawhirunpat, for his invaluable advices, guidance, attention and encouragement throughout my study.

To my co-advisor, Associate Professor Dr. Praneet Opanasopit, I would like to thank for her guidance and meaningful advices, and for her help in making all contacts possible and successful.

I would like to acknowledge the Graduate School, Silpakorn University for the financial support (Annual Government Budget Expenditures for the fiscal year 2010).

I would like to sincere thanks to all teachers, follow graduate students, researchers and the staffs in Faculty of Pharmacy, Silpakorn University, for giving me the place, equipments, knowledge and friendship.

I am also grateful to L.B.S. Laboratory Co., Ltd and Mr Anurak Lorwongngam from Medicine Supply Co., Ltd. for the kindness supporting of ketoprofen raw material.

I would like to pay greatly thanks to National Blood Centre, Thai Red Cross Society, Dr Soisa-ang Pikulsod (Director), Mrs Pintira Tantien (Assistant Director), Miss Thitiphorn Bhakbhumpong (Chief of Quality Control Section) to give me a precious opportunity and helpfulness to my study.

To my laboratory brothers, sisters and friends, thanks for their assistance and kindness, and to my friends, I would like to thank for their assisting and entertaining me through the years.

Finally, I would like to express my most thankfulness to my beloved family for constant supportive and encouragement. They give me all worthwhile oppotunity, I would like to thank for their kind understanding, warm and memorable time. Furthermore, thanks for taking care of my physically and believing in my ability always.

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CHAPTER 1
INTRODUCTION

1. Statement and significance of the research problem

Transdermal drug delivery (TDD) offers an advantageous mode of drug administration by eliminating first pass hepatic metabolism, providing sustained drug release for a prolonged period of time, painless and therefore offers superior patient compatibility. However, the natural function of the skin is the protection of the body against the loss of endogenous substances such as water and undesired influences from the environment caused by exogenous substances. This implies that the skin acts as a barrier against diffusion of substances through the underlying tissue. Several technological advances have been made to overcome this challenge such as chemical penetration enhancer, liposome, microemulsion, iontophoresis, sonophoresis, electroporation, magnetic energy, microneedle applications and stratum corneum ablation (Barry 2006: 3-14).

Microemulsions are isotropic, thermodynamically stable transparent systems of oil, water and surfactant. They are frequently in combination with a co-surfactant with a droplet size usually in the range of 20 to 200 nm. They can be classified as oil-in-water (o/w), water-in-oil (w/o) or bicontinuous systems depending on their structure (Lawrence and Rees 2000: 89-121). Microemulsions as drug delivery tool show favourable properties such as thermodynamic stability (long shelf-life), easy formation (zero interfacial tension and almost spontaneous formation), optical isotropy, ability to be sterilized by filtration, high surface area (high solubilization capacity) and very small droplet size. Microemulsions can be introduced into the body orally, topically on the skin, or nasally, as an aerosol for direct entry into the lung (Kogan and Garti 2006: 58-66). Microemulsions have been subjected to numerous studies because of their great potential in many applications. Although various systems to prepare microemulsions for transdermal drug delivery

were reported in the literature, the novel system for preparation of microemulsions is still important to investigate to obtain a stable and effective drug delivery system.

2. Objective of this research

2.1 To prepare microemulsion from various surfactant and cosurfactant systems.

2.2 To characterize the microemulsion properties.

2.3 To study the effect of isopropyl myristate, surfactant and water ratio to drug loading capacity and ketoprofen shed snake skin permeation.

3. The research hypothesis

3.1 The studied system is able to form microemulsion.

3.2 Isopropyl myristate, surfactant and water ratio plays an important role on the characteristic of microemulsion and skin permeation of ketoprofen.

CHAPTER 2

LITERATURE REVIEWS

1. Introduction to transdermal drug delivery

1.1 Structure and function of human skin

The skin is the largest organ of the body, which acts as a protective barrier against the entry of foreign material and possible invasion of pathogens. The structure of human skin is portrayed in Figure 1. The skin is about 0.5 mm thick and is made up of two distinct layers, the inner dermis and the overlaying epidermis.

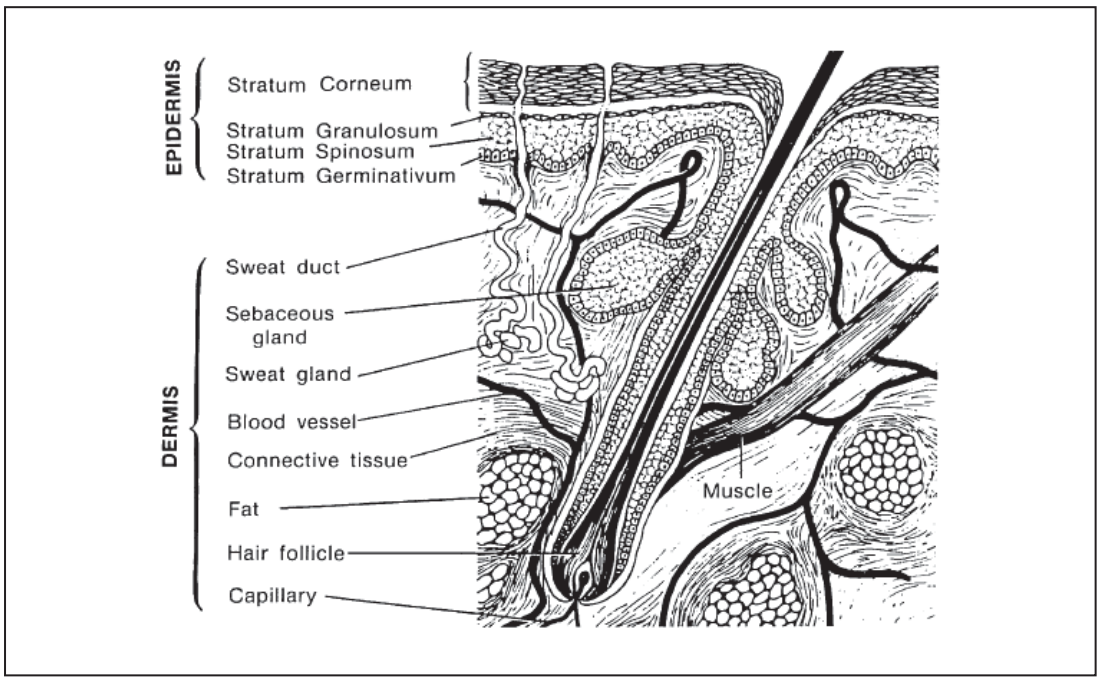

Figure 1 Schematic cross section of the upper most layers of human skin, showing the stratum corneum, the epidermis and the dermis, along with sweat glands and hair follicles

Source: Venkatraman Subbu and Gale Robert, "Skin adhesives and skin adhesion: 1. Transdermal drug delivery systems," Biomaterials 19 (1998): 1120.

1.1.1 The epidermis

The epidermis is divided into four anatomical layers namely stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC) as shown in Figure 1. The SC is the heterogeneous outermost layer of the epidermis and is approximately 10 - 20 μm thick. It is non-viable epidermis and consists of 15 - 25 flattened, stacked, hexagonal and cornified cells embedded in a mortar of intercellular lipid. Each cell is approximately 40 μm in diameter and 0.5 μm thick. The thickness varies and may be a magnitude of order larger in areas such as the palms of the hands and soles of the feet. The SC is a composite of corneocytes (terminally differentiated keratinocytes) and secreted contents of the lamellar bodies (elaborated by the keratinocytes), that give it a 'bricks-and-mortar' structure. This arrangement creates a tortuous path through which substances have to tranverse in order to cross the SC. The protein-enriched corneocytes (bricks) impart a high degree of tortuosity to the path of water or any other molecule that traverses the SC, while the hydrophobic lipids, organised into tight lamellar structures (mortar) provide a water-tight barrier property to the already tortuous route of permeation in the interfollicular domains. The barrier nature of the stratum corneum depends on its unique constituents; 75-80% protein, 5-15% lipid with 5-10% unidentified on a dry weight basis. (Walters and Roberts 2002: 1-39; Williams 2003: 1-25).

1.1.2 The dermis

The dermis (or corium), at 3 to 5 mm thick and is the major component of human skin. The dermis is composed of a network of connective tissue, predominantly collagen fibrils providing support and elastic tissue providing flexibility, embedded in mucopolysaccharide gel. Blood vessels, nerves and lymphatic vessels cross this matrix and skin appendages (endocrine sweat glands, apocrine glands and pilosebaceous units) penetrate it. (Williams 2003: 1-25; Murthy and Shivakumar 2010: 1-36)

1.2 Routes of drug permeation across the skin

Stratum corneum is the rate-limiting barrier to delivery for most molecules. There are essentially three pathways by which a molecule can traverse intact stratum corneum (Roberts, Cross and Pellett 2002: 89-179)

1.2.1 Transcellular pathway

It was originally believed that transcellular diffusion mechanisms dominated over the intercellular and transappendageal routes during the passage of solutes through the stratum corneum. However, transport by the transcellular route would involve the repeated partitioning of the molecule between lipophilic and hydrophilic compartments, including the almost impenetrable corneocyte intracellular matrix of keratin and keratohyaline. The pathway is directly across the stratum corneum and hence the pathlength for permeation is usually regarded as the thickness of the stratum corneum.

1.2.2 Intercellular pathway

The intercellular lipid route provides the principal pathway for the small, uncharged molecules. The intercellular route is highly tortuous, with permeants moving through the continuous domains between the keratinocytes. The pathlength taken by the molecule is considerably greater than the stratum corneum thickness. Various estimates have been proposed for the intercellular permeation distance, ranging from 150-500 μm.

1.2.3 Transappendageal pathway

The penetrant transverses the SC *via* a 'shunt' pathway: *e.g.,* a hair follicle or a sweat gland. These shunts are known to be important at short times prior to steady state diffusion. The available diffusional area of the shunt route is approximately 0.1% of the total skin area and therefore the contribution to drug permeation compared to the former is significantly less. Despite their small fractional area, the skin appendages may provide the main portal of entry into the subepidermal layers of the skin for ions and large polar molecules. The appendageal pathway has been reported to be the major contributor to the initial phase of SC permeation.

1.3 Mathematics of skin permeation (Watkinson and Brain 2002: 61-88; Williams 2003: 27-49)

1.3.1 Fick's first law of diffusion

In transport, the flow (or flux, Ji in mol cm^{-2} s^{-1}) is related to the velocity of molecular movement (υ in cm s^{-1}) and the concentration (C_i in mol cm^{-3}) of the molecules in motion in equation (1).

$$J_i = C_i v \qquad (1)$$

A fundamental principle of irreversible thermodynamics is that the flow, at any point in the system, at any instant, is proportional to the appropriate potential gradient. It can be expressed mathematically for a species *i* as shown in equation (2) where $\partial\mu_i/\partial x_i$ is the gradient and L_i is the proportionality constant.

$$J_i = -L_i (\partial\mu i/\partial x) \qquad (2)$$

Equation (2) is the general form of Fick's first law of diffusion.

If constant temperature and pressure is assumed equation (2) can be expressed as equation (3) where *Di* is the diffusion co-efficient.

$$J_i = -D_i (\partial C_i/\partial x) \qquad (3)$$

Equation (3) is the classic form of Fick's first law of diffusion.

1.3.2 Fick's second law of diffusion

Fick's second law relates the rate of change in concentration with time at a given point in a system to the rate of change in concentration gradient at that point.

$$\partial C/\partial t = D\partial^2 C/\partial x^2 \qquad (4)$$

Fick's laws are more applicable if certain parameters or boundaries are specified. Most of in-vitro experimental design aim to mimic as closely as possible the in-vivo situation. The most common in-vitro design is one where a membrane separates two compartments. One compartment contains the permeant in a vehicle (donor solution) and the other compartment contains a receptor solution that provided sink conditions. After sufficient time, steady-state permeation across the membrane is achieved when the concentration gradient of the permeant across the membrane is constant. Under these conditions, equation (4) can be simplified to (5), where Q is the cumulative mass of the permeant that passes through a unit area of a membrane in a time (t), C_0 is the concentration of the permeant in the first layer of the membrane and h is the thickness of the membrane.

$$dQ/dt = J = DC_0/h \qquad (5)$$

In practical terms, *Co* (the concentration of permeant in the outer layer of the membrane), that is very difficult to measure. The value *Co* is replaced with a term that

links it to the concentration in the vehicle *Cv* through the partition co-efficient *K*, which rearranges to give equation 6.

$$dQ/dt = J = DKC_v/h \qquad (6)$$

The term DK/h in equation 6 is called permeability coefficient (*P*). It can be substituted into equation 6 to give

$$dQ/dt = J = PC_v \qquad (7)$$

The permeation profile can be plotted of Q, the cumulative amount of drug passing through a unit area of membrane against time (t). The slope of the straight line is flux (dQ/dt). The lag time can be obtained from extrapolation of the pseudo-steady state portion of the permeation profile to the intercept on the time axis and can be related to the diffusion coefficient by equation (8)

$$t_{lag} = h^2/6D \qquad (8)$$

1.4 In-vitro perceutaneous absorption study

In vitro techniques to assess skin penetration and permeation are used extensively in industry and academia. Some from of in vitro diffusion cell experiment is often the most appropriate method for assessment of percutaneous penetration in a developmental drug delivery program or in a dermal toxicology screen.

1.4.1 In Vitro skin diffusion Cells

In vitro diffusion systems range in complexity from a simple two compartment “static” diffusion cell to multijacketed “flow-through” cells. Construction materials must be inert, and glass is most common. Excised skin is always mounted as a barrier between a donor chamber and a receptor chamber and the amount of compound permeating from the donor to the receptor side is determined as a function of time, efficient mixing of the receptor phase is essential. Static diffusion cells are usually of the upright (Franz) (Figure 2) or side-by-side type (Figure 3). The main difference in the application of these two static cell types is that side-by-side cells can be used for the measurement of permeation from one stirred solution, through a membrane, and into another stirred solution. This is of particular adventage when examining flux from saturated solutions in the presence of excess solid if accumulation of solid on the membrane surface must be prevented. Upright cells are

particularly useful for studying absorption from semisolid formulations spread on the membrane surface and are optimal for simulating in vivo performance. Flow-through cells can be useful when the permeant has a very low solubility in the receptor medium. Sink conditions are maximized as the fluid is continually replaced using a suitable pump (Brain, Walters and Watkinson 2002: 197-202). In common with the static system, flow-through diffusion cells are temperature controlled (water bath, usually at 37°C to maintain surface skin temperature at 32°C as an in vivo mimic) (Williams 2003: 62-64).

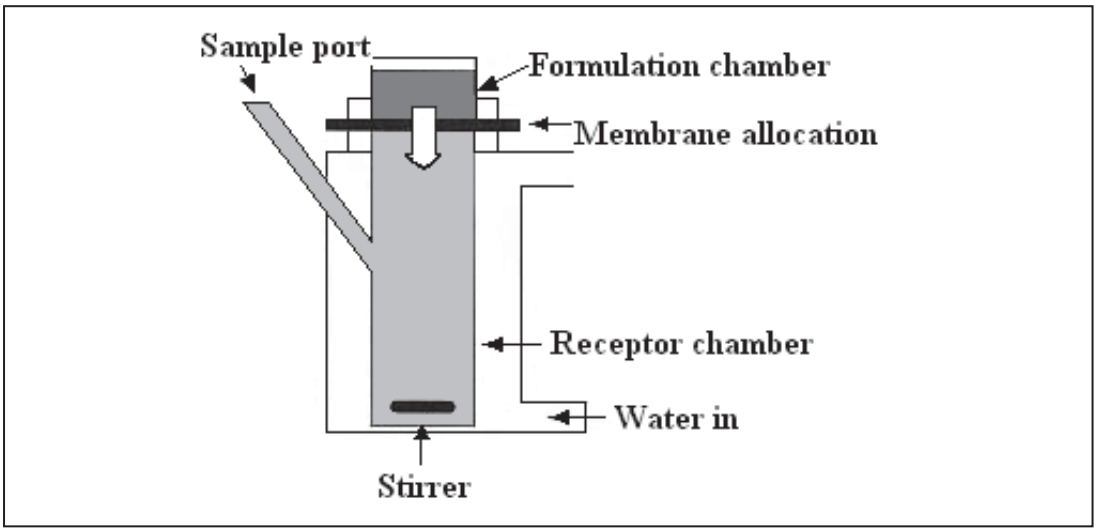

Figure 2 Franz diffusion cell

Source: Franz diffusion cell [Online], accessed 15 March 2011. Available from http://www.medbc.com/annals/review/vol_...1p14.htm

1.4.2 Skin sources

The difference of skin sources gave the different results in perceutaneous absorption, these differences were due to the physiology of the skin.

Human skin

Human skin is the most appropriate choice to predict the percutaneous absorption. Human skin samples can be obtained from a variety of sources, for example, from skin banks or by donation from patient undergoing a surgical procedure. However, there are considerable ethical and legal constraints on obtaining and using human material. A concern with experiments using skin donated

from a particular surgical procedure arises from the use of skin from one body site, or from one sex because the variation in skin permeability are evident between different body sites.

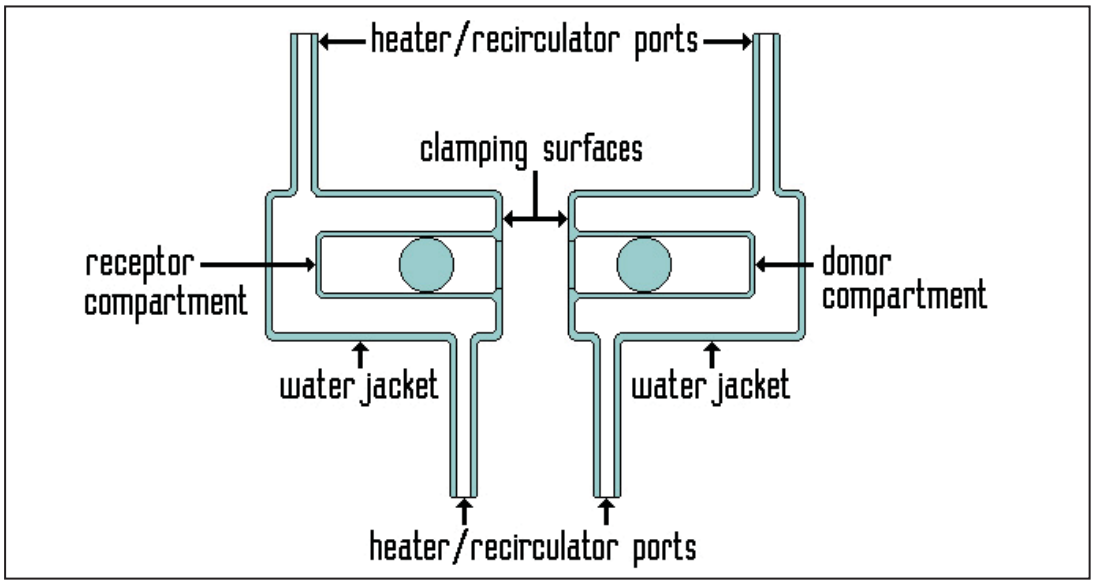

Figure 3 Side by side diffusion cell

Source: Harizontal cell, PermeGear Horizontal cell [Online], accessed 15 March 2011. Available from http://www.permegear.com/sbsparts.htm

Animal skin

A wide range of different animal skin has been proposed as model for human skin in vitro. Pig skin is the best natural model membrane for permeation studies. The stratum corneum of the pig is similar in thickness to the human membrane, and through its lipid content does differ from that of human tissue it has remarkably similar permeability properties.

Snake skin offers considerable advantages over human material as it is relatively abundant (Williams 2003: 54-60). Shed snake skin usually consists of three distinctive layers; beta, mesos and alpha-layers. Shed snake skin is composed of two very different regions; scales and separating these, hinges. The scales are rigid while the hinge is elastic. The scales on the dorsal part are much smaller and usually thinner than the scales on the ventral part. The behaviour of the shed snake skin is also very similar to that of human stratum corneum. There are two parallel permeation

pathways: lipid and pore pathways in the shed snake skin as in human stratum corneum. The lipid content of the shed snake skin is similar to the human stratum corneum, that is, neutral lipids are a major lipid component in both skins and fatty acids, with carbon chain lengths of C16 and C18 predominant (Priprem et al 2008: 444-450; Itoh et al 1990: 1042-1047).

Artificial skins

The artificial skin is produced to avoid the problems with wide variability in the permeability of real skin. Artificail skin offers the advantages of reproducibility and control. The membrane is isotropic, no regional variability and permeation can be described by simple mathematical rules. The artificial membrane has disadvantages in term of poorly representing human tissue in vivo: no metabolism, no pores or shunt routes (Williams 2003: 54-60).

1.5 Enhancing transdermal drug delivery

1.5.1 Chemical penetration enhancers

Several chemicals are known to interact with the skin and disrupt the highly ordered lipid bilayer structure that forms the primary barrier to diffusion of exogenous molecules. Permeation enhancers enhance transport of drugs across skin by a variety of complex mechanisms (Figure 4). They can directly exert their effect on skin structure by acting on intercellular lipids or corneocytes, the dead cells of the stratum corneum. Permeation enhancers can be divided broadly in two categories based on their action on intercellular lipids of the skin. Chemical enhancers can either extract lipids from the skin thereby creating diffusion pathways for the drug to permeate through or they can partition themselves into the lipid bilayers thereby disrupting the highly ordered lipid lamellae and causing their fluidization. Lipid extraction or fluidization in presence of chemical enhancers could occur through a variety of different mechanisms. Alternately, chemical enhancers can increase skin transport of a drug by enhancing its thermodynamic activity in the formulation. Chemical permeation enhancers have traditionally been classified based on their chemical structures rather than their mechanisms of action on skin. The examples of chemical enhancers are

1. Water
2. Hydrocarbons
3. Alcohols, fatty alcohols and glycols
4. Fatty acid
5. Amines
6. Amides
7. Esters of fatty acids
8. Surfactants
9. Terpenes, terpenoids and essential oils
10. Sulfoxides
11. Lipids and phospholipids
12. Azone
13. Pyrrolidones
14. Urea

(Williams and Barry 2004: 603-618; Karande and Mitragotri 2009: 2362-2373)

1.5.2 Stratum corneum bypassed or removed

Microneedle array

The stratum corneum can be bypassed by injection. Microneedles are needles that are 10 to 200 μm in length and 10 to 50 μm in width. They are solid or hollow and are connected to a reservoir which contains the active principle. Microneedle arrays are applied to the skin surface so that they pierce the upper epidermis far enough to increase skin permeability and allow drug delivery, but too short to cause any pain to the receptors in the dermis (Barry 2006: 11; Cevc and Vierl 2010: 285; Prausnitz, Mikszta and Devens 2006: 239-254).

Stratum corneum ablation

Adhesive tape can remove stratum corneum prior to drug application. Tape stripping is also popular for assessing bioavailability by measuring drug uptake into skin (Barry 2006: 11).

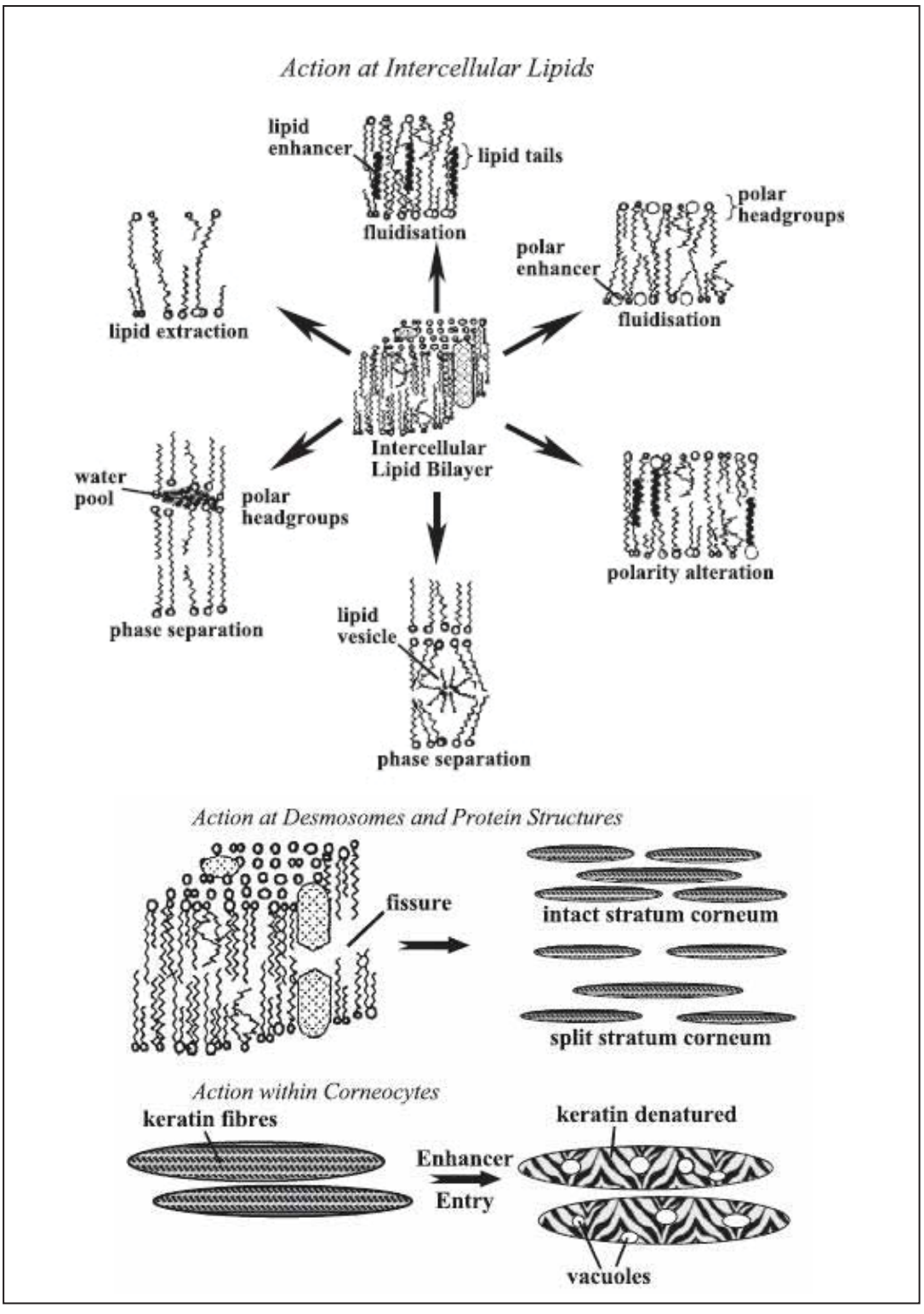

Figure 4 Actions of penetration enhancers

Source: Adrian C. Williams and Brian W. Barry, "Penetration enhancers," Advanced Drug Delivery Reviews 56 (2004): 605, 615.

1.5.3 Electrically Assisted Techniques

Iontophoresis

Drugs in the ionic form, contained in some reservoir, can be '*phoresed*' out with a small direct current (approximately 0.5 mA/cm^2). Three main mechanisms promote drug entry: (a) charged species are driven mainly by electrical repulsion from the driving electrode; (b) the electric current may increase the permeability of skin; and (c) electroosmosis may promaote passage of uncharged molecules. Efficiency of transport depends mainly on polarity, valency and mobility of the charged species as well as electrical duty cycles and formulation components (Abla et al. 2006: 177-209; Barry 2006: 13).

Electroporation

Electroporation or electropermeabilization is the transitory structural perturbation of lipid bilayer membranes due to the application of high voltage pulses (approximately 100 to 1000 V/cm) and is also described as the simultaneous creation of a transient, high permeability state and electrically driven transport in bilayer membranes by the application of high voltage for a short period of time (Barry 2006: 13-14; Medi and Singh 2006: 221-238).

Ultrasound (Phonophoresis, Sonophoresis)

Phonophoresis (or sonophoresis) uses ultrasound energy in order to enhance the skin penetration of active substances. It is the movement of drugs through living intact skin and into soft tissue under the influence of an ultrasonic perturbation. When skin is exposed to ultrasound, the waves propagate to a certain level and cause several effects that assist skin penetration. The propagation of an ultrasonic wave within the skin has two main physical consequences, namely heating and cavitation. (Barry 2006: 13).

Magnetophoresis

Magnetic fields can move diamagnetic materials through skin (Barry 2006: 14).

Radio waves

This technique uses the energy of radiofrequency waves to from microchannels through the stratum corneum, with the possibility of feedback control.

A densely spaced array of microelectrodes takes microseconds to from the holes; applied drug the easily passes into the skin (Barry 2006: 14).

1.5.4 Supersaturation of drug

Skin absorption can be enhanced using supersaturated solutions that have greater thermodynamic activity or chemical potential than the saturated solutions. The degree of saturation can be increased by increasing the drug concentration in the vehicle or reducing the drug solubility in the vehicle. Thermodynamic activity of saturated solution is unity and a transient increase of the degree of saturation to greater than 1 can be achieved by supersaturation (Murthy and Shivakumar 2010: 1-36).

1.5.5 Eutectic systems

A eutectic mixture is a physical mixture of two components that do not interact to form a new chemical substance but at certain ratios inhibit each other's crystallization, resulting in a substance with a lower melting point than that of either of the components (Stott, Williams and Barry 1998: 297-308). A eutectic mixture is formed only when the two components are miscible in the liquid state but remain completely immiscible in the solid state.The melting point of a drug influences the solubility and hence the skin penetration. The permeant melting point was found to be inversely related to the lipophilicity. It has been postulated that the lower the melting point of the permeant, the greater is the solubility in a given solvent, including the skin lipids. (Murthy and Shivakumar 2010: 1-36).

1.5.6 Prodrug approach

Prodrugs have been utilized to enhance the dermal and transdermal permeation of drugs with unfavorable partition coefficients (Sloan 1989: 67-101). The prodrug approach involves addition of a promoiety to increase the partition coefficient and hence the permeation of the parent molecule across the skin. On reaching the viable epidermis, esterases release the parent molecule by hydrolysis of the prodrug (Murthy and Shivakumar 2010: 1-36).

1.5.7 Vehicle and particles

Liposomes

Liposomes are colloidal systems comprised of bilayered vesicles made of phospholipids. They may consist of a single (unilamellar) or few (oligolamellar) or many (multilamellar) concentric phospholipid bilayer(s). Depending on the size of the vesicles they are further categorized as small unilamellar (SUV) or large unilamellar (LUV) vesicles. Liposomes are capable of encapsulating both hydrophilic and lipophilic molecules in their concentric bilayers. The hydrophilic drugs are usually entrapped in the inner aqueous compartment, while the lipophilic or amphiphilic and sometimes charged molecules are associated with the phospholipid bilayers. When the vesicular bilayers are made up of non-ionic surfactants they are termed niosomes. The physicochemical properties like size, charge, lamellarity, and elasticity are governed by the composition of the vesicles. These properties are known to have a significant influence on the effectiveness of liposomes as drug delivery systems. Liposomes are used as carriers to deliver the entrapped drugs into the skin. They act as permeation enhancers by virtue of the phospholipids that penetrate into the stratum corneum and subsequently alter the skin lipid bilayers. They are known to act as a depot for sustained release of actives into the skin, and also modulate the rate and extent of systemic drug absorption. Liposomal formulations are known to favor drug deposition in the skin, reduce irritation potential of drugs, and improve drug stability. (Barry 2006: 7-8; Murthy and Shivakumar 2010: 1-36)

High velocity particles

The PowerJect system fires solid nanoparticles through the horny layer into viable tissues, driven by a supersonic shock wave of helium (Barry 2006: 8).

Microemulsions

Microemulsions are known to increase the drug absorption on topical application. This could be due to the penetration enhancement effect of the carrier, usually composed of saturated or unsaturated fatty acids serving as the oil phase (Pappinen and Urtti 2006: 109-116; Murthy and Shivakumar 2010: 1-36).

2. Microemulsion

Microemulsions are clear, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a cosurfactant. The microemulsion concept was introduced as early as the 1940s by Hoar and Schulman who generated a clear single-phase solution by titrate a milky emulsion with hexanol (Lawrence and Rees 2000: 89-121). The mixture of oil, water and surfactants is able to form a wide variety of structures and phases. Besides microemulsions, structural examinations can reveal the existence of regular emulsions, anisotropic crystalline hexagonal or cubic phases, and lamellar structures depending on the ratio of the components. Most of these different phases and structures are easily recognised by simple visual inspection of the compositions due to their physical appearance (e.g., emulsions are nontransparent and phases separate after a while, lamellar structures and cubic phases are high viscous) or can be revealed by inspection with polarised light (crystalline phases), and thereby discerned from actual microemulsions. Depending on properties of the involved components, microemulsions can potentially appear over a wide range of oil–water–surfactant compositions. However, with given oil–water–surfactant components, microemulsions are usually only formed in narrow specific concentration ranges. The region of existence is typically presented in pseudo-ternary phase diagrams, as ratios between oil, water and a fixed mixture of surfactant–co-surfactant (Kreilgaard 2002: 77-98). The most commonly microemulsion microstructures are micellar (oil-in-water), inverted micellar (water-in-oil) and bicontinuous structures (Figure 5).

2.1 Method of Preparation

2.1.1 Phase Titration Method

Microemulsions are prepared by the spontaneous emulsification method (phase titration method) and can be depicted with the help of phase diagrams. Construction of phase diagram is a useful approach to study the complex series of interactions that can occur when different components are mixed. Microemulsions are formed along with various association structures (including emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) depending on the chemical composition and concentration of each component. As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo

ternaryphase diagram is often constructed to find the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular component.

2.1.2 Phase Inversion Method

Phase inversion of microemulsions occurs upon addition of excess of the dispersed phase or in response to temperature. During phase inversion drastic physical changes occur including changes in particle size that can affect drug release both *in vivo* and *in vitro*. These methods make use of changing the spontaneous curvature of the surfactant. For non-ionic surfactants, this can be achieved by changing the temperature of the system, forcing a transition from an o/w microemulsion at low temperatures to a w/o microemulsion at higher temperatures (transitional phase inversion). During cooling, the system crosses a point of zero spontaneous curvature and minimal surface tension, promoting the formation of finely dispersed oil droplets. This method is referred to as phase inversion temperature (PIT) method. Instead of the temperature, other parameters such as salt concentration or pH value may be considered as well instead of the temperature alone (Talegaonkar et al. 2008: 238-257).

2.2 Microemulsion characterization

Microemulsions have been evaluated using a wide range of different techniques over the years, but a complementarity of methods is generally required in order to fully characterise these systems. At the macroscopic level viscosity, conductivity methods provide useful information. Viscosity measurements for example can indicate the presence of rod-like or worm like reverse micelles and conductivity measurements provide a means of determining whether a microemulsion is oil-continuous or water-continuous, as well as providing a means of monitoring percolation or phase inversion phenomena (Lawrence and Rees 2000: 89-121; Talegaonkar et al. 2008: 238-257).

Scattering techniques such as static and dynamic light scattering (SLS, DLS), small-angle neutron and X-ray scattering (SANS, SAXS), cryo transmission electron microscopy and pulsed field gradient spin echo (self-diffusion) NMR have been instrumental in the original structural characterisation of microemulsions, and

they are still widely employed in order to determine the structural features of microemulsions with an Å resolution, in order to obtain a detailed picture of the build-up of the microemulsion structure. (Gradzielski 2008: 263-269; Talegaonkar et al. 2008: 238-257).

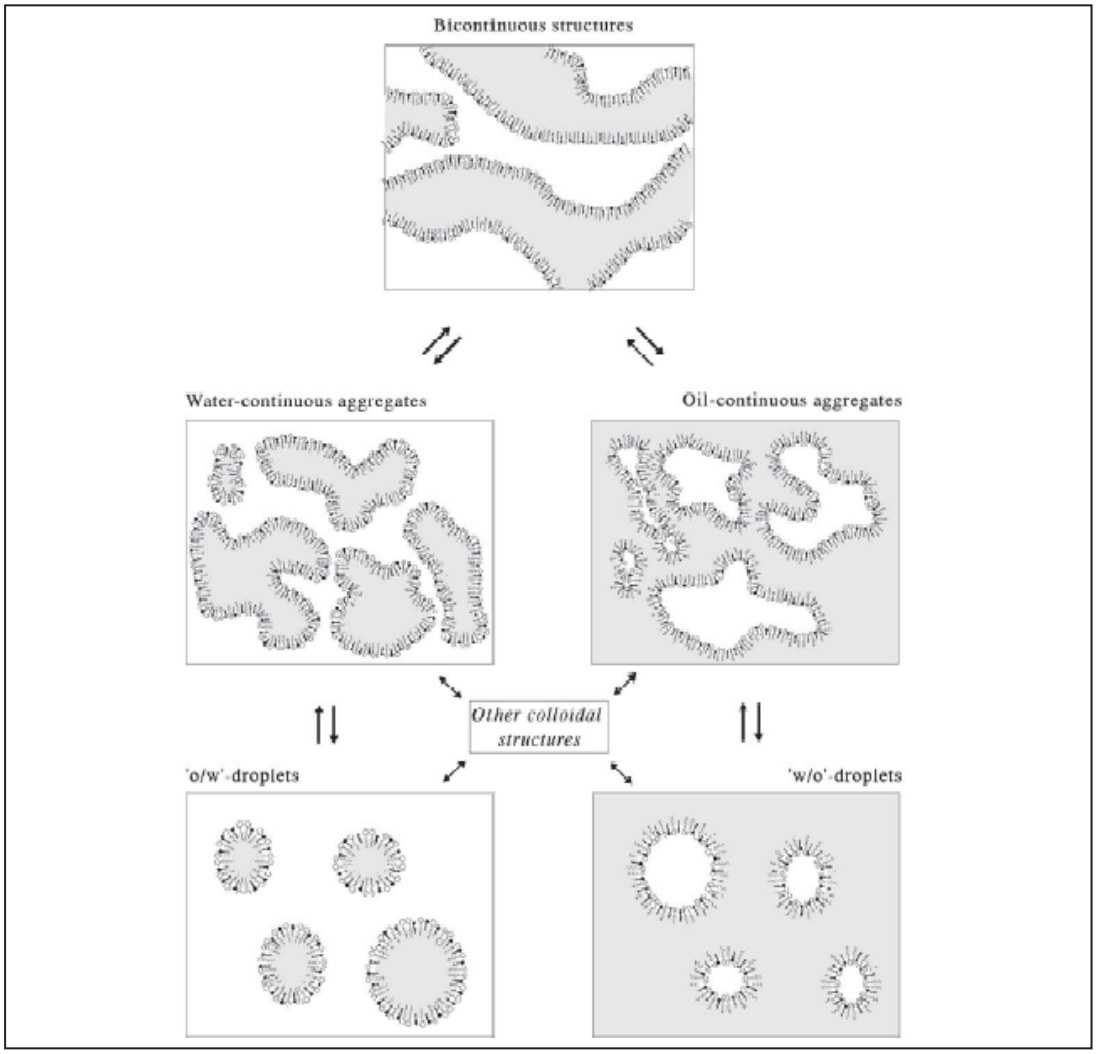

Figure 5 Basic dynamic microemulsion structures formed by oil phase (grey), aqueous phase (white) and surfactant / co-surfactant interfacial film, and plausible transitions between the structures (indicated by arrows) by increase of oil fraction (clockwise from left to right) and water fraction (anti-clockwise from right to left), respectively.

Source: Mads Kreilgaard, "Influence of microemulsions on cutaneous drug delivery," Advanced Drug Delivery Reviews 54 Suppl., 1 (2002): S81.

Microemulsions seem to be ideal liquid vehicles for drug delivery since they provide all the possible requirements of a liquid system including thermodynamic stability (long shelf life), easy formation (zero interfacial tension and almost spontaneous formation), low viscosity with Newtonian behavior, high surface area (high solubilization capacity), and very small droplet size. The small droplets have better chance to adhere to membranes and to transport bioactive molecules in a more controlled fashion. Using the microemulsion vehicles, water-insoluble and oil-soluble components from different plant extracts can be co-solubilized in order to attain synergistic effect for a specific therapeutic goal (Kogan and Garti 2006: 369-385). Microemulsions can be introduced into the body orally, parenterally, topically on the skin, or nasally, as an aerosol for direct entry into the lungs.

2.3 Parenteral microemulsions

Microemulsions have evolved as a novel vehicle for parenteral delivery of the hydrophobic drugs. Their interesting features such as spontaneity of formation, ease of manufacture, high solubilization capacity and self-preserving property make them the vehicle of choice. The nanostructure of the micromeulsions ensures that the probability of emboli formation in the blood is insignificant. Furthermore, the small size of the microemulsions may result in higher blood circulation time which would be useful in certain cases. Microemulsions were found to be less painful on injection as compared to the co-solvent based formulations. Microemulsions have also been shown to reduce the toxicity potential of the certain drugs like Amphotericin B by means of encapsulation (Date and Nagarsenker 2008: 19-30). The overview of some of the important investigations related to the parenteral microemulsions and their *in vivo* advantages are listed in Table 1.

2.4 Nasal microemulsions

The water-in-fluorocarbon (FC) reverse microemulsions stabilized by semi-fluorinatedamphiphiles derived from the dimorpholinophosphate polar head group are being investigated as new delivery systems for drugs or genetic materials into the lung (Courrier et al. 2003: 689-696; Vandamme and Krafft 2004: 141-148).

Table 1 Overview of parenteral micromeulsions and their *in vivo* advantages

Drug	Composition	*In vivo* advantages
Paclitaxel	- Lecithin, Poloxamer 188, Cremophore EL, Ethanol - Cremophore EL, Glycofurol, Labrafil 1944 CS, PLGA - Lecithin, Poloxamer 188, Ethanol, Tricaproin, Tributyrin - Capmul MCM, Myvacet 9-45 Lecithin, Butanol	- Less hyersensitivity reaction, higher AUC value and prolongedcirculation as compared to Taxol - Anti-tumor effects observed for prolonged time - Higher AUC values and mean residence time as compared to Taxol - Higher AUC values and mean residence time as compared to Taxol
Vincristine	PEGylated phospholipid, Vitamin E, Cholesterol, Oleic acid	Higher efficacy, survival rate and lesser side-effects as compared to free drug
Norcanthridine	- Ethyl oleate, lecithin, ethanol - Isopropyl myristate, lecithin, Tween 80	- Higher concentrations in liver and AUC values as compared tocommercial formulations. The drug is used for liver metastases. Hence, higher liver concentrations would be useful - Higher efficacy, survival rate and lesser nephrotoxicity as compared to Fungizone
Amphotericin B	Solutol HS 15, Peceol, Myrj-52	Higher LD50 value as compared to Fungizone
Itraconazole	POE-50-hydrogenated castor oil, benzyl alcohol, MCT, Ethanol	Higher AUC values as compared to the cyclodextrin based Formulation
Flurbiprofen	PEGylated phospholipid, Ethanol, Ethyl oleate, lecithin	Prolonged circulation and higher AUC values as compared to the solution
Clonixic acid	Tween 85 and 20, castor oil	Less painful as compared to marketed formulation

Table 1 (continue)

Drug	Composition	*In vivo* advantages
Ibuprofen eugenol ester	Solutol HS 15, ethanol, MCT	Prolonged circulation and higher AUC values as compared to the solution
Propofol	Solutol HS 15, Tween 80	Less painful as compared to marketed emulsion of propofol (propovan)
Artemether	Lecithin, labrasol, ethanol, Poloxamer 188, ethyl oleate	Significant improvement in anti-malarial activity as compared to the conventional oily solution
Quercetin	Tween 20, clove oil	Significant improvement in anti-leishmanial activity as compared to the free drug
Bassic acid	Tween 20, clove oil	Significant improvement in anti-leishmanial activity as compared to the free drug

Source: Abhijit A. Date and M.S. Nagarsenker, "Parenteral microemulsions: An overview," International Journal of Pharmaceutics 355 (2008): 24.

2.5 Ocular microemulsions

Water-continuous microemulsions for ocular application composed of lecithin and Macrogol- 1500-glyceroltriricinoleate as surfactants, polyethylene glycol 200 and propylene glycol as cosurfactants, isopropylmyristate as lipophilic component. These microemulsions have favourable features for ocular use. They show an acceptable physicochemical behaviour, especially pH value, refractive index and viscosity, and a good physiological compatibility. A prolonged pilocarpine release from microemulsions with lecithin was shown in in vitro experiments. The miotic activity was measured on albino rabbits (Haße and Keipert 1997: 179-183).

2.6 Oral microemulsions

Insulin loaded microemulsions were developed adopting a low shear reverse micellar approach using didoceyldimethylammonium bromide (DMAB) as the surfactant, propylene glycol (PG) as the co-surfactant, triacetin (TA) as the oil phase and insulin solution as the aqueous phase. The microemulsions displayed a 10-fold enhancement in bioavailability compared with plain insulin solution administered per

oral in healthy rats. The short-term in vivo efficacy in streptozotocin induced diabetic rats provided the proof of concept by a modest glucose reduction at a dose of 20 IU/kg (Sharma et al. 2010: 159-169).

2.7 Transdermal microemulsions

Microemulsions have several advantages as drug delivery systems, such as enhanced drug solubility, good thermodynamic stability, ease of manufacturing and enhancement of drug permeation effects upon transdermal administration. They have reported to enhance the transdermal permeation of drugs significantly compared to conventional formulations such as solutions, gels or creams. They are able to incorporate both hydrophilic and lipophilic drugs. Several plausible mechanisms have been proposed to explain the advantages of microemulsion for the transdermal delivery of a drug:

1. A large amount of drug can be incorporated in the formulation due to the high solubilizing capacity that might increase thermodynamic activity towards the skin.

2. The permeation rate of the drug from microemulsion may be increased, since the affinity of a drug to the internal phase in microemulsion can be easily modified to favour partitioning into stratum corneum, using different internal phase, changing its portion in microemulsion.

3. The surfactant and co surfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as penetration enhancers.

4. The percutaneous absorption of drug will also increase due to hydration effect of the stratum corneum if the water content in microemulsion is high enough.

Due to the small droplet size and large amount of inner phase in microemulsions, the density of droplets and their surface area are assumed to be high. Therefore, droplets settle down to close contact with the skin providing high concentration gradient and improved drug permeation. Moreover, low surface tension ensures good contact to the skin. Also, the dispersed phase can act as a reservoir making it possible to maintain an almost constant concentration gradient over the skin for a long time (Talegaonkar et al. 2008: 238-257).

Components of the transdermal formulations (Kogan and Garti 2006: 369-385)

Oil phase

Saturated and unsaturated fatty acids can be used as effective penetration enhancer for a variety of drugs. The most popular enhancer is oleic acid. Another compound that is often used as an oil phase and as a permeation enhancer in transdermal formulations is isopropyl myristate (IPM).

Surfactants

There is wide use of low toxicity substances such as phospholipids as penetration enhancers. Absorption of phospholipids on skin can increase tissue hydration, consequently increasing drug permeation. When phospholipids are applied to skin as vehicles due to their physico-chemical properties and structures they can fuse with stratum corneum lipids, perturb its structure and facilitate drug delivery. Non-ionic surfactants such as polysorbates (ethoxylated sorbitan esters) are very common use in topical formulations as solubilizing agents. Some study results indicate that a nonionic surfactant may also affect the skin barrier function. In the experiments conducted in vitro on rat skin it was found that Span 20®, Tween 20®, and Azone® have different mechanisms of enhancement. Span 20® and Azone® affect the intercellular lipids of the stratum corneum by making them more fluid. This way the diffusion of lipophilic compounds through the stratum corneum (lipophilic pathway) is enhanced. Tween 20® enhances penetration by allowing the polar molecule to partition across the barrier more easily. It is also possible that since the experiments were conducted in aqueous medium, large micelles were formed. These micelles have the potential to extract lipids from the skin. This modifies the composition of the membrane and favors permeation of the hydrophilic compounds. Recently, new low-irritant surfactants based on caprylocaproyl macrogolglycerides for microemulsions as drug delivery vehicles for topical application were studied. Other surfactants such as dioctyl sodium sulphosuccinate, Plurol Isostearique®, and Plurol Oleique®, are used very often.

Cosurfactants

Short-chain alkanols are widely used as permeation enhancers. Ethanol is very common among transdermal formulations and its addition is known to enhance the flux of several drugs. Sometimes when using ethanol-water-based vehicles, the effect of ethanol is concentration-dependent, and, therefore, under certain conditions it can even decrease the permeation. Various mechanisms have been suggested for the enhancing activity of ethanol. It can increase the drug solubility in the vehicle or it can alter the structure of the membrane and increase the permeability of the drug. Another mechanism is based on the fact that ethanol is volatilized from the applied formulation and, consequently, increases the drug concentration to a supersaturated state with a greater driving force for permeation. In addition, ethanol may extract some of the lipid fraction from the stratum corneum and, thus, can improve the drug flux through it. Propylene glycol (PG) has been found to act as an enhancer by a mechanism similar to that of ethanol.

Aqueous phase

In most of the studies water served as the aqueous phase. In some of the cases phosphate buffer of pH 7.4 was used.

3. Ketoprofen

Ketoprofen is an anionic non-steroidal anti-inflammatory drug (NSAID). It is a derivative of propionic acid. It has been described chemically in a number of ways:

1. (2*RS*)-2-(3-benzoylphenyl) propanoic acid (British Pharmacopoeial Commission 2010: 846)
2. 2-(3-benzoylphenyl) propionic acid (Moffat 1986: 697-698; Lund 1994: 933-935; Winholz 1983: 762)
3. 2-(benzoyl-3-phenyl) propionic acid (Lund 1994: 933-935; Liversidge 1981: 443-471)
4. 3-benzoyl-α-methylbenzeneacetic acid (Winholz 1983: 762)
5. α-(benzoylphenyl) propionic acid (Liversidge 1981: 443-471)
6. α-(3-benzoylphenyl) propionic acid (Liversidge 1981: 443-471)

7. m-benzoylhydratropic acid (Winholz 1983: 762)
8. (*RS*)-2-(3-benzoylphenyl) propionic acid (Sweetman 2002: 47-48).

Ketoprofen is a white or almost white, crystalline odourless powder with a sharp bitter taste (Sweetman 2002: 47-48). It is prepared by chemical synthesis as a racemate and contains not less than 99.0% and not more than the equivalent of 100.5% of (2*RS*)-2-(3-benzoylphenyl) propanoic acid, calculated with reference to the dried substance (British Pharmacopoeial Commission 2010: 846).

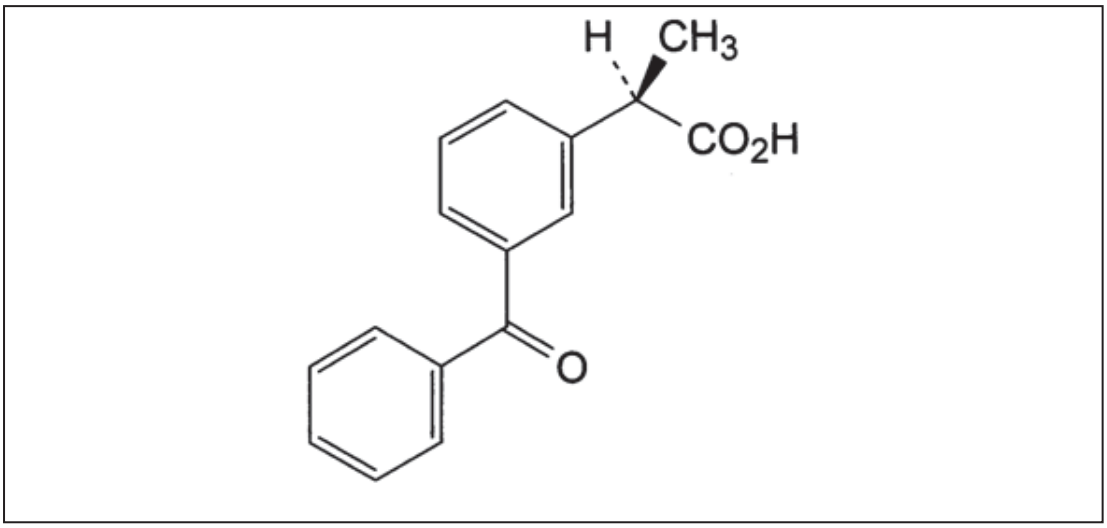

Figure 6 Ketoprofen structure

Source: British Pharmacopoeial Commission, The British Pharmacopoeia, London: The Pharmaceutical Press, 2010: 846.

Molecular weight

254.3 (BP 2010: 846)

pKa (Liversidge 1981: 443-471)

The pKa in dioxan: water (2:1) is 7.2, acetonitrile: water (3:1) is 5.02 and methanol: water (3:1) is 5.94.

Melting point

Ketoprofen has been reported to melt in the range of 94°C to 97°C (BP 2010: 846; Sweetman 2002: 47-48).

Solubility

Ketoprofen is practically insoluble in water, freely soluble in acetone, ethanol and methylene chloride (British Pharmacopoeial Commission 2010: 846). It is also soluble in chloroform, ether and benzene (Lund 1994: 933-935).

Ketoprofen formulations

Ketoprofen is currently marketed throughout the world in a variety of forms: capsules, tablets, injectable solutions, suppositories and gels.

Route	**Dosage form**	**Strength**
Oral	Tablets	50 mg
	Enteric coated	100 mg
	Capsules	50 mg, 75 mg, 100 mg
	Extended (Controlled) Release	100 mg, 200 mg
Parenteral	Intramuscular	100 mg/2 mL
Rectal	Suppository	100 mg
Topical	Gel	2.5 g/100 g

Ketoprofen can be used as a model drug to develop the transdermal drug delivery system such as effect of the amount of solvent in gel and cream preparation on drug release (Simon, Lionel and Charles 2003: 37-45), bioadhesive gels of ketoprofen by using gelling polymers like sodium carboxymethylcellulose, xanthan gum, poloxamer 407 and carbopol 934P as bioadhesive polymer with and without penetration enhancer (oleic acid) (Singh et al 2009: 193-198), forming ketoprofen-cyclodextrin complexes and entrapment in the liposome (Maestrelli et al. 2006: 53-60), encapsulation by hydroxypropyl-β-cyclodextrin and pH adjustment to enhance transdermal delivery of ketoprofen (Sridevi and Diwan 2002: 151-154), transdermal iontophoretic delivery in cadaver skin and healthy volunteers (Panus et al. 1997: 113-121).

The development of microemulsion composed of triglycerides as oil phase, a mixture of lecithin and *n*-butanol as a surfactant/co-surfactant system and an aqueous solution as the external phase for perceutaneous delivery of ketoprofen

showed an enhanced permeation through human skin with respect to emulsion and gel (Paolino et al. 2002: 21-31). The addition of enhancer such as limonene, menthol, cineol and camphor affected the perceutaneous absorption of ketoprofen loaded microemulsion formulation consisted of 3% ketoprofen, 6% oleic acid, 30% Labrasol/Cremophor RH 40 (1:1) and water (Rhee et al. 2001: 161-170).

CHAPTER 3
MATERIALS AND METHODS

1. Materials

1. Isopropyl myristate
2. Polyethelene glycol 400
3. Ethanol absolute
4. n-butanol
5. PEG-40- hydrogenated castor oil (Cremophor® RH40)
6. Water
7. Potassium chloride
8. di-basic Sodium phosphate (Na_2HPO_4)
9. mono-basic Potassium phosphate (KH_2PO_4)
10. Sodium chloride
11. Phosphoric acid
12. Methanol HPLC grade
13. Ketoprofen
14. Indomethacin
15. 2.5% Ketoprofen gel

2. Equipments

1. Analytical balance (Satorius CP224S, Sartorius CP3202S; Scientific promotion Co., Ltd.)

2. pH meter (HORIBA compact pH meter B-212, Sartorius Professional Meter PP-15)

3. Conductivity meter (EC Testr 11^+)

4. Viscometer (Brookfield DV-III ULTRA)

5. Zetasizer Nano ZS (Malvern instruments Ltd., Malvern, UK)

6. High performance liquid chromatography (HPLC) with diode array detector (Agillent technology, USA)

7. Franz diffusion cell

8. Cellulose acetate membrane filter 0.45 μm (VertiSep™)
9. Magnetic stirrer and magnetic bar
10. Measuring pipettes (1, 2, 5, 10 mL)
11. Micropipette 20-100 μL, 100-1000 μL, 1-5 mL (Biohit®; Gibthai Co., Ltd.)
12. Micropipette tip
13. TERUMO® Syringe 5 mL
14. Water bath
15. HPLC column: C18, 5μm, 4.6x 150 mm (VertiSep™)

3. Methods

3.1 Construction of pseudoternary phase diagram

The system consisted of isopropyl myristate as oil phase, water as aqueous phase, PEG40 hydrogenated castor oil (Cremophor® RH40) as surfactant and polyethylene glycol 400 (PEG400), ethanol absolute and n-butanol as co-surfactant. The surfactant/cosurfactant ratio was 1:1, 2:1, 3:1, 4:1, respectively, for the mixture of Cremophor® RH40: ethanol absolute and n-butanol. The surfactant/cosurfactant ratio was 3:1, 2:1, 1:1, 1:2, 1:3, respectively, for the mixture of Cremophor® RH40 and PEG400

Pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature. The mixture of IPM and surfactant/co-surfactant mixture was prepared at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, respectively. These mixtures were titrated dropwise with water under magnetic stirring. After being equilibrated, the systems were visually characterized. Transparent fluid systems were characterized as microemulsion.

3.2 Selection of microemulsion formulations

The criteria for selection of microemulsion formulations in the skin permeation studies were the good physicochemical property and stability of the formulations. In this research, the microemulsion formulations composed of IPM, water, Cremophor® RH40:PEG400 ratio 1:1 (Table 2) was selected. To study the effect of oil, surfactant mixture and water ratio to the characteristic, loading capacity

and drug skin permeability the formulation, the concentration of surfactant mixture at 50%w/w were used in the formulation A1-A6; IPM concentration was kept at 30%w/w in formulation B1-B4 and the water concentration was kept constant at 20%w/w in formulation C1-6.

Table 2 Microemulsion formulations

Formulation Number	%IPM	%Surfactant Mixture	%Water
A1	45	50	5
A2	40	50	10
A3	35	50	15
A4	30	50	20
A5	25	50	25
A6	20	50	30
B1	30	60	10
B2	30	55	15
B3	30	50	20
B4	30	45	25
C1	10	70	20
C2	15	65	20
C3	20	60	20
C4	25	55	20
C5	30	50	20
C6	35	45	20

3.3 Drug loading capacity

Drug loading capacity was studied by adding excess amount of ketoprofen to the microemulsion formulations and stirring for 48 h at 25 °C. The formulations were then centrifuged, and the supernatants were collected. About 0.1 g of supernatant with 10 mL of methanol were mixed together and then centrifuged.

The supernatants were collected, appropriately diluted, and ketoprofen concentration was determined by high performance liquid chromatography (HPLC).

3.4 Loading of 2.5 % ketoprofen into microemulsion formulations

Microemulsion formulations were prepared by mixing surfactant mixture, IPM and water by weight ratio using magnetic stirrer at ambient temperature. 0.25 g of ketoprofen was accurately weighed and adjusted to 10 g with the microemulsion formulations, following by stirring with magnetic stirrer at ambient temperature.

3.5 Drug content analysis in microemulsion formulations

Drug content in microemulsion formulations was determined by dissolving 2.5% ketoprofen in microemulsion formulations. 0.5 g of 2.5% ketoprofen loading microemulsion was accurately weighed and adjusted to 10 mL with methanol. The mixture was centrifuged, then the supernatants were collected and appropriately diluted. Ketoprofen was determined by high performance liquid chromatography (HPLC).

3.6 Characterization of microemulsion formulations

The characteristics of microemulsion both before and after loading with 2.5% w/w ketoprofen were studied as followed,

3.6.1 Conductivity

The conductivity of the microemulsion formulations was determined using conductivity meter (EC Testr 11^{+}) at 25ºC. The measurements were performed in triplicate.

3.6.2 Viscosity

The viscosity was measured using a Brookfield DV-III ULTRA rheometer (Brookfield Engineering Laboratories, USA) fitted with a CP-52 spindle. Brookfield Rheocalc operating software was used to control the rheometer. The measurements were performed in triplicate at 25 ºC.

3.6.3 Particle size

The droplet size of the microemulsion formulations was measured using photon correlation spectroscopy (Zetasizer Nano series, Malvern).

The measurement was performed using a He-Ne laser at 633 nm with clear disposable zeta cell. The measurements were performed in triplicate at 20 ºC.

3.6.4 pH

The pH was determined using pH meter (Sartorius Professional Meter PP-15). The measurements were performed in triplicate.

3.7 Skin permeation studies

Shed snake skin of *Naja kaouthia* was used as a model membrane because it was reported to be similar in permeability with human skin (Panomsuk et al 2004). It was stored at -10°C prior to use. After thawing, the skin was cut to a round section of 1 $inch^2$ and was then immediately placed on the diffusion cell. The skin sample was mounted between two half-cells of franz diffusion cell (6.0 mL volume and 2.31 cm^2 effective diffusion area) with a water jacket connected to a water bath at 32°C. The dorsal surface of skin was placed in contact with the drug loading microemulsion formulations, 2.5% ketoprofen dissolved in PEG400: water ratio 7: 3 and 2.5% ketoprofen gel available in the market (ketoprofen gel A and ketoprofen gel B). The receiver was filled with phosphate buffer pH 7.4 (PBS) and stirred at 1200 rpm with magnetic bar. A part of the receiver solution (1 mL) was collected at 1, 2, 4, 6, 8, 10, 12 h, respectively, and the same volume of PBS was replaced to keep the volume constant. The samples were stored at 4°C until analysis. The cumulative amount of KP was plotted against time, and the pseudo-steady state flux was determined from the slope of linear regression analysis. 2.5% ketoprofen dissolved in PEG400: water ratio 7: 3 was used as the control vehicle. The enhancement ratio (ER) is determined by the equation 9:

$$ER = \frac{\text{flux from microemulsion formulation}}{\text{flux from control vehicle}} \quad (9)$$

3.8 HPLC analysis

The HPLC system consisted of a pump, HPLC column C18, 4.6 mm x 150 mm, a diode array detector and an integrator. The flow rate of mobile phase was

1.0 mL/min. The mobile phase composed of 0.1% (v/v) phosphoric acid: methanol (25:75, v/v) was used. The detection wavelength was set at 254 nm.

3.8.1 Standard curve for drug loading capacity and drug content assay

0.5 g ketoprofen was dissolved in 20 mL methanol and diluted to 50 mLwith mobile phase. The stock solution concentration was 10 mg/mL of ketoprofen. The stock solution was diluted with mobile phase to 10, 25, 50, 75, 100 µg/mL, respectively.

3.8.2 Standard curve for skin permeation studies

Standard ketoprofen

0.05 g ketoprofen was dissolved in 20 mL methanol and diluted to 50 mLwith mobile phase. The stock solution concentration was 1 mg/mL of ketoprofen. The stock solution was diluted with mobile phase to 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 µg/mL, respectively.

Internal standard indomethacin

0.025 g indomethacin (IDM) was dissolved in 20 mL methanol and diluted to 50 mLwith mobile phase. The stock solution concentration was 500 µg/mL of indomethacin. The stock solution was diluted with mobile phase to 10 mL (50µg/mL). The solution was then mixed with ketoprofen solution to make 5µg/mL concentration of IDM before analysis.

3.9 Statistical analysis

All experimental measurements were triplicately performed. Result values were expressed as mean value ± standard deviation (SD). Statistical significance of differences in steady state flux of all microemulsion formulations, 2.5% ketoprofen solution and 2.5% ketoprofen gel available in the markets were examined using analysis of variance (ANOVA) with post hoc test.

CHAPTER 4
RESULTS AND DISCUSSION

1. Pseudo-ternary phase diagram

The pseudo-ternary phase diagram of microemulsion system composed of isopropyl myristate (IPM) as oil phase, water, PEG40-hydrogenated castor oil (Cremophor® RH40) as surfactant with different co-surfactant and surfactant:co-surfactant ratio are shown in Figure 7-9. The sheded area of the phase diagram indicated microemulsion region while the outside area referred to the turbid regions.

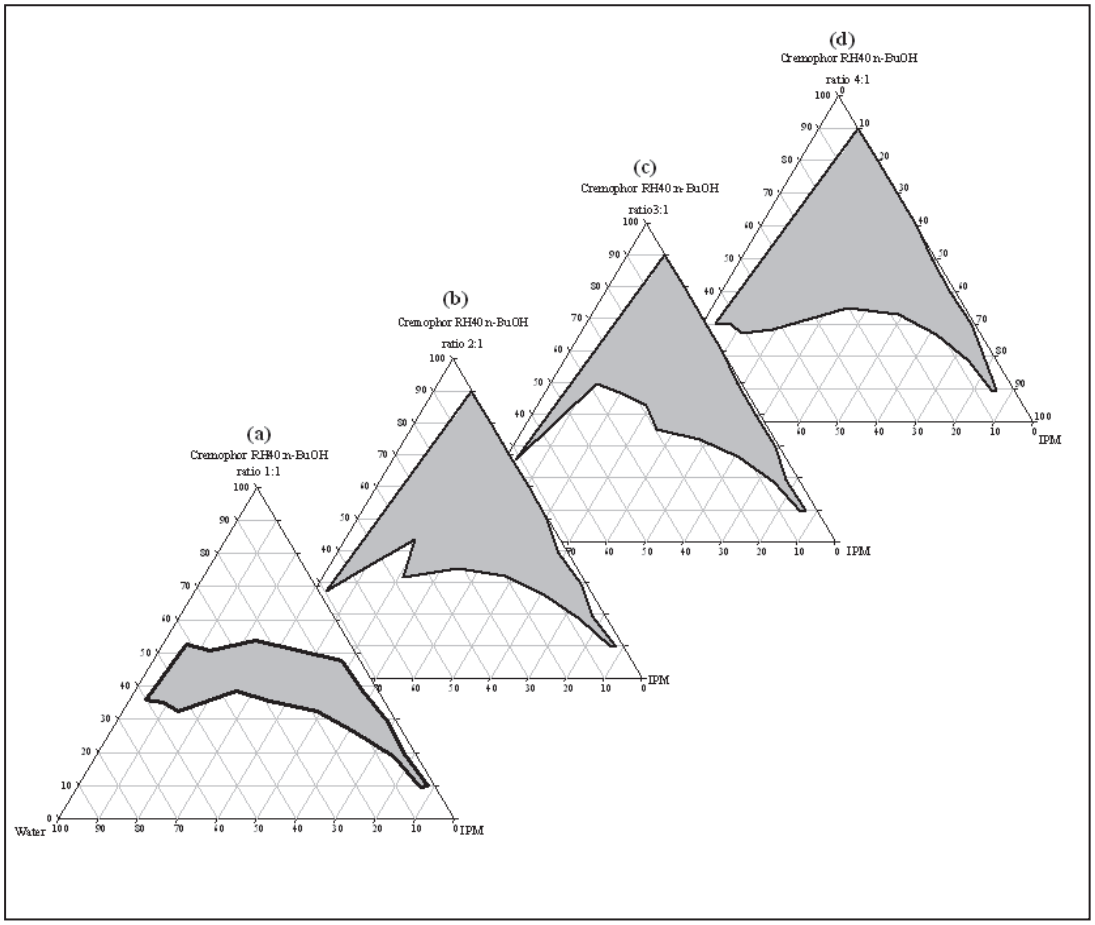

Figure 7 Pseudo-ternary phase diagram of isopropyl myristate (IPM), water, Cremophor® RH40: n-Butanol ratio (a) 1:1, (b) 2:1, (c) 3:1, (d) 4:1

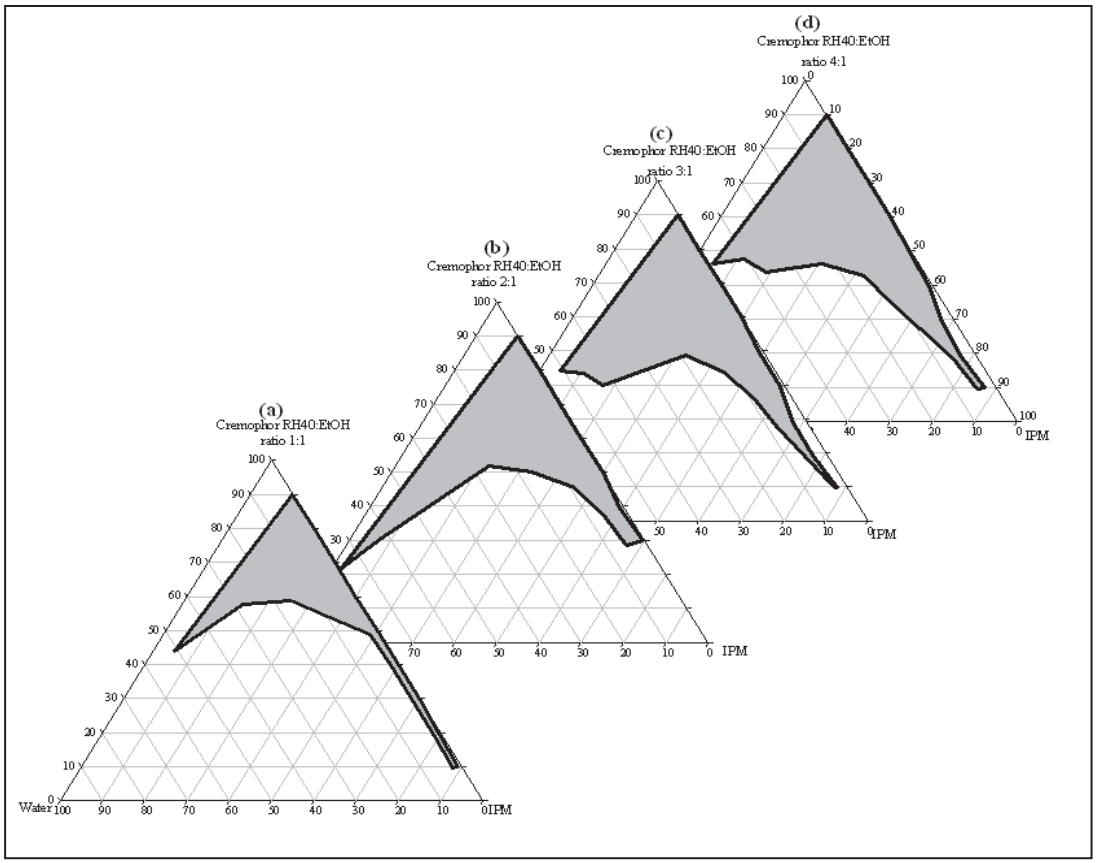

Figure 8 Pseudo-ternary phase diagram of isopropyl myristate (IPM), water, Cremophor® RH40: ethanol ratio (a) 1:1, (b) 2:1, (c) 3:1, (d) 4:1

1.1 Cremophor® RH40: n-Butanol

The pseudo-ternary phase diagrams with various weight ratios of Cremophor® RH40 to n-Butanol are shown in Figure 7. The smallest microemulsion area was found in the formulation containing the ratio of Cremophor® RH40: n-Butanol (1:1) (Figure 7a). As the ratio of Cremophor® RH40 in the surfactant mixture (SM) was increased from 2:1 (Figure 7b) to 3:1 (Figure 7c), and to 4:1 (Figure 7d), the microemulsion area increased.

1.2 Cremophor® RH40: ethanol

The pseudo-ternary phase diagrams with various weight ratios of Cremophor® RH40 to absoute ethanol are shown in Figure 8. The microemulsion area of the system composed of IPM, water, Cremophor® RH40: ethanol increased when the ratio of Cremophor® RH40 in the surfactant mixture was increased. However, the

microemulsion area of this system was smaller than that of the previous system compared at the same surfactant to co-surfactants ratio.

1.3 Cremophor® RH40: PEG400

The pseudo-ternary phase diagrams with various weight ratios of Cremophor® RH40 to PEG400 are shown in Figure 9. The largest microemulsion area was observed in the system composed of Cremophor® RH40: PEG400 ratio 1:1 (Figure 9c). The microemulsion area decreased when the ratio of Cremophor® RH40 in the SM was increased from ratio 1:1 (Figure 9c) to 1:0.5 (Figure 9b) and to 1:0.3 (Figure 9a), respectively.

As the ratio of PEG400 in the SM was increased from 1:1(Figure 9c) to 1:2 (Figure 9d), and to 1:3 (Figure 9e), respectively, the microemusion area also decreased. By increasing ratio of PEG400, the microemulsion area was smaller than increasing ratio of Cremophor® RH40, suggesting that PEG400 had more effect on microemulsion forming than Cremophor® RH40.

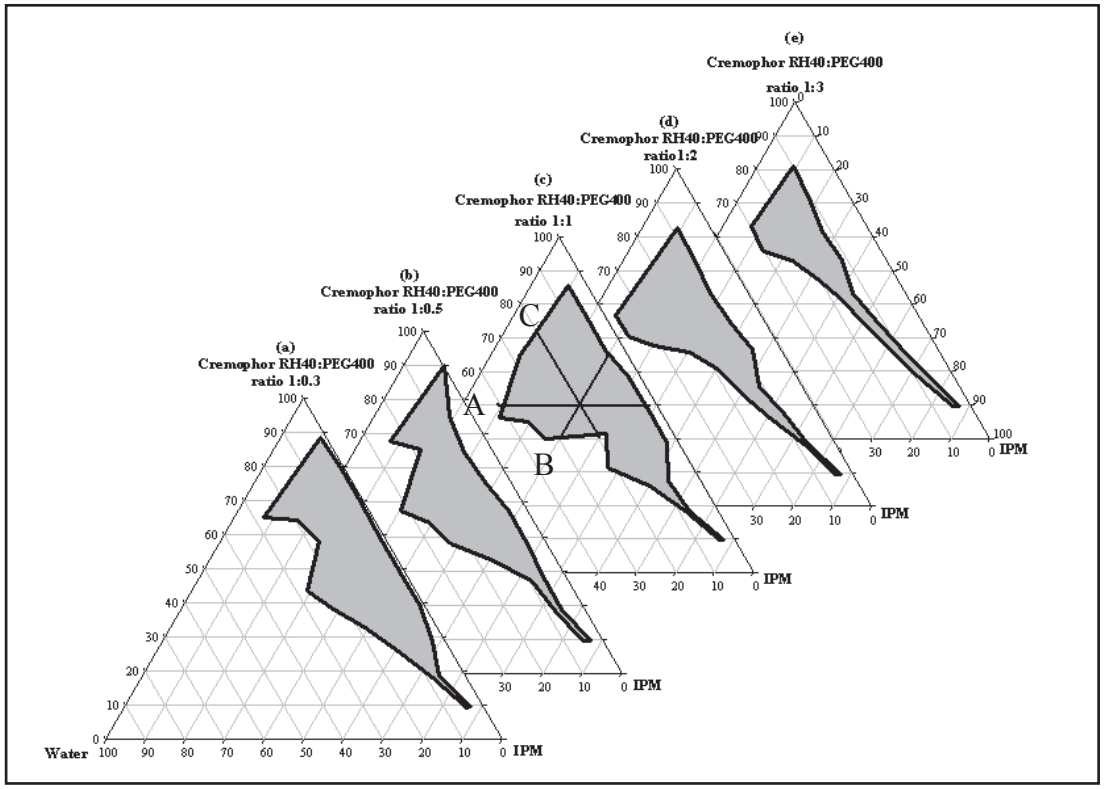

Figure 9 Pseudo-ternary phase diagram of isopropyl myristate (IPM), water, Cremophor® RH40: PEG400 (a) 1:0.3, (b) 1:0.5, (c) 1:1, (d) 1:2, (e) 1:3

2. Selection of microemulsion formulations

In this study, the microemulsion formulations of Cremophor® RH40: PEG400 ratio 1:1 (Figure 9c) was selected for further study since it was composed of non-volatile co-surfactant (PEG400). In the formulation containing volatile co-surfactant such as ethanol and n-butanol, the viscosity of microemulsion was changed when kept at room temperature for 1 month. This might be due to the volatile property of ethanol and n-butanol. Moreover, butanol has stink odor, which makes unpleasant property for use as dermal preparation.

The concentration of surfactant mixture, IPM, and water used in this study was selected from the approximately middle range of concentration of the microemulsion area. The concentration of surfactant mixture was fixed to 50% w/w in A1-A6 formulation. The IPM concentration was fixed to 30% w/w in B1-B4 formulation and the water concentration was fixed to 20% w/w in C1-6 formulation.

3. Drug loading capacity

Drug loading capacity of microemulsions formulations were between 9.13 and 21.08 % w/w (Table 3). The loading capacity of formulations A1-A6 increased by increasing the amount of IPM, and decreased when the amount of water increased (Figure 10). These results suggested that IPM significantly affected the loading capacity of KP in microemulsions. In formulations B1-B4; the loading capacity increased by increasing the amount of surfactant, however it decreased when the amount of water was increased, indicating that surfactant could significantly increase the solubility of drug in the formulations. In the case of formulations C1-C6; water amount was kept constant at 20%, drug loading capacity tended to increase when amount of surfactant mixture increased. The result indicated that surfactant could increase the solubility of drug in the formulations. However, the effect of surfactant mixture on loading capacity of ketoprofen was more than IPM (Figure 11), as the solubility of KP in IPM (1.90% w/w) was significantly lower than that of surfactant mixture (29.54 % w/w). However the solubility of KP in these two solvents was extremely higher than in water (0.03% w/w).

Table 3 Maximum KP loading capacity of microemulsion formulations

Formulation	Drug loading capacity±SD	
	(mg/g)	(% w/w)
A1	189.03 ± 1.47	18.90 ± 0.15
A2	181.75 ± 2.21	18.18 ± 0.22
A3	171.87 ± 1.22	17.19 ± 0.12
A4	166.29 ± 2.04	16.63 ± 0.20
A5	157.38 ± 0.97	15.74 ± 0.10
A6	91.37 ± 0.69	9.14 ± 0.07
B1	210.84 ± 1.82	21.08 ± 0.18
B2	204.45 ± 1.70	20.45 ± 0.17
B3	166.29 ± 2.04	16.63 ± 0.20
B4	114.92 ± 1.41	11.49 ± 0.14
C1	205.13 ± 3.52	20.51 ± 0.35
C2	205.47 ± 1.68	20.55 ± 0.17
C3	175.52 ± 3.03	17.55 ± 0.30
C4	173.76 ± 1.57	17.38 ± 0.16
C5	166.29 ± 2.04	16.63 ± 0.20
C6	138.92 ± 0.18	13.89 ± 0.02
Water	0.27 ± 0.00	0.03 ± 0.00
IPM	19.05 ± 0.13	1.90 ± 0.01
Cremophor RH40:PEG400 1:1	295.39 ± 1.36	29.54 ± 0.14

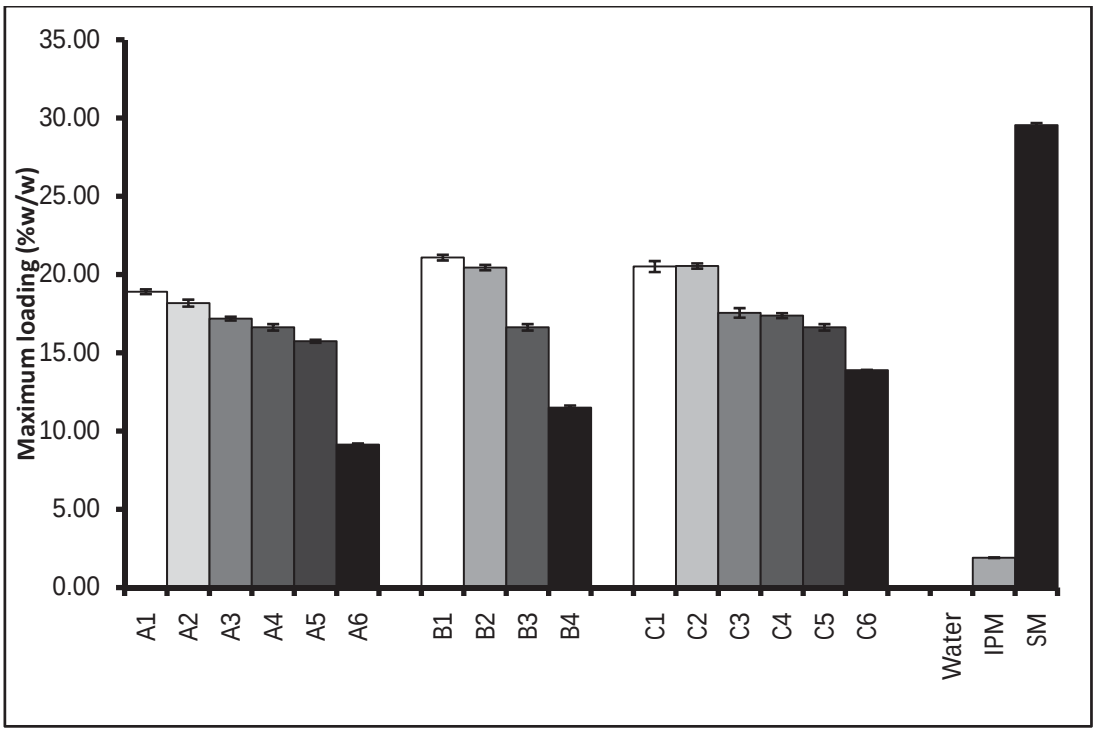

Figure 10 Maximun loading capacity (% w/w) of microemulsion formulation, water, IPM and surfactant mixture

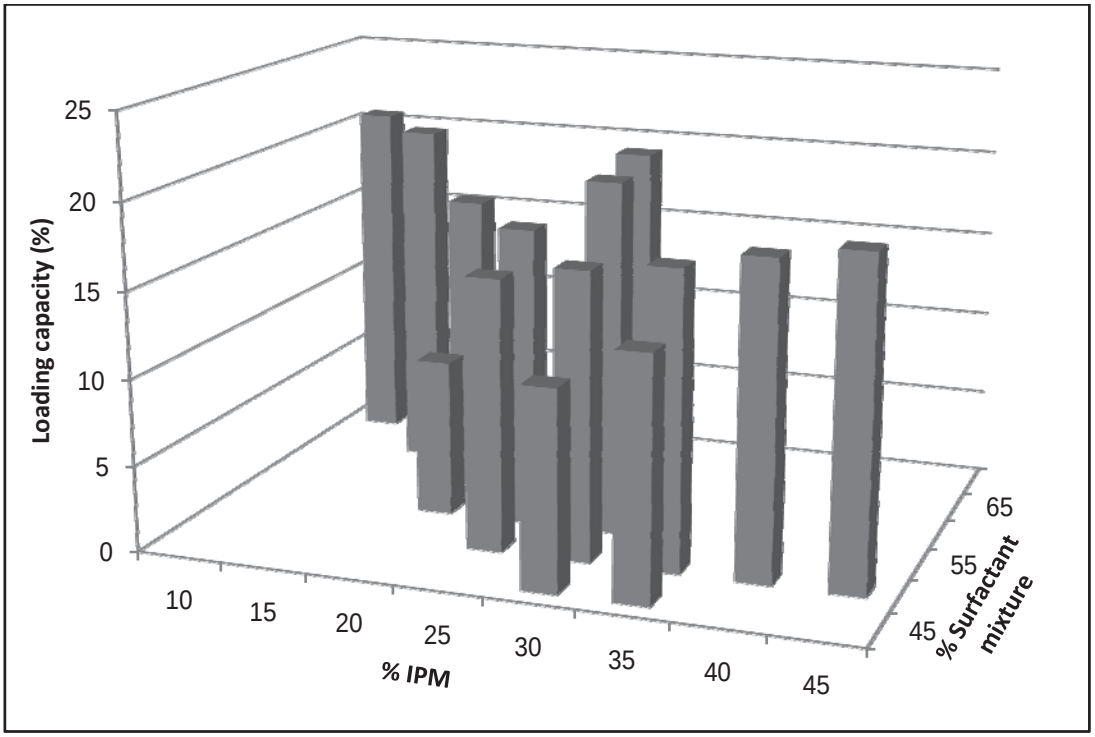

Figure 11 Maximum loading capacity (% w/w) of microemulsion formulations

4. Formulation of 2.5% KP microemulsion

4.1 Drug content

The KP content in ketoprofen loading microemulsion formulations was between 97.17 and 102.05% (Table 4). The results indicated that the preparation process did not effect on the content of KP.

Table 4 KPcontent in microemulsion formulations

Formulation	KP content ± SD	
	(mg/g)	(% content)
A1	24.76 ± 0.09	99.04 ± 0.37
A2	24.73 ± 0.05	98.94 ± 0.19
A3	25.23 ± 0.09	100.92 ± 0.35
A4	24.58 ± 0.12	98.32 ± 0.47
A5	25.46 ± 0.12	101.84 ± 0.49
A6	24.87 ± 0.09	99.49 ± 0.35
B1	24.29 ± 0.12	97.17 ± 0.49
B2	24.74 ± 0.04	98.95 ± 0.16
B3	24.58 ± 0.12	98.32 ± 0.47
B4	25.51 ± 0.03	102.05 ± 0.13
C1	24.62 ± 0.08	98.49 ± 0.33
C2	24.60 ± 0.01	98.40 ± 0.06
C3	25.18 ± 0.03	100.72 ± 0.13
C4	24.30 ± 0.02	97.18 ± 0.07
C5	24.62 ± 0.03	98.48 ± 0.13
C6	24.76 ± 0.09	99.04 ± 0.37

4.2 Physicochemical properties

The concentration of KP in this study was 2.5% w/w. It has been reported that at this concentration KP can permeate into the skin for the therapeutic concentration for anti-inflammatory effect (Patel and Leswell 1996: 497-507). Moreover, the 2.5 % KP gel is available in the markets and can be used to compare the skin permeation. In this study, 2.5 % KP could be loaded in the microemusion without any precipation, as this concentration was lower than the maximum loading of all formulations. The characteristics of microemulsion formulations before and after loading of 2.5% KP are shown in Table 5.

4.2.1 Droplet size

The droplet size in both before and after loaded with 2.5 % microemulsion was in the nano-size range (45.67-467.97 nm) (Figure 12). The loading of ketoprofen in the formulation did not significantly influence on the droplet size of microemulsions.

4.2.2 Viscosity

The viscosity tended to increase when the water amount in the formulations increased (Figure 13). It is expected that the hydrophilic chain of nonionic surfactant are strongly hydrated and connected together with hydrogen bonds allowing strong interaction (Podlogar et al. 2004: 115-128; Boonme et al. 2006: E1-E6). In case of formulation C1-C6, when the amount of water was fixed at 20%, the formulation viscosity increased as the amount of surfactant mixture decreased. It is expected that in the high amount of surfactant with limited amount of water, the hydrophilic chain of the surfactant can not be strongly hydrated. However, when the amount of surfactant decreased and the surfactant/water ratio increased, hydrophilic chain of surfactant were more hydrated, the viscosity then increased.

KP loaded into the microemulsions did not influence on the viscosity of microemulsion when water/surfactant ratio was less than 20/50. When water/surfactant ratio was more than 20/50, the microemulsion containing 2.5% KP had lower viscosity than blank microemulsion (Figure 14). KP structure contains a large lipophilic (apolar) and small hydrophilic (polar) domain (-COOH), therefore, it could act as a co-surfactant. It does not only remain in the oil phase but is also be

incorporated in the surfactant film, preventing the formation of strongly hydration and hydrogen bonds interaction of nonionic surfactant in high water amount formulation (Podlogar, Rogač and Gašperlin 2005: 68-77).

4.2.3 Conductivity

The conductivity of the microemulsion formulation before loading of 2.5% KP was between 0.2 and 37.27 μS/cm. It has been previously reported that the conductivity of w/o microemulsions was at lower than 10 μS/cm, and the o/w microemulsion have relatively high conductivity as compared with w/o microemulsions (about 10-100 μS/cm) (Baroli et al 2000: 209-218; Park et al 2005: 243-248), moreover, the o/w microemulsion have higher viscosities than w/o system (Boonme et al. 2006: E1-E6). According to the conductivity and viscosity measurement, it could be suggested that formulation A1, A2, A3, B1, C1, C2 and C3 were w/o, however other formulations were o/w microemulsions. The electrical conductivity of microemulsion formulations increased as the water amount increased (Figure 15). KP loaded did not significantly (p-value>0.05) affect the conductivity of microemulsions (Figure 16). The results indicated that ketoprofen did not change the microemulsions type. Junyaprasert et al. reported that the model drugs in free-base form did not affect the conductivity as compared to the blank counterparts while the drugs in salt form led to an increased conductivity, as salt forms were expected to dissociate in the presence of water and thereby causing an increase in conductivity of the nonionic microemulsions (Junyaprasert et al. 2007: 288-298).

4.2.4 pH

The pH of microemulsion tended to decrease when the ratio of water/surfactant increased (Figure 17). The pH of surfactant mixture was 8.9, and the pH of water was 6.3. When the surfactant mixture was diluted with water the pH of the microemulsion was gradually decreased. When KP was loaded into microemulsion, the pH of the microemulsions significantly decreased (Figure 18). KP is a weak acid, and the pH of saturated ketoprofen in water was 4.0. The result indicated that almost of ketoprofen molecules in the microemulsion formulation was in the ionized-form since pH of microemulsion system was higher than pKa of KP.

Table 5 Physicochemical properties of microemulsion formulation

Formulation	Size±SD(nm)		Viscosity±SD (cP)		Conductivity±SD (μS/cm)		pH±SD	
	Blank ME	2.5% KP loaded ME	Blank ME	2.5% KP loaded ME	Blank ME	2.5% KP loaded ME	Blank ME	2.5% KP loaded ME
A1	77.86±4.60	94.73±3.79	253.56±0.95	229.31±0.95	1.70±0.00	0.40±0.00	8.48±0.02	6.76±0.01
A2	257.60±2.13	206.13±9.62	81.03±0.00	223.25±1.66	1.73±0.06	2.50±0.00	8.08±0.02	6.71±0.02
A3	106.03±15.16	207.47±17.46	308.68±1.91	361.05±2.53	9.70±0.00	8.83±0.06	7.79±0.01	6.55±0.02
A4	172.10±2.62	176.40±5.62	951.61±2.55	732.02±8.16	20.87±0.15	18.73±0.06	7.36±0.02	6.35±0.02
A5	170.87±4.52	152.27±11.50	1119.53±0.00	914.48±3.31	24.00±0.00	23.30±0.00	7.15±0.02	6.17±0.01
A6	105.73±1.66	98.68±3.41	1137.62±1.50	792.11±1.66	34.50±0.10	33.10±0.00	6.84±0.02	6.09±0.01
B1	45.67±0.85	50.40±3.75	110.80±0.00	131.74±0.95	0.20±0.00	0.60±0.00	7.81±0.02	6.77±0.00
B2	204.27±9.64	194.33±12.76	189.07±0.95	120.72±1.66	13.23±0.06	15.50±0.00	7.79±0.01	6.70±0.01
B3	172.10±2.62	176.40±5.62	951.61±2.55	732.02±8.16	20.87±0.15	18.73±0.06	7.36±0.02	6.35±0.02
B4	253.30±18.56	275.87±6.58	1158.67±2.53	892.43±8.16	37.27±0.12	35.50±0.00	7.08±0.03	6.25±0.02
C1	467.97±32.59	285.70±6.06	384.20±0.95	280.57±2.52	6.80±0.10	9.20±0.00	7.77±0.02	6.65±0.01
C2	233.70±51.73	381.03±16.56	234.82±0.00	283.33±2.52	8.90±0.10	7.87±0.06	7.63±0.02	6.58±0.01
C3	225.73±33.42	167.30±26.97	277.82±1.66	266.24±7.21	8.77±0.06	8.87±0.06	7.49±0.02	6.53±0.00
C4	204.27±8.17	131.80±16.61	483.97±0.95	428.30±5.96	16.47±0.06	14.03±0.06	7.44±0.02	6.44±0.01
C5	172.10±2.62	176.40±5.62	951.61±2.55	732.02±8.16	20.87±0.15	18.73±0.06	7.36±0.02	6.35±0.02
C6	172.10±16.87	176.40±9.42	1067.35±0.96	952.51±5.96	18.60±0.00	20.97±0.06	7.31±0.02	6.04±0.01

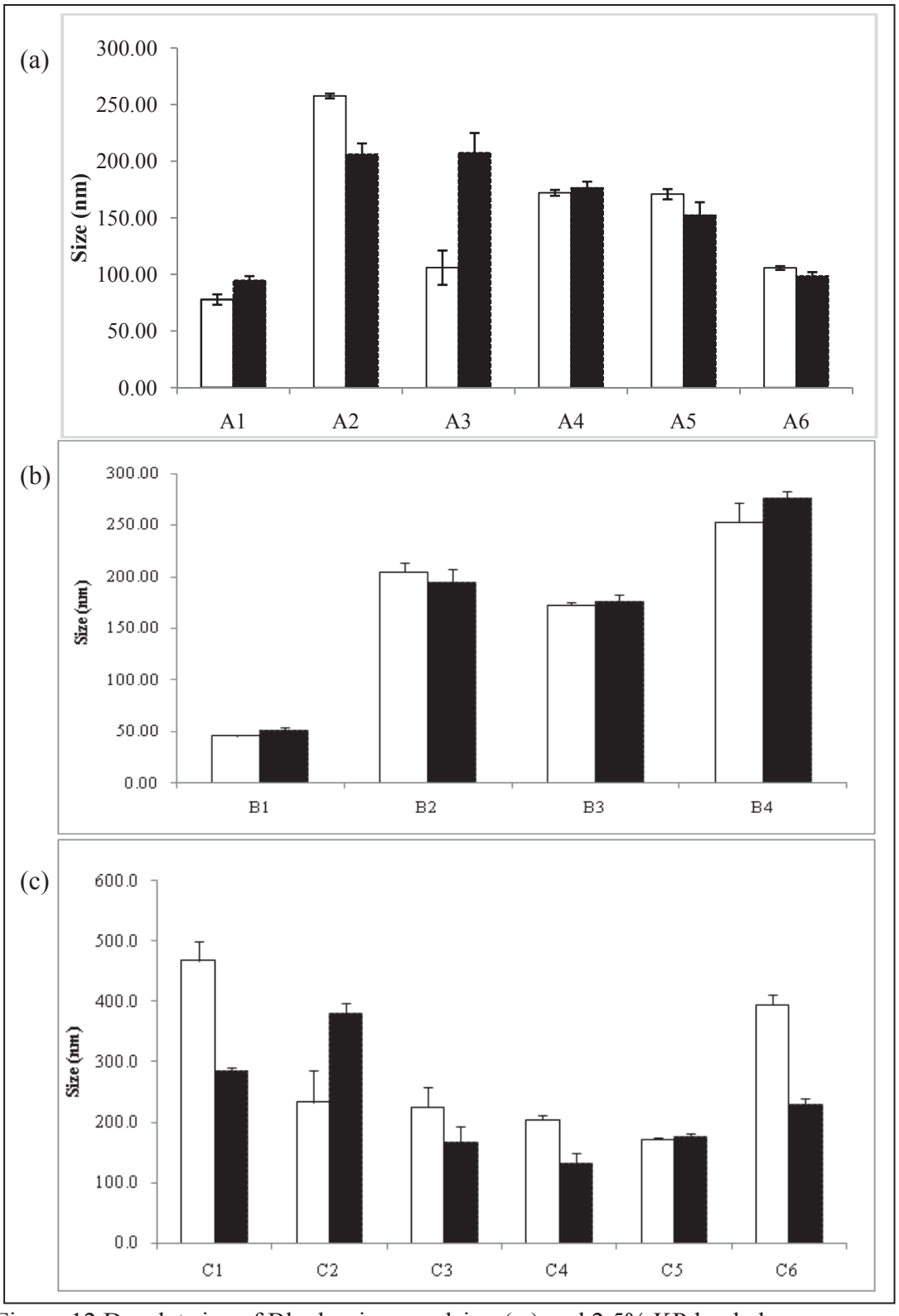

Figure 12 Droplet size of Blank microemulsion (□) and 2.5% KP loaded microemulsion (■): formulation (a) A1-A6, (b) B1-B4, (c) C1-C6

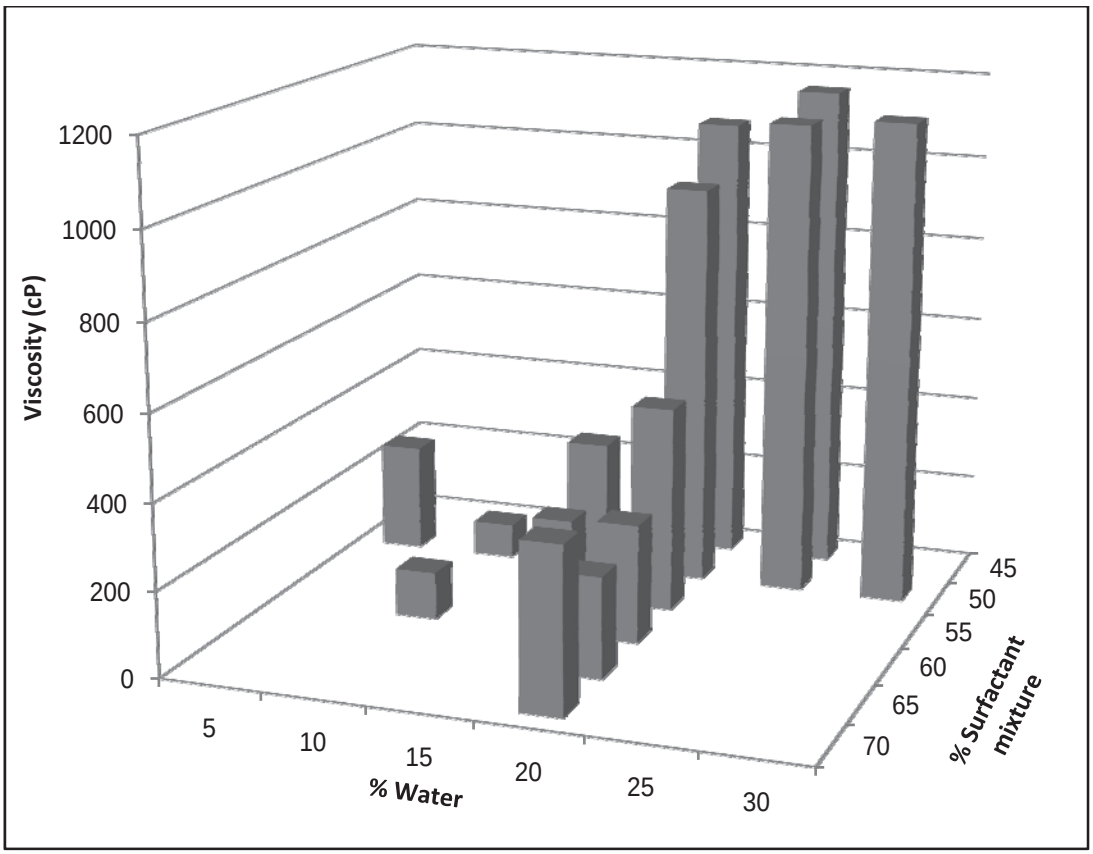

Figure 13 Viscosity of blank microemulsion

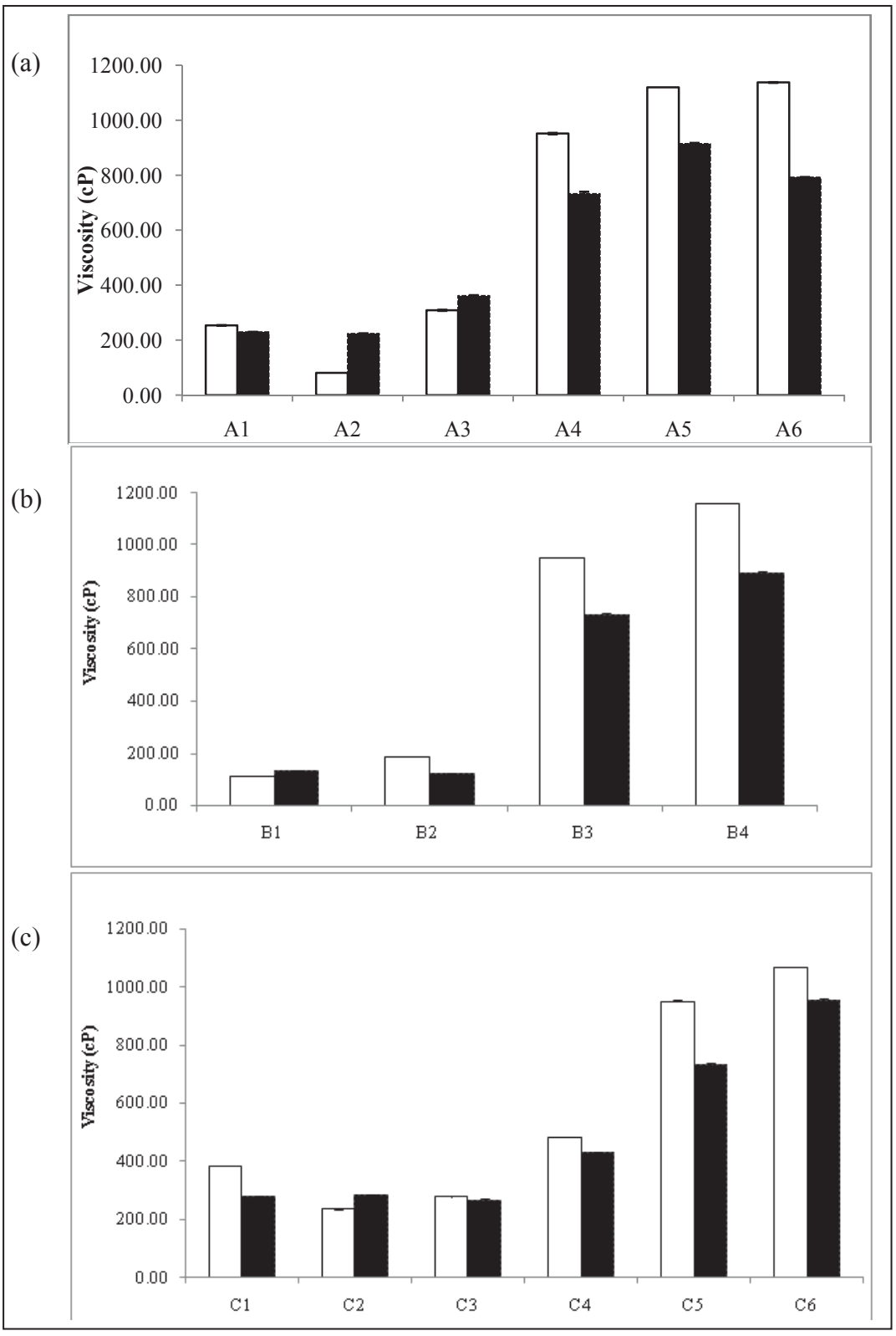

Figure 14 Viscosity of Blank microemulsion (□) and 2.5% KP loaded microemulsion (■): formulation (a) A1-A6, (b) B1-B4, (c) C1-C6

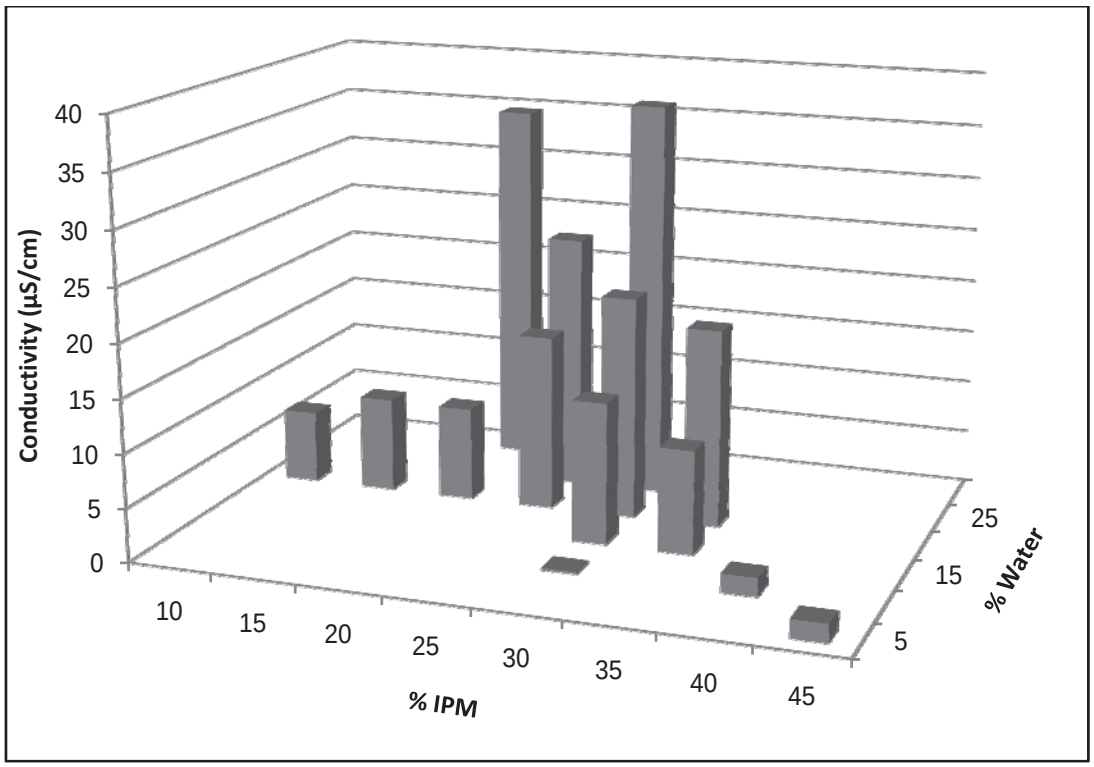

Figure 15 Conductivity of blank microemulsion

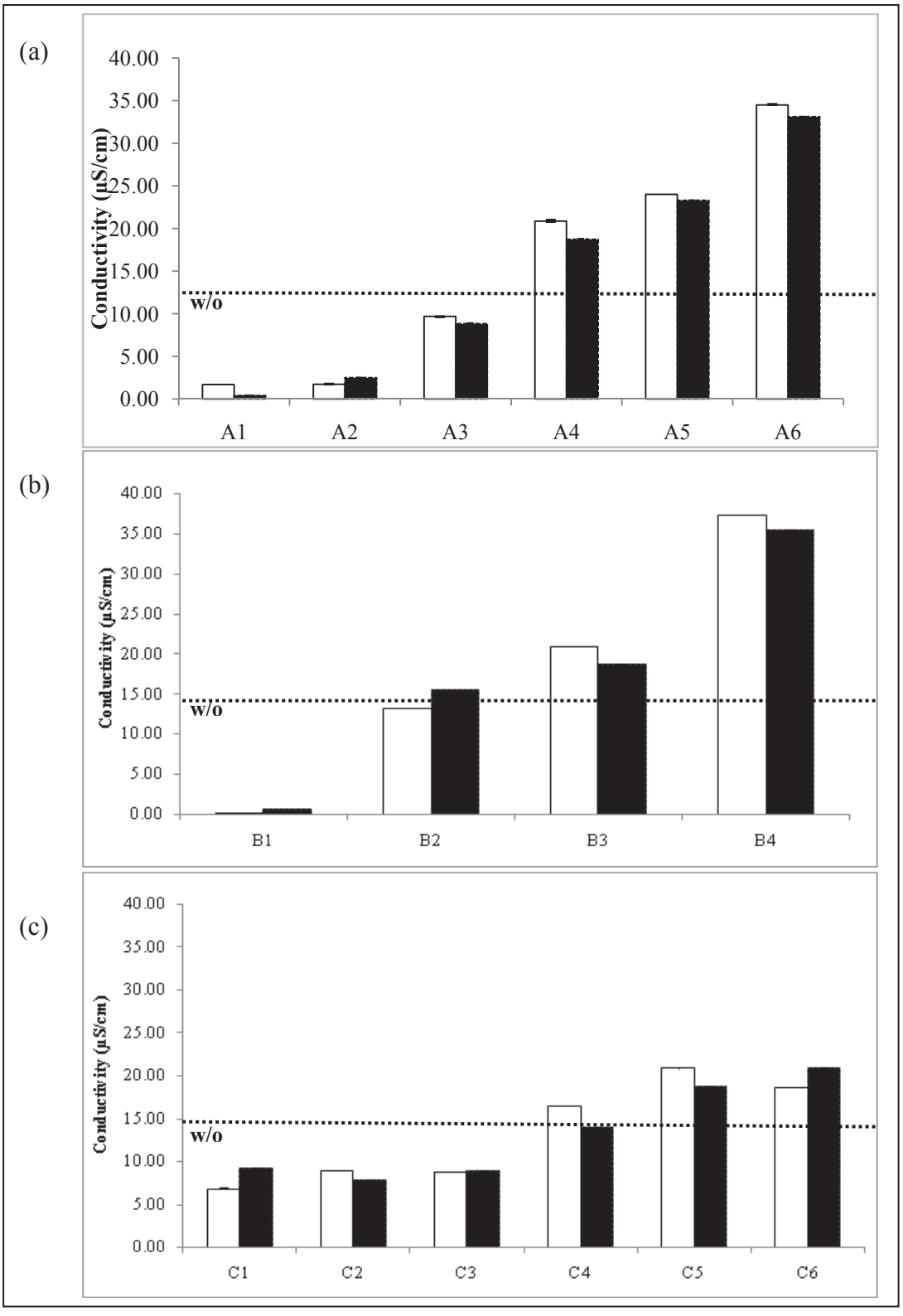

Figure 16 Conductivity of Blank microemulsion (□) and 2.5% KP loaded microemulsion (■): formulation (a) A1-A6, (b) B1-B4, (c) C1-C6

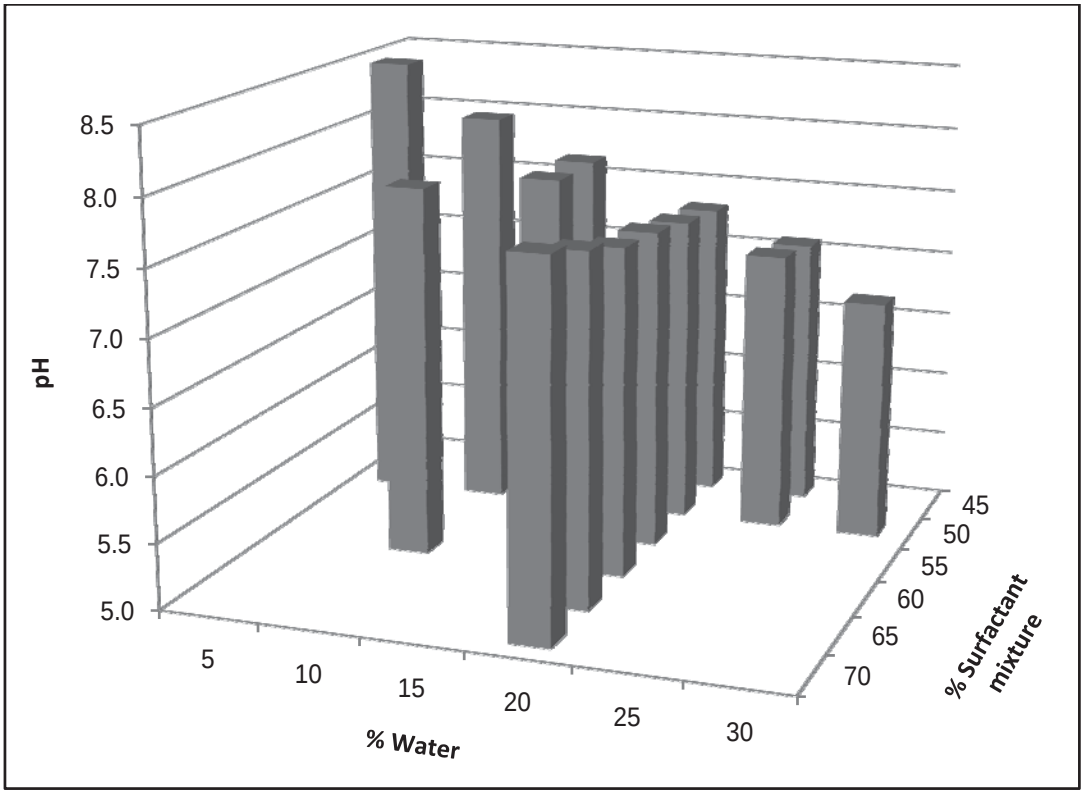

Figure 17 pH of blank microemulsion

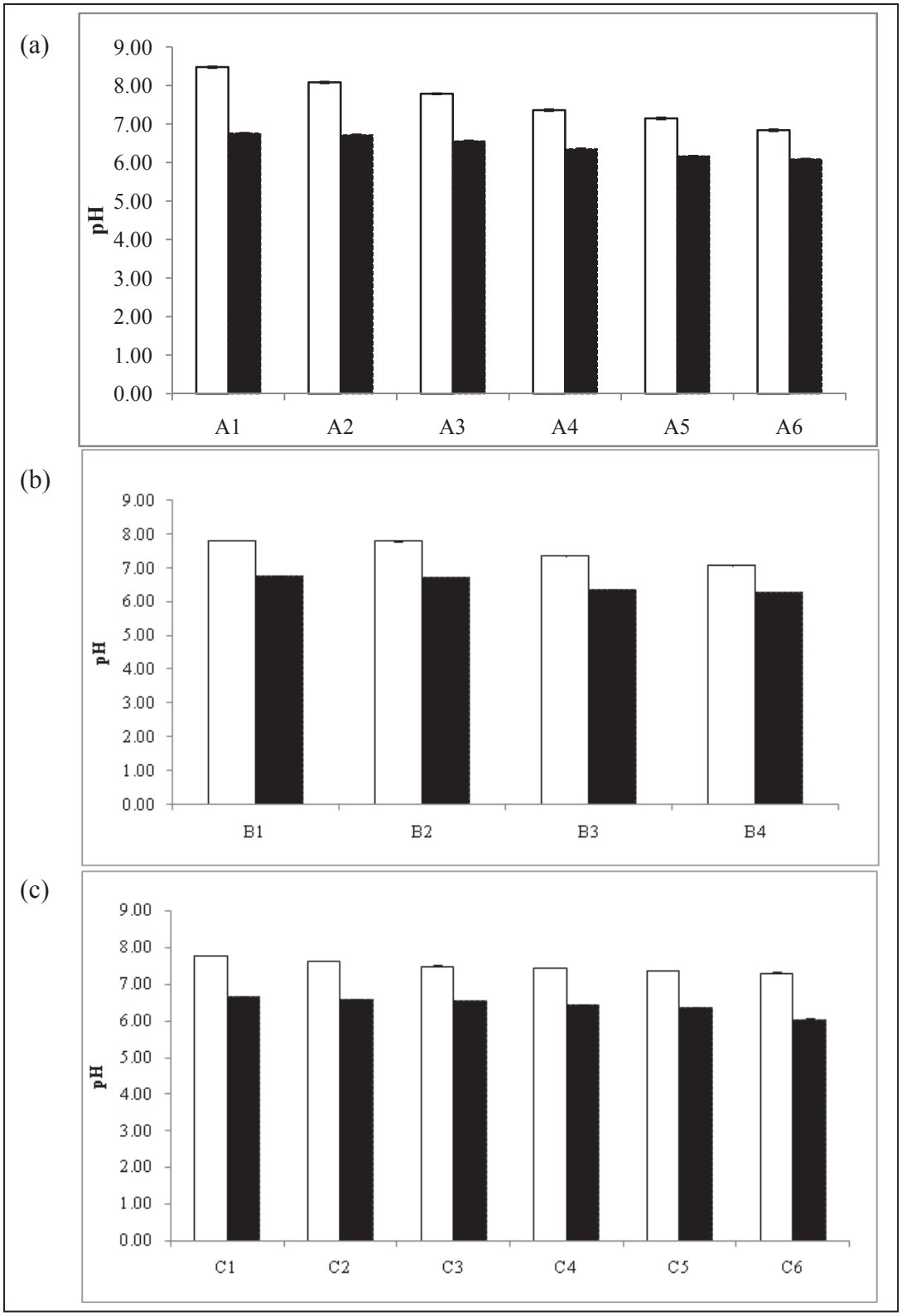

Figure 18 pH of blank microemulsion (□) and 2.5% KP loaded microemulsion (■): formulation (a) A1-A6, (b) B1-B4, (c) C1-C6

4.3 Skin permeation

Skin permeation profiles of all formulations, 2.5% commercial KP gel and 2.5% KP solution are shown in Figure 19. The skin permeation parameter; flux and lag time, and enhancement ratio are shown in Table 6. The flux of all prepared microemulsion formulations and commercial KP gel (company A, B) was significantly higher than 2.5% ketoprofen solution about 2-26 folds (Figure 20-24). The possible mechanisms of action of microemulsion in the skin permeation enhancement have been previously discussed in the literature. Firstly, microemulsions act as drug reservoirs where loaded drug is released from the inner pseudophase to the outer pseudophase and finally further into the outer. Secondly, microemulsion droplets might breakdown on the surface of stratum corneum and then release their content into skin. Thirdly, skin permeation of loaded drug occurs directly from the droplets to the stratum corneum without microemulsion fusion at the stratum corneum. The last mechanism has been frequently supported by findings of other groups and indicates that the enhancement effect of microemulsion is caused by the nano-sized droplets dispersed in continuous phase which can move easily into the stratum cormeum and carry the drug through the skin barrier (Peltola et al 2003: 99-107; Kogan and Garti 2006: 369-385; Junyaprasert et al 2007: 288-298; Karande and Mitragotri 2009: 2362-2373). Therefore, the skin permeation enhancement was obtained in the microemulsion formulations.

According to the conductivity, it could be suggested that formulation A1, A2, A3, B1, C1, C2 and C3 were w/o, however other formulations were o/w microemulsions. The flux of w/o microemulsion was lower than o/w microemulsion. The results were in agreement with previous reports which indicated that the o/w microemulsions provided higher membrane fluxes of diclofenac diethylamine (Djordjevic, Primorac and Stupar 2005: 73-79) and KP (Podlogar, Rogac and Gasperlin 2005: 68-77) than the w/o micromeulsions. According to the permeation of drug-loaded microemulsion droplets attribute to the permeation enhancement effect, the oil droplets of the o/w type might permeate into the epidermis easier than the water droplets of the w/o type at the same surfactant concentration owing to the lipophilic nature of the SC. The oil can enter the hydrophobic tail of the stratum

corneum bilayer, perturb it by creating separate domains, and induce highly permeable pathways in the SC. In addition, a hydrophobic drug is preferentially encapsulated in the oil droplet and the highly drug loaded dropletsfavor partitioning into the epidermis, resulting in the higher flux (Junyaprasert et al 2007: 288-298). Moreover, it might be that the stronger interaction between microemulsion components in w/o microemulsion led to slower KP released from the formulation and permeated through the skin (Podlogar, Rogac and Gasperlin 2005: 68-77).

In the case of o/w microemulsion, the skin permeation flux tended to increase when amount of water increased and amount surfactant mixture decreased (Figure 20). The results were in agreement with previous reports of theophylline and ketoprofen which poorly water-soluble drug, but soluble in the surfactant mixture. The thermodynamic activity of drug in the microemulsion at the lower content of surfactant was a significant driving force for the release and the penetration of drug into skin, thus the skin permeation flux of KP increased (Rhee et al. 2001: 161-170; Zhao et al. 2006: 58-64).

In the case of w/o microemulsion, formulation A1-A3, IPM was significantly affected the skin permeation flux of microemulsion formulations (Figure 22). The skin permeation flux significantly increased about 2 times while the amount of IPM decreased from 45% to 35%. IPM enhances skin permeation by acting as a fluidizer of intercellular lipids, and affects the lipid-rich phase in the stratum corneum, thereby reducing its barrier function (Ren et al 2009; 129-135). However, the higher content of IPM resulted in the higher solubility of KP. The thermodynamic activity of KP in microemulsion at the higher content of IPM was lower than the lower content. Therefore, the driving force for the skin permeation of KP of microemulsion at the higher concentration of IPM was lower than the lower content of IPM. In formulations C1-C6, when the water amount was fixed to 20% and the amount of surfactant mixture increased, the skin permeation tended to decrease (Figure 24). The result indicated that the ratio of oil/surfactant mixture affected the skin permeation flux of drug. The skin permeation results suggested that the skin permeation flux was affected by surfactant mixture more than oil.

KP solubility in IPM was lower than in the surfactant mixture. The thermodynamic activity of the drug in the microemulsion at the lower concentration of surfactant mixture was a significant driving force for the skin permeation of the drug. Therefore, the increased content of surfactant in microemulsion might decrease the skin permeation of drug (Rhee et al. 2001: 161-170; Zhao et al. 2006: 58-64).

The appropriate microemulsion formulation was o/w microemulsion. Surfactant mixture amount in the formulations was less than 50%, and the appropriate oil/water ratio was nearly 1:1, which was shown in formulation B4 (composed of 25% water, 45% surfactant mixture and 30% IPM).

The ratio of surfactant mixture, IPM and water had no effect on lag time of the microemulsion formulations (Figure 25). The results ware in agreement with previous report that the lag time of hydrocortisone was not significantly different in microemulsion formulations. The lag time is a permeation parameter depending mainly on the diffusivity of drug through the skin with the lag time being reduced with increasing diffusivity. However, the lag time can thus indirectly depend on the drug release. Considering this with no significant difference between the lag time values obtained after application of different formulations will futher indicate that the skin permeation of drug did not depend on drug release (Maghraby 2008: 285-292).

Table 6 Skin permeation flux and lag time of microemulsion formulations

Formulation	Flux±SD ($\mu g/cm^2/h$)	Lag time±SD (hr)	ER folds
A1	0.58 ± 0.08 *	3.45 ± 0.44	10.5
A2	0.78 ± 0.01 *	2.85 ± 0.15	14.0
A3	1.02 ± 0.21 *	2.74 ± 0.45	18.3
A4	1.02 ± 0.26 *	3.86 ± 0.05	18.2
A5	1.13 ± 0.14 *	3.42 ± 0.14	20.3
A6	1.19 ± 0.16 *	3.05 ± 0.14	21.4
B1	0.49 ± 0.16 *	3.28 ± 0.20	8.7
B2	0.44 ± 0.01 *	3.09 ± 0.25	7.9
B3	1.02 ± 0.26 *	3.86 ± 0.05	18.2
B4	1.58 ± 0.10 *	3.16 ± 0.34	28.4
C1	0.14 ± 0.03 *	4.06 ± 0.39	3.0
C2	0.41 ± 0.05 *	3.12 ± 0.19	7.5
C3	0.67 ± 0.06 *	3.51 ± 0.10	12.0
C4	0.74 ± 0.01 *	3.28 ± 0.27	13.2
C5	1.02 ± 0.26 *	3.86 ± 0.05	18.2
C6	1.25 ± 0.28 *	2.55 ± 0.24	22.5
2.5% KP Solution	0.06 ± 0.01	0	1.0
2.5% KP gel A	1.21 ± 0.13 *	3.56 ± 0.14	20.2
2.5% KP gel B	0.64 ± 0.03 *	1.04 ± 0.13	10.7

* $p<0.05$ compared with 2.5% KP solution

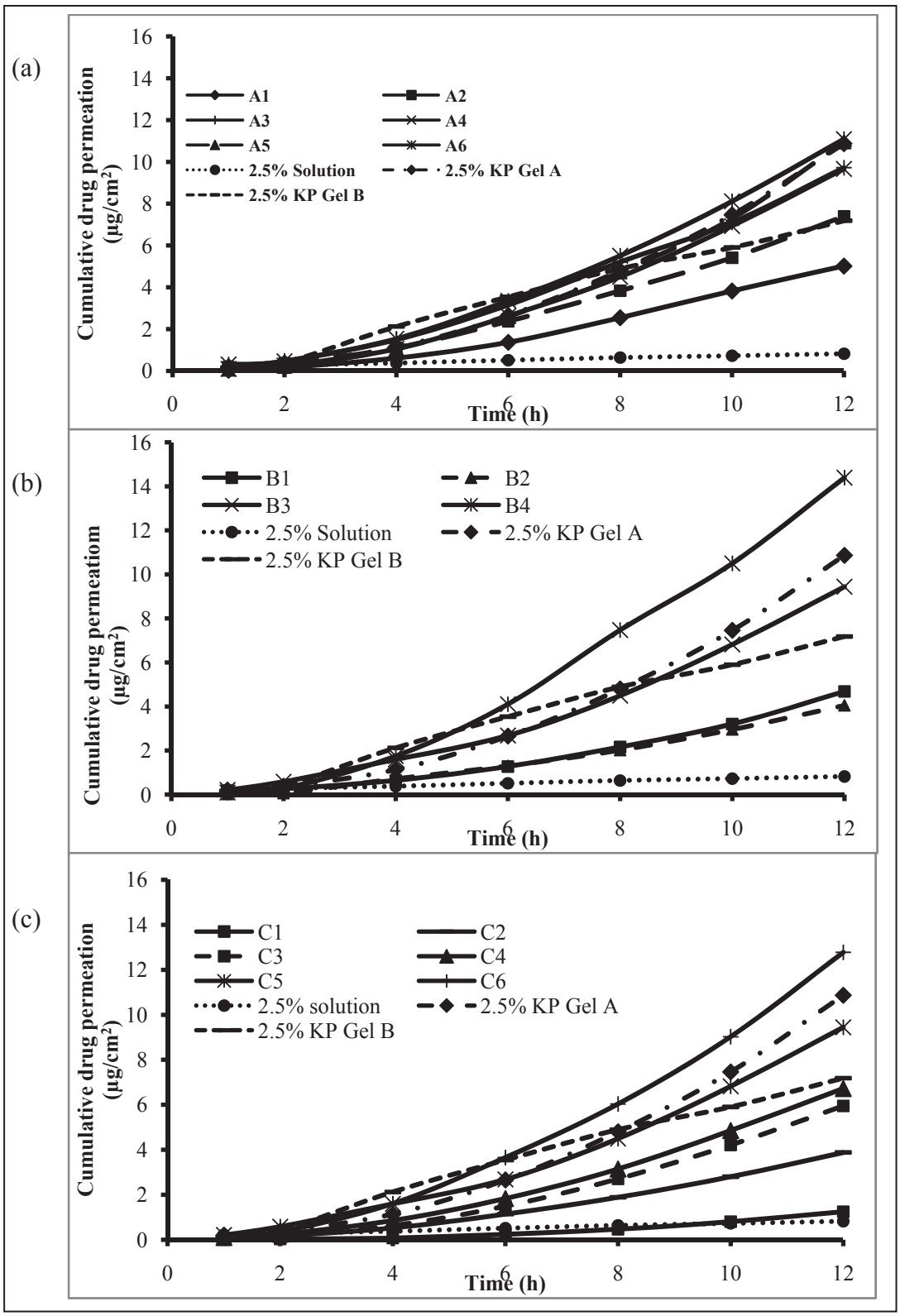

Figure 19 Permeation profiles of microemulsion formulations (a) A1-A6, (b) B1-B4, (c) C1-C6

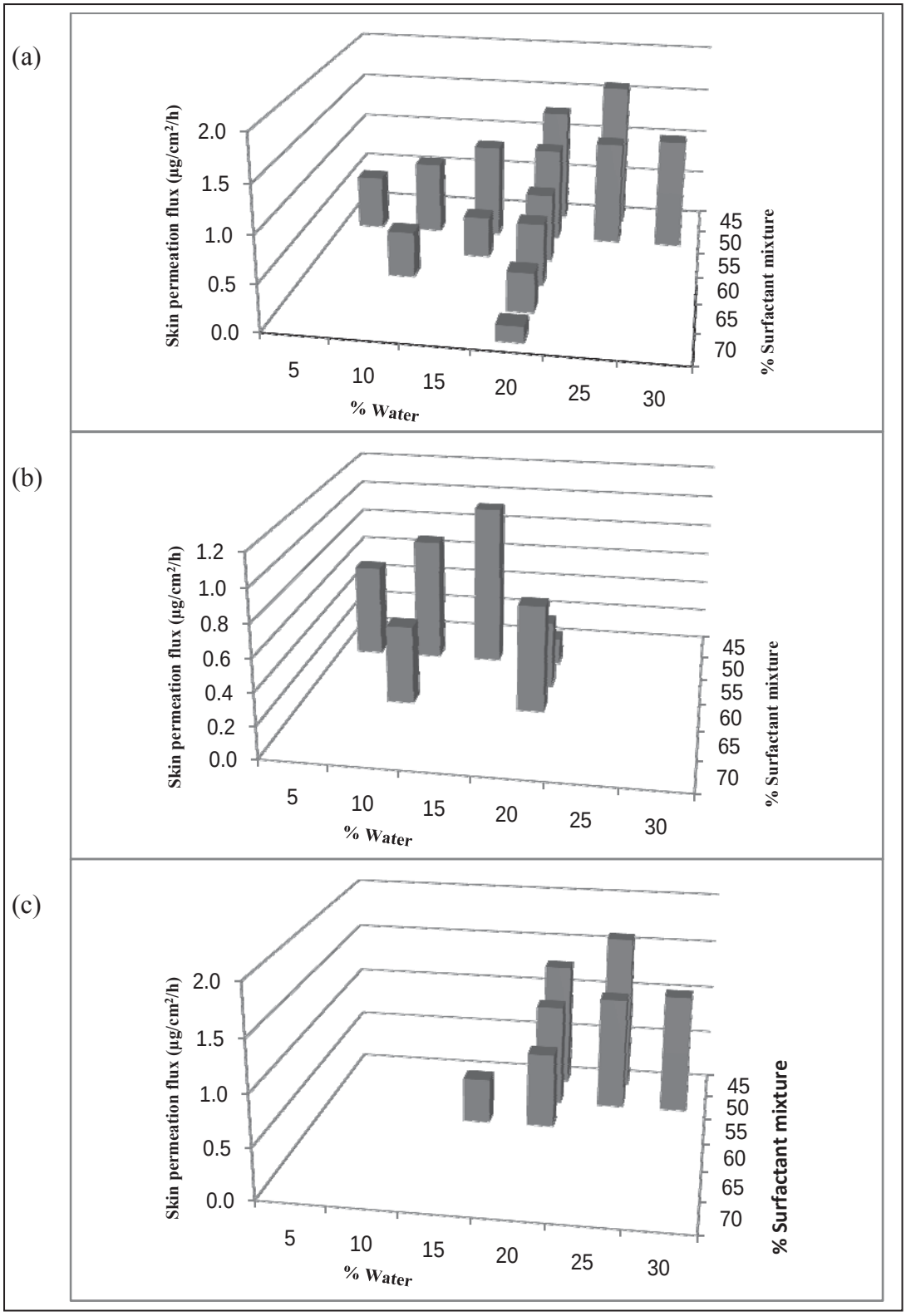

Figure 20 Skin permeation flux of (a) microemulsion formulations, (b) w/o and (c) o/w microemulsion

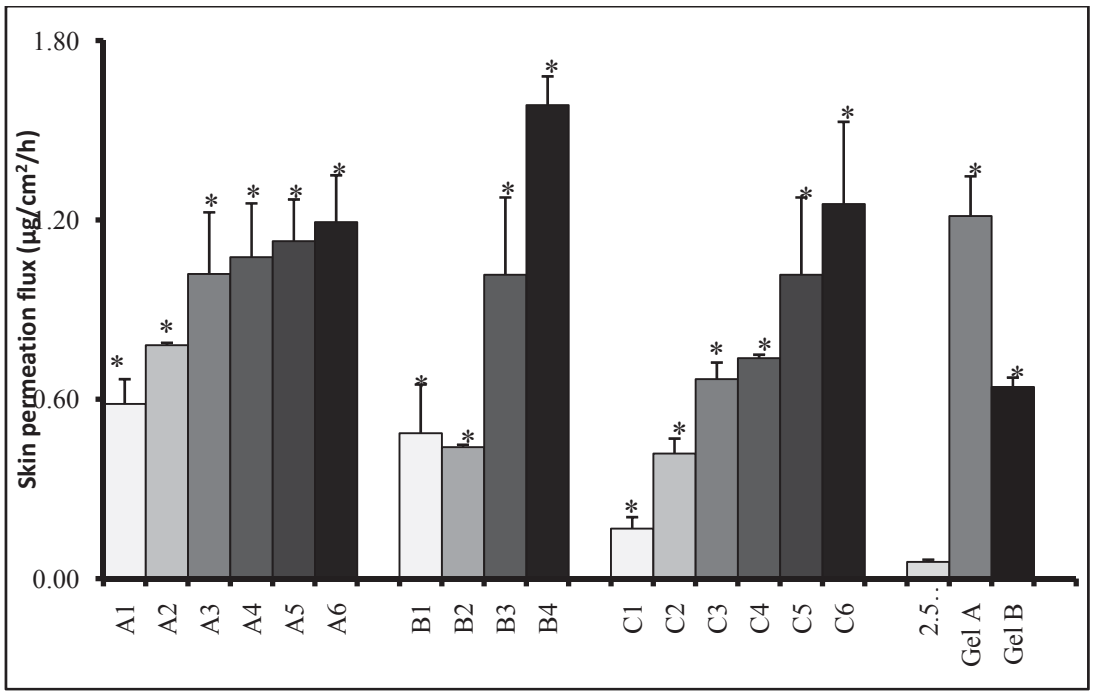

Figure 21 Skin permeation flux of microemulsion formulations

* $p<0.05$ compared with 2.5% KP solution

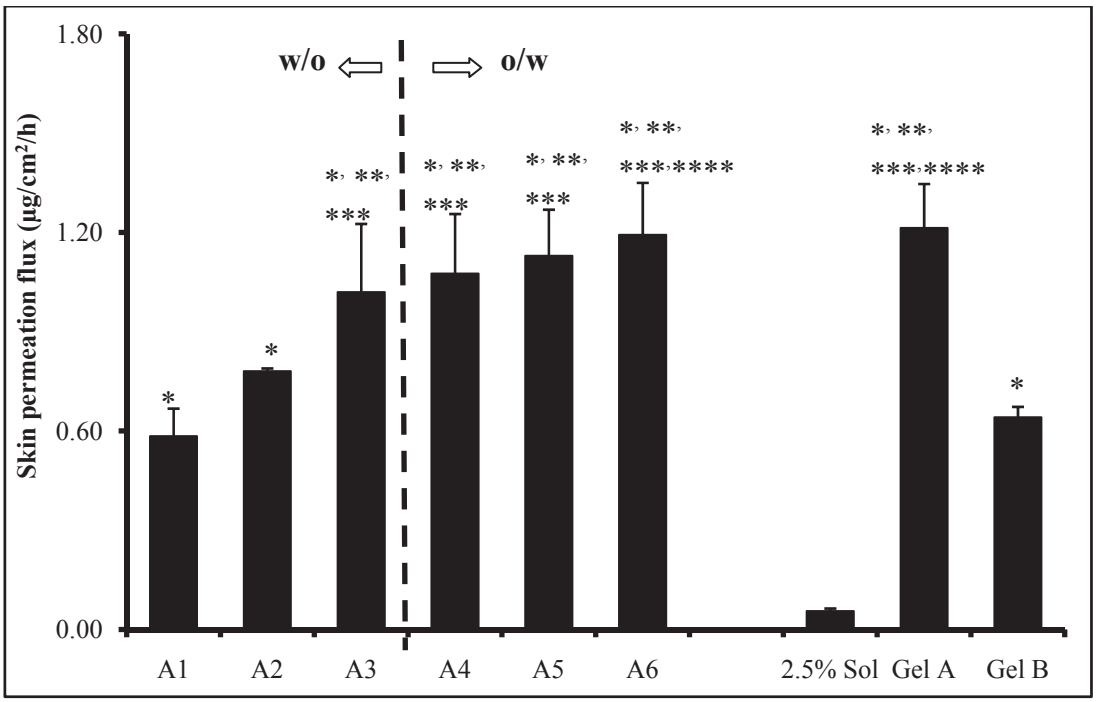

Figure 22 Skin permeation flux of microemulsion formulations A1-A6

* $p<0.05$ compared with 2.5% KP solution

** $p<0.05$ compared with 2.5% KP gel B

*** $p<0.05$ compared with A1

**** $p<0.05$ compared with A2

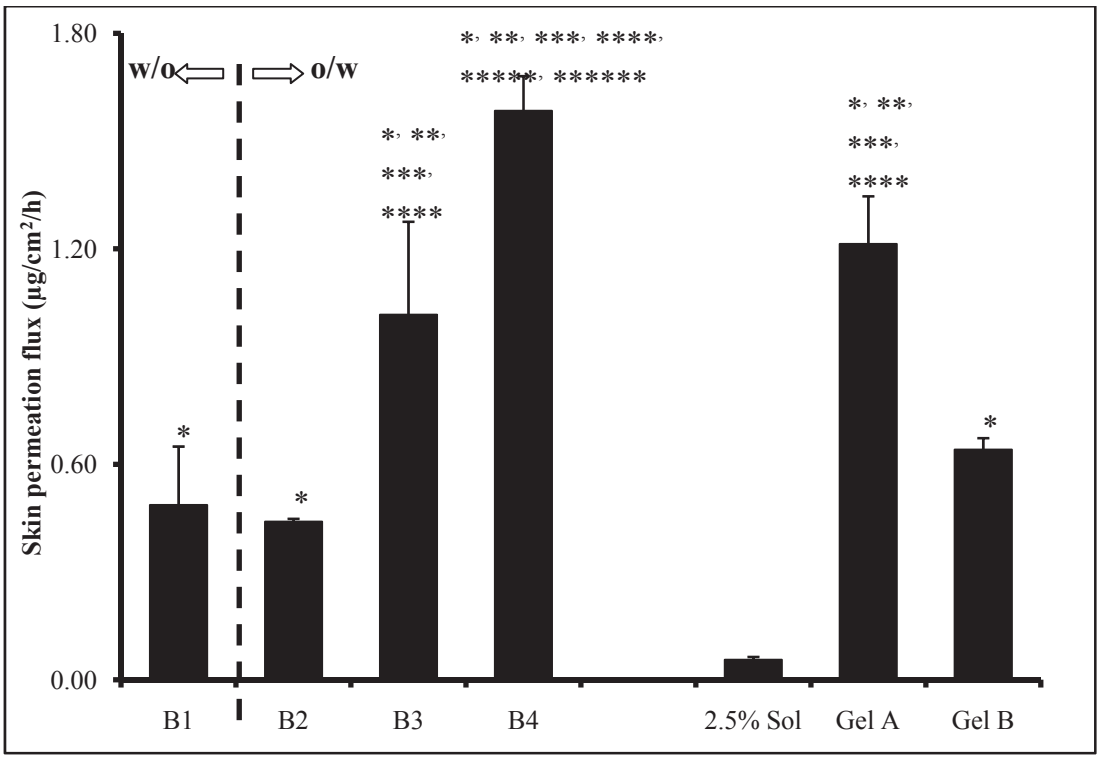

Figure 23 Skin permeation flux of microemulsion formulations B1-B4

* p<0.05 compared with 2.5% KP solution

** p<0.05 compared with 2.5% KP gel B

*** p<0.05 compared with B1

**** p<0.05 compared with B2

***** p<0.05 compared with B3

****** p<0.05 compared with 2.5% KP gel A

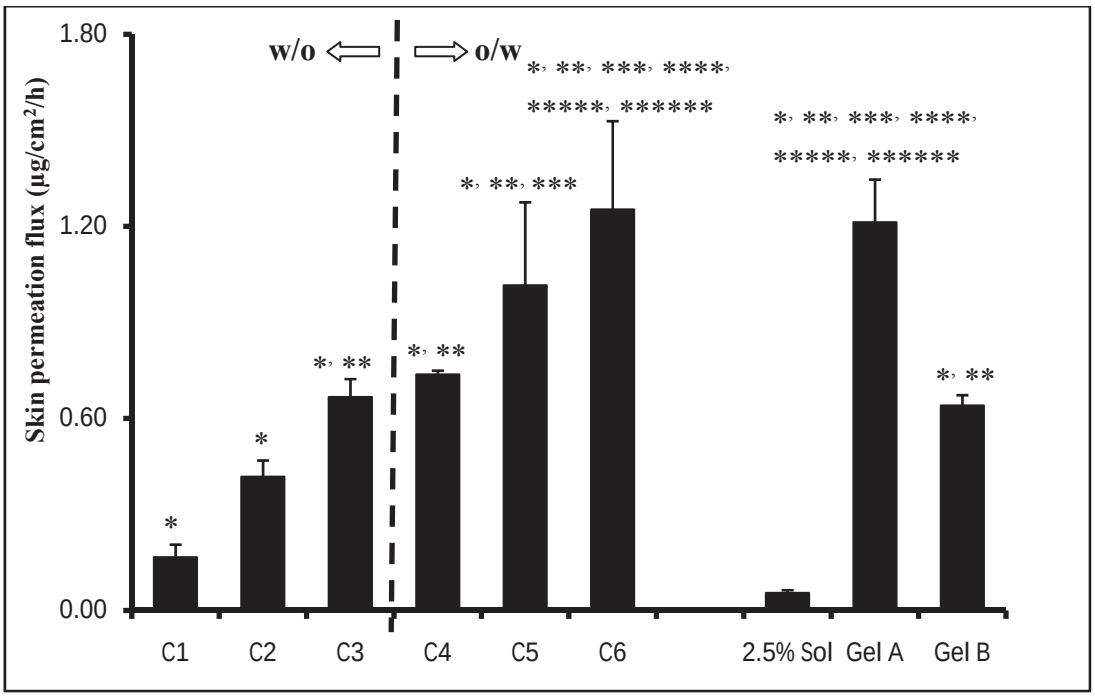

Figure 24 Skin permeation flux of microemulsion formulations C1-C6

* $p<0.05$ compared with 2.5% KP solution

** $p<0.05$ compared with C1

*** $p<0.05$ compared with C2

**** $p<0.05$ compared with C3

***** $p<0.05$ compared with C4

****** $p<0.05$ compared with 2.5% KP gel B

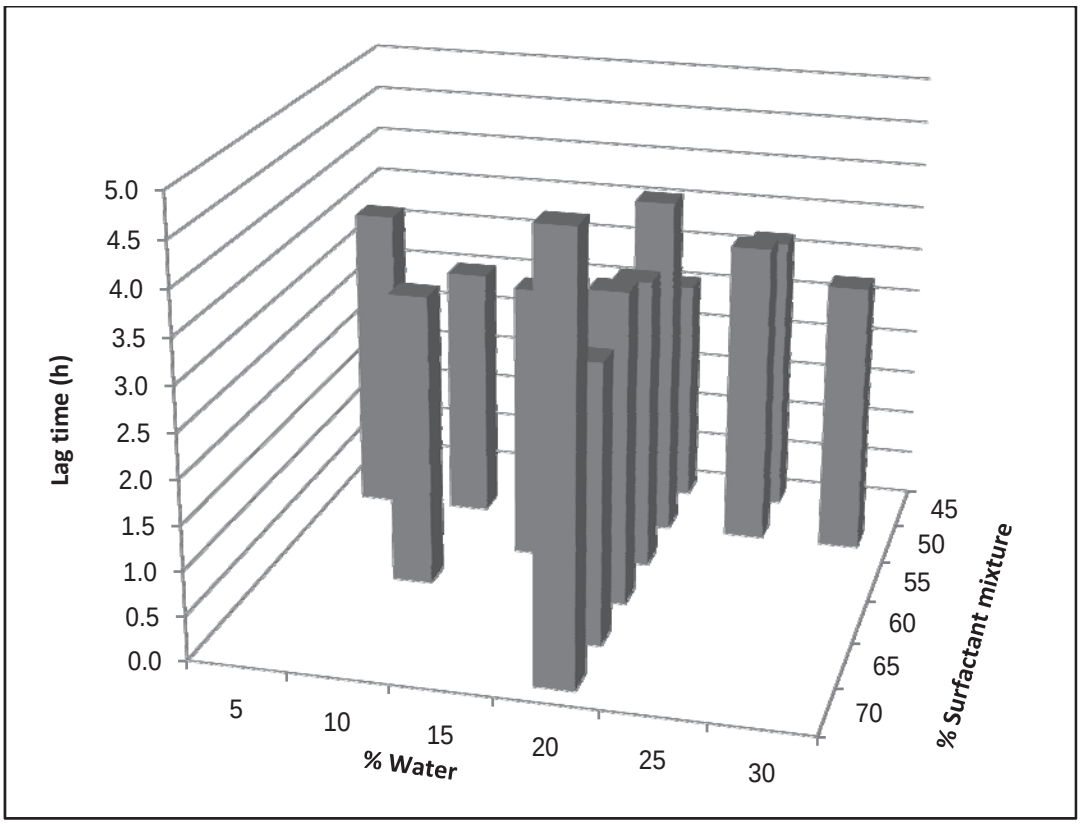

Figure 25 Lag time of microemulsion formulations

CHAPTER 5
CONCLUSIONS

1. The microemulsion systems composed of isopropyl myristate (IPM) as oil phase, water, PEG40-hydrogenated castor oil (Cremophor® RH40) as surfactant and PEG400, ethanol, and butanol as cosurfactant has been successfully prepared. However, because of the suitability in physical stability, the systems consisting of PEG40-hydrogenated castor oil (Cremophor® RH40) as surfactant and PEG 400 as co-surfactant with surfactant: co-surfactant ratio was 1:1 were used to study the characteristic, drug loading and skin permeation of KP in this study.

2. The ratio of IPM, water and surfactant mixture played an important role on loading capacity, physicochemical properties and skin permeation of KP in both o/w and w/o microemulsions as follows,

2.1 As the amount of water increased, conductivity of the microemulsion formulation and skin permeation of KP microemulsion increased, however, loading capacity of KP in the microemulsions and pH of the microemulsion decreased.

2.2 As the amount of surfactant increased, the loading capacity of KP in the microemulsions and pH of the system increased, however, the skin permeation of KP decreased.

2.3 As the amount of IPM increased, the loading capacity increased, however, the conductivity of the microemulsion formulation and the skin permeation of KP decreased.

3. The highest KP loading capacity was obtained from the formulation composed of 30% IPM, 60% surfactant and 10% water whereas the highest skin permeation flux was found in that consisting of 30% IPM, 45% surfactant and 25% water.

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APPENDIX

APPENDIX A

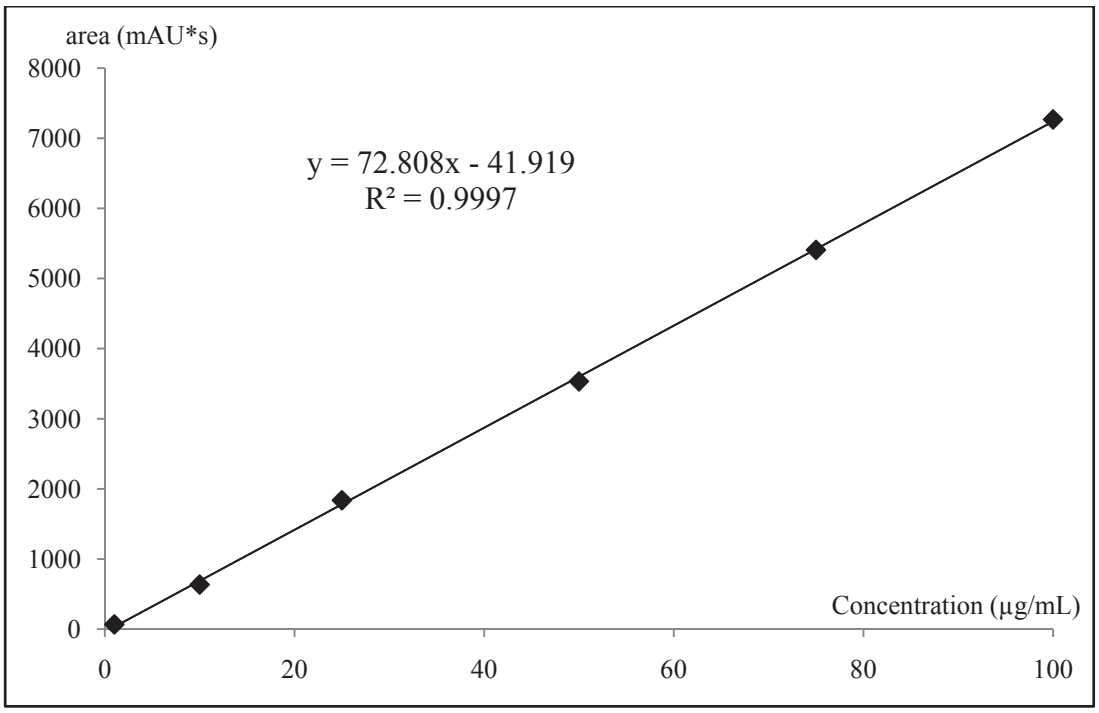

Figure 26 The calibration curve of ketoprofen for drug loading capacity and drug content assay

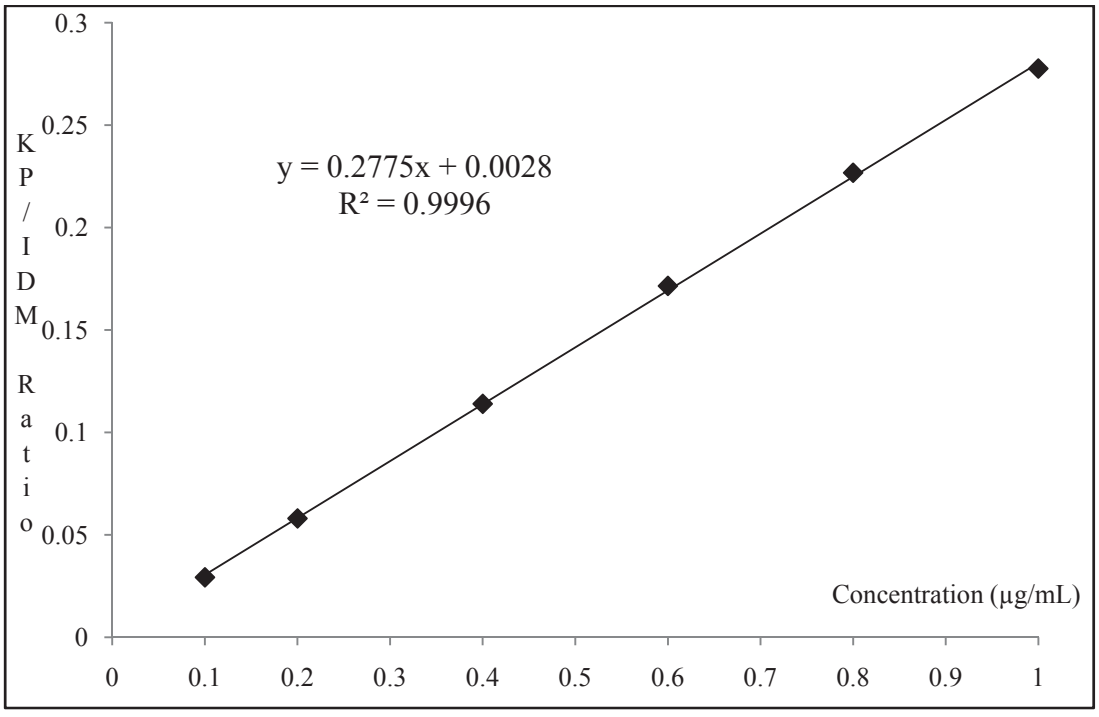

Figure 27 The calibration curve of ketoprofen for skin permeation studies

APPENDIX B

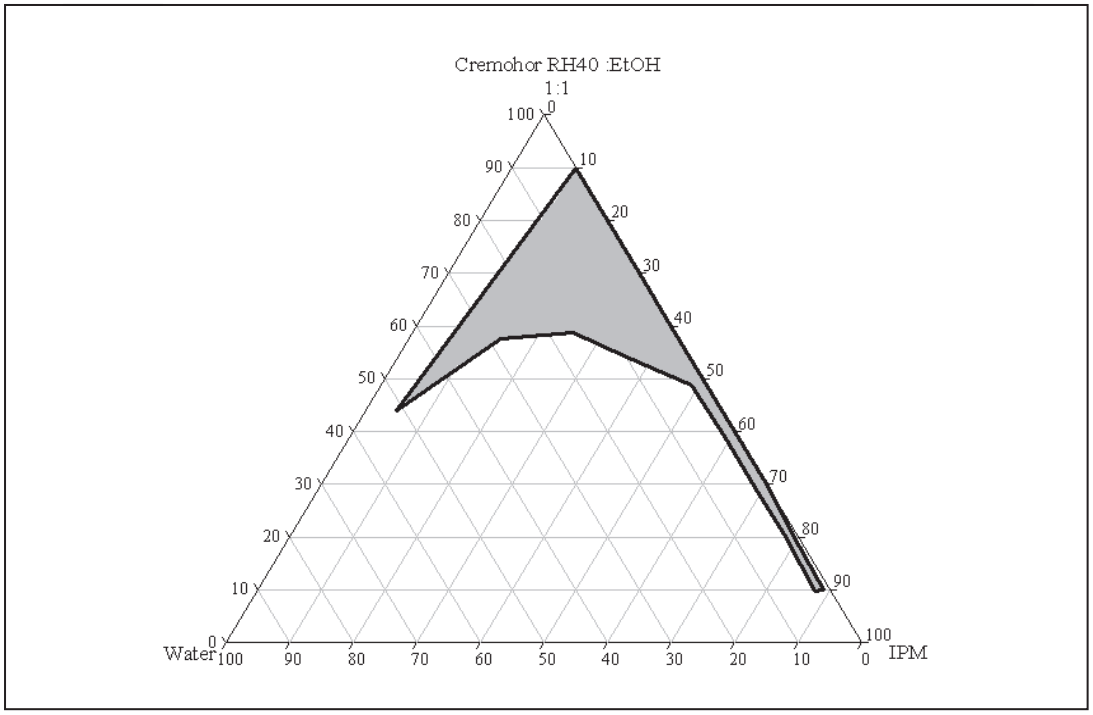

Figure 28 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: ethanol absolute ratio 1:1

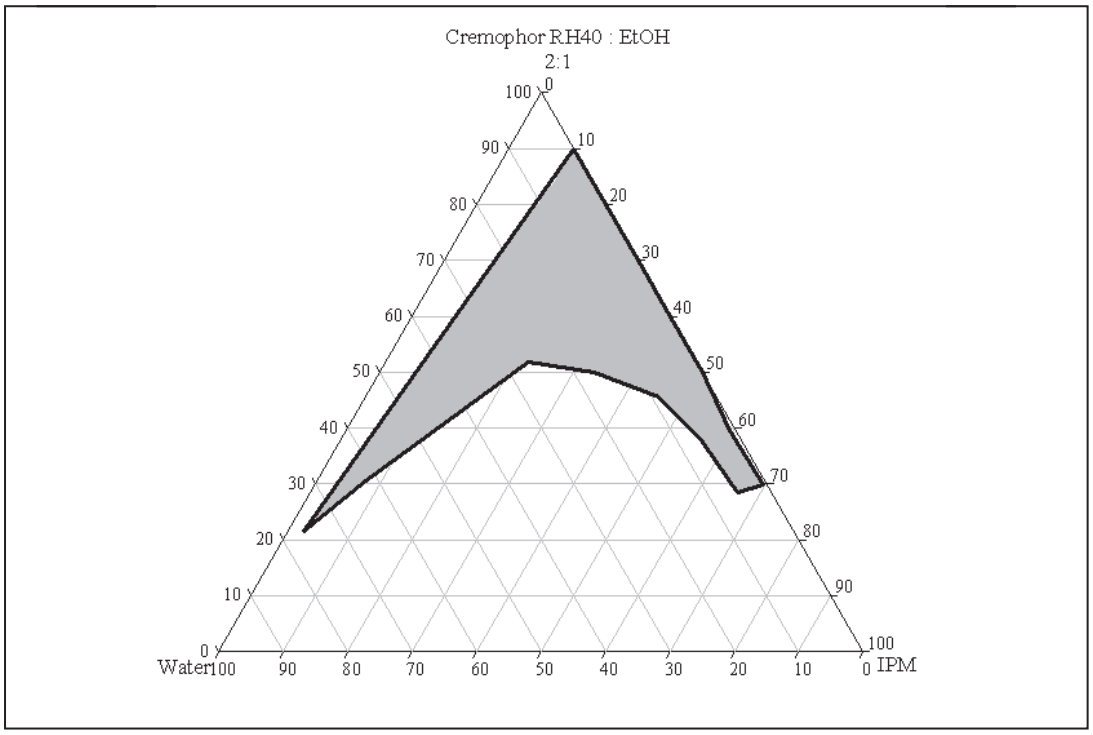

Figure 29 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: ethanol absolute ratio 2:1

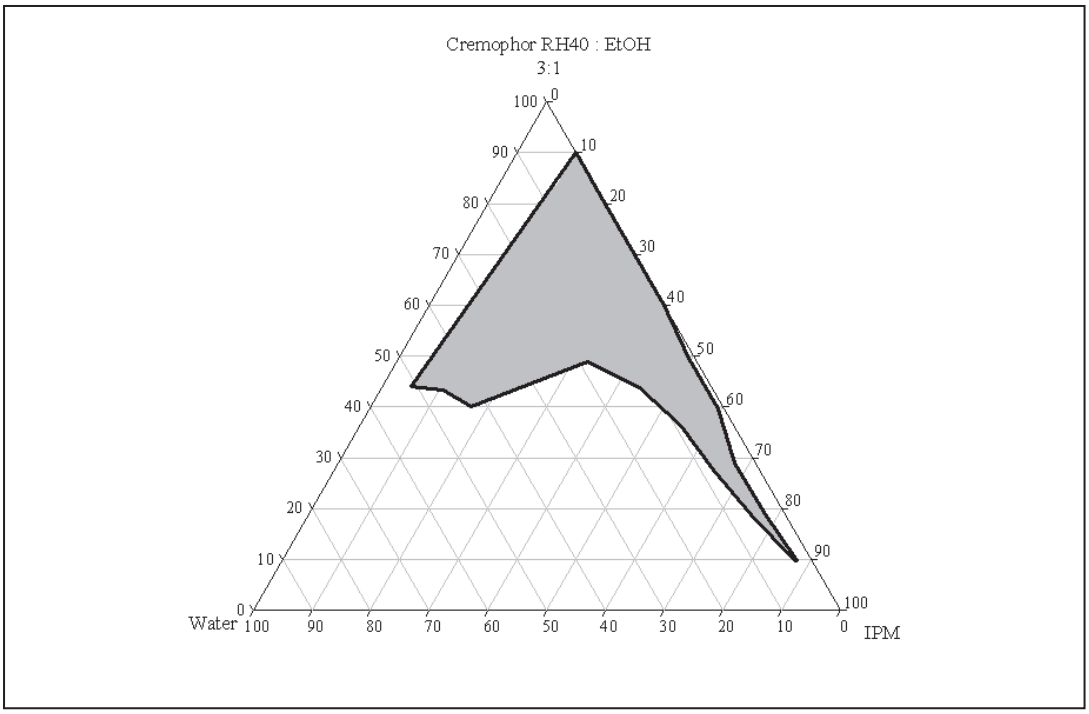

Figure 30 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: ethanol absolute ratio 3:1

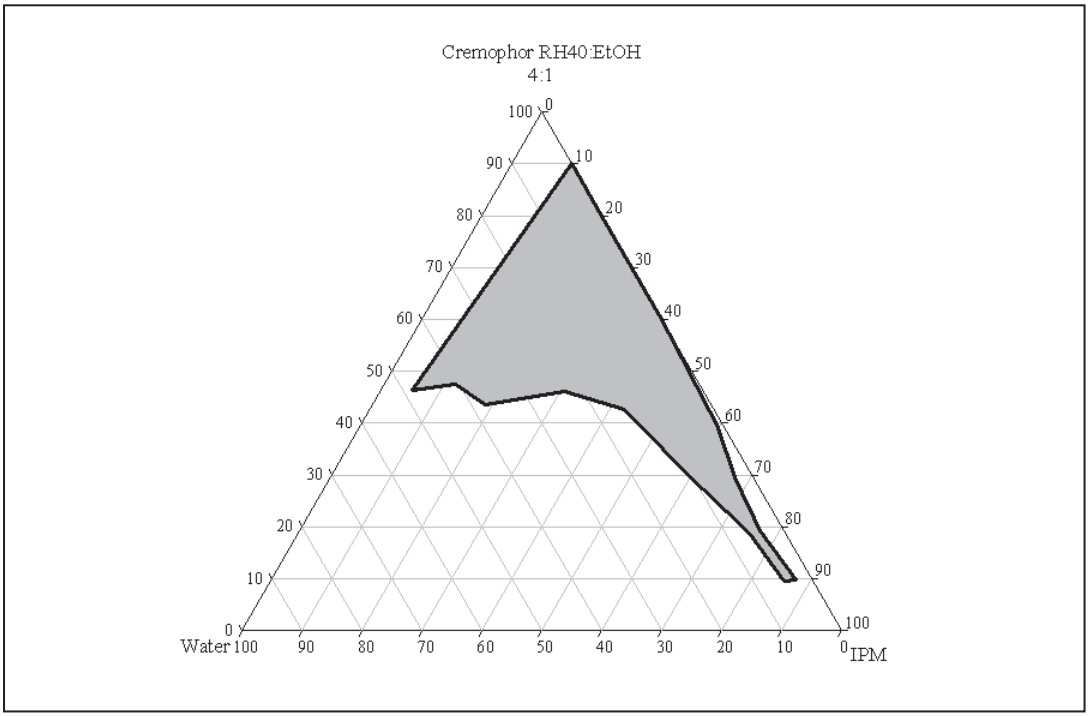

Figure 31 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: ethanol absolute ratio 4:1

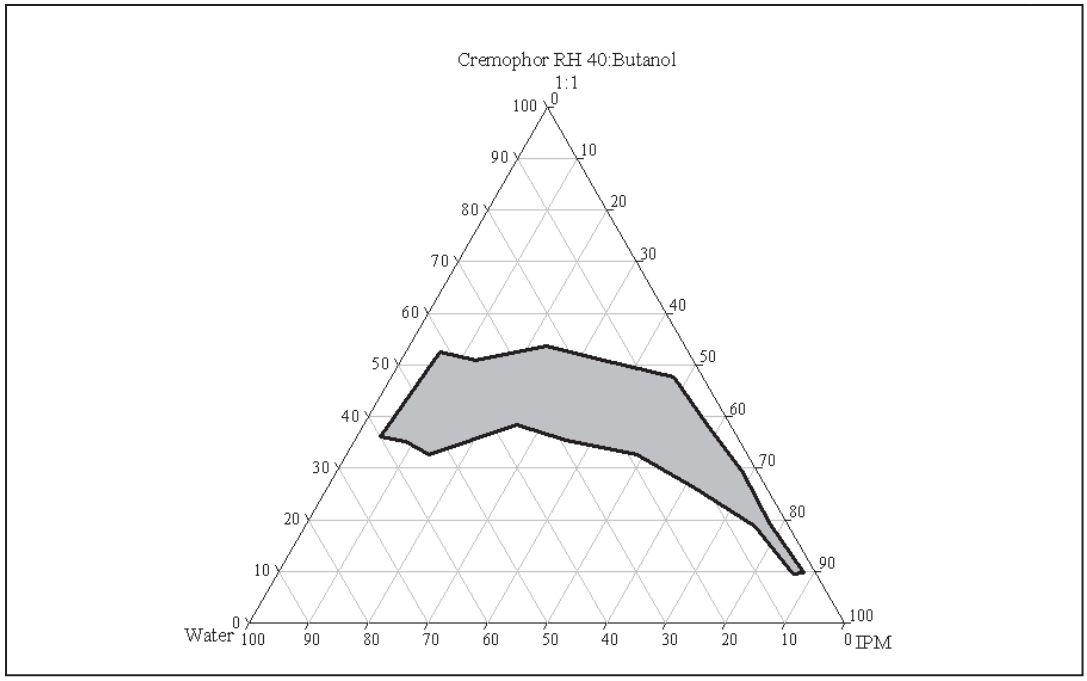

Figure 32 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: n-butanol ratio 1:1

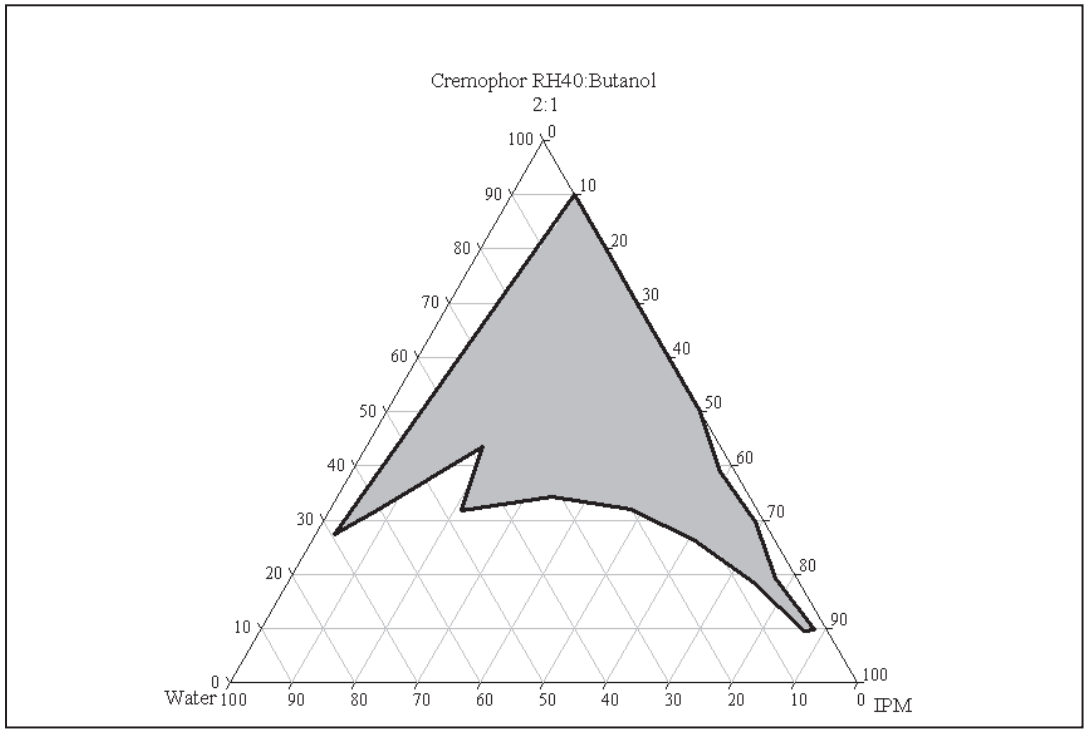

Figure 33 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: n-butanol ratio 2:1

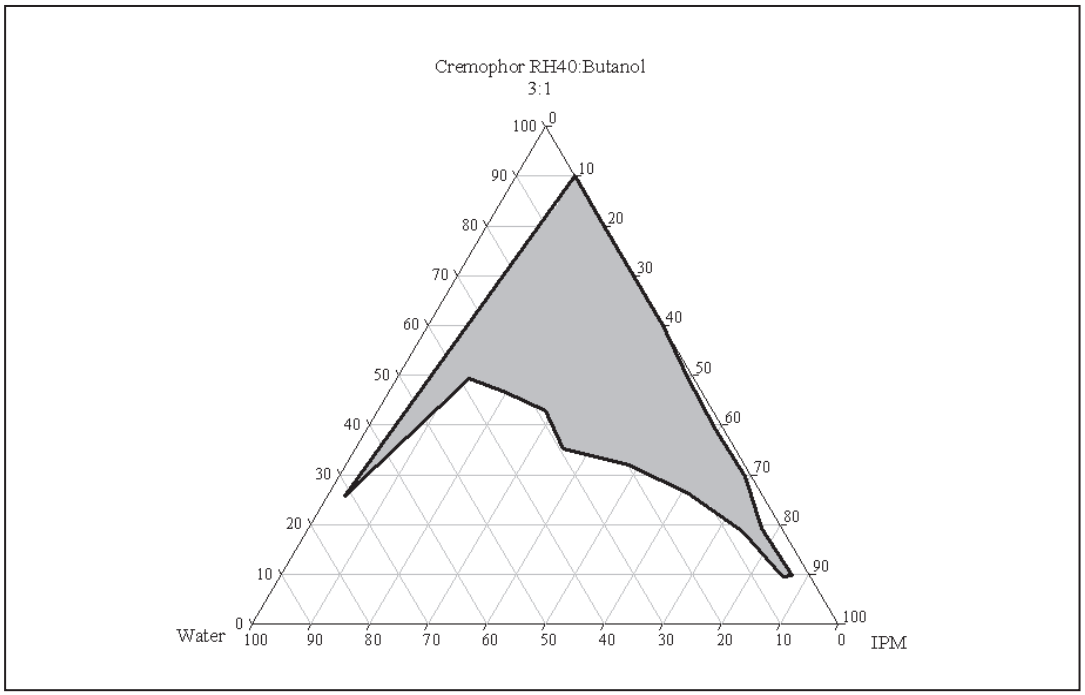

Figure 34 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: n-butanol ratio 3:1

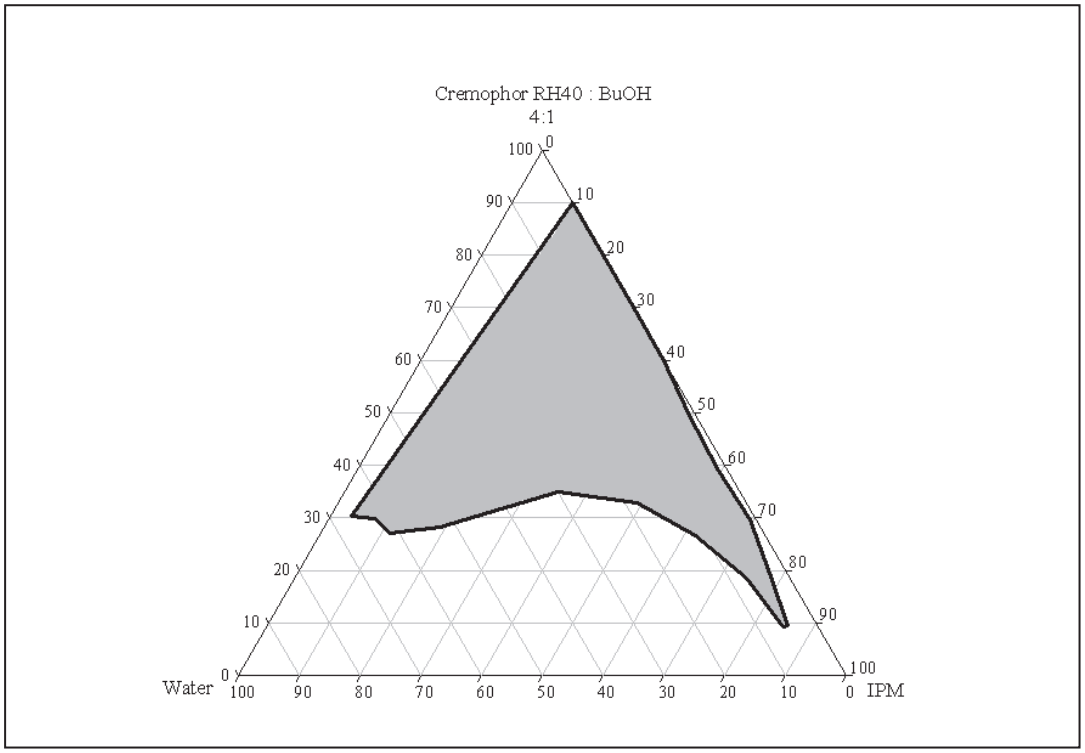

Figure 35 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: n-butanol ratio 4:1

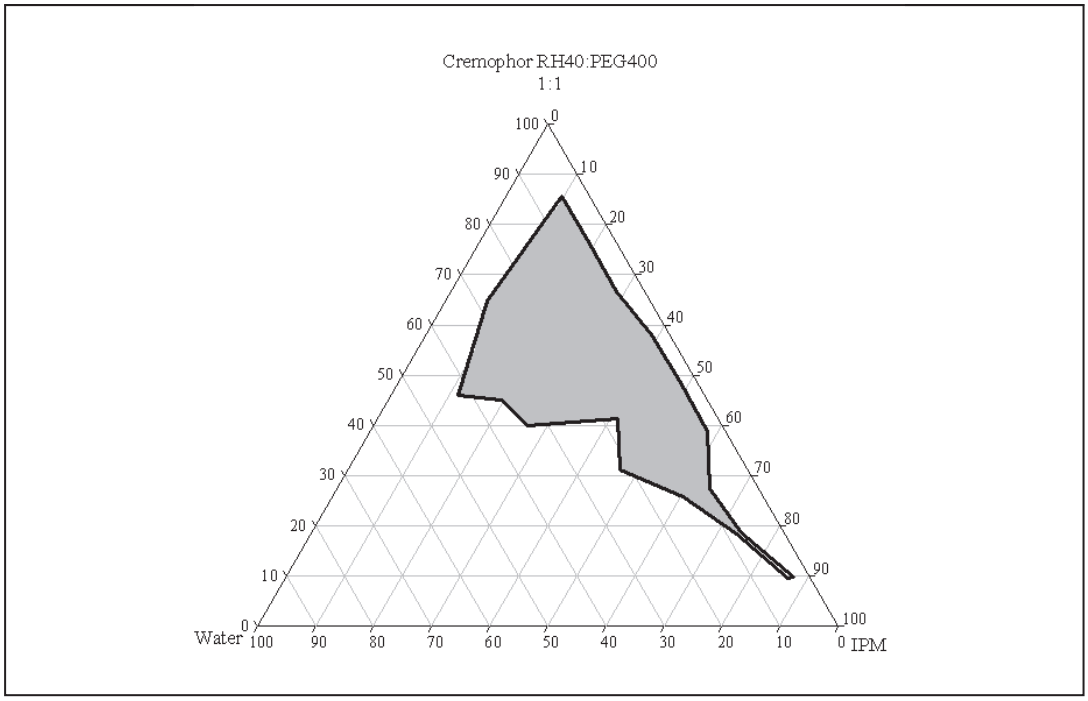

Figure 36 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: PEG400 ratio 1:1

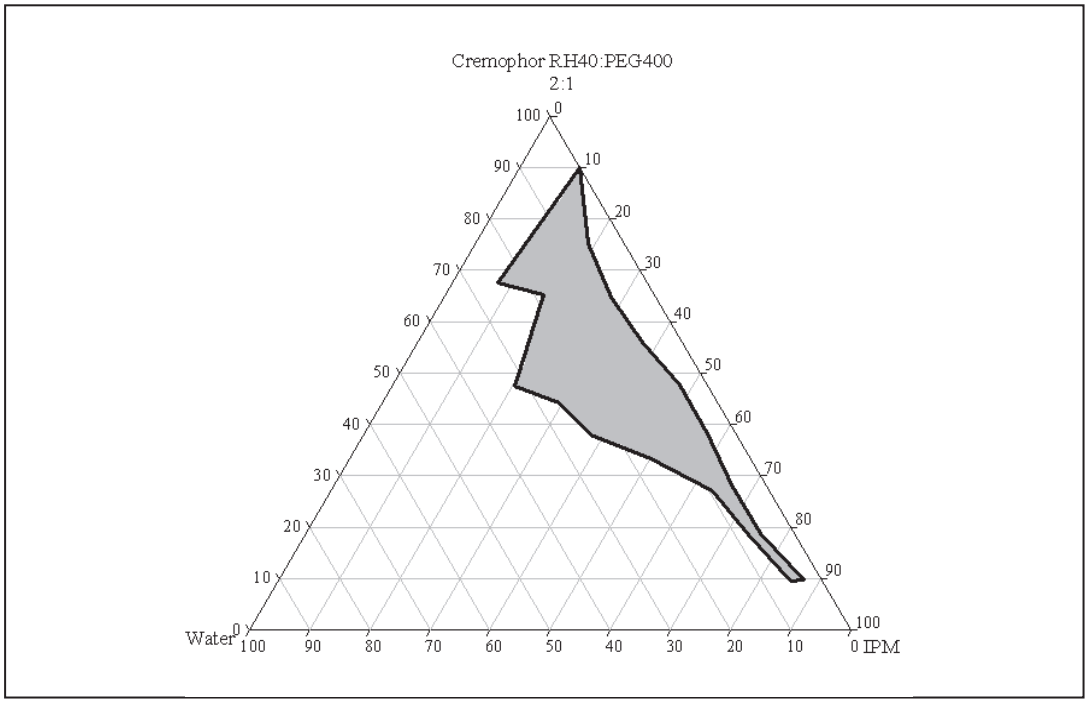

Figure 37 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: PEG400 ratio 2:1

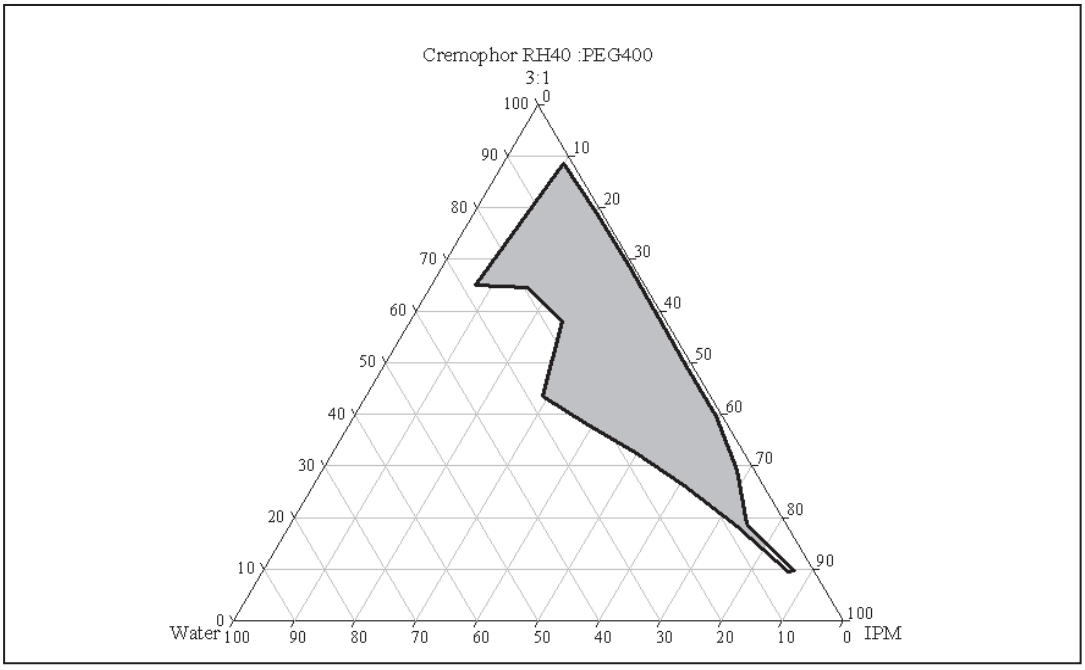

Figure 38 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: PEG400 ratio 3:1

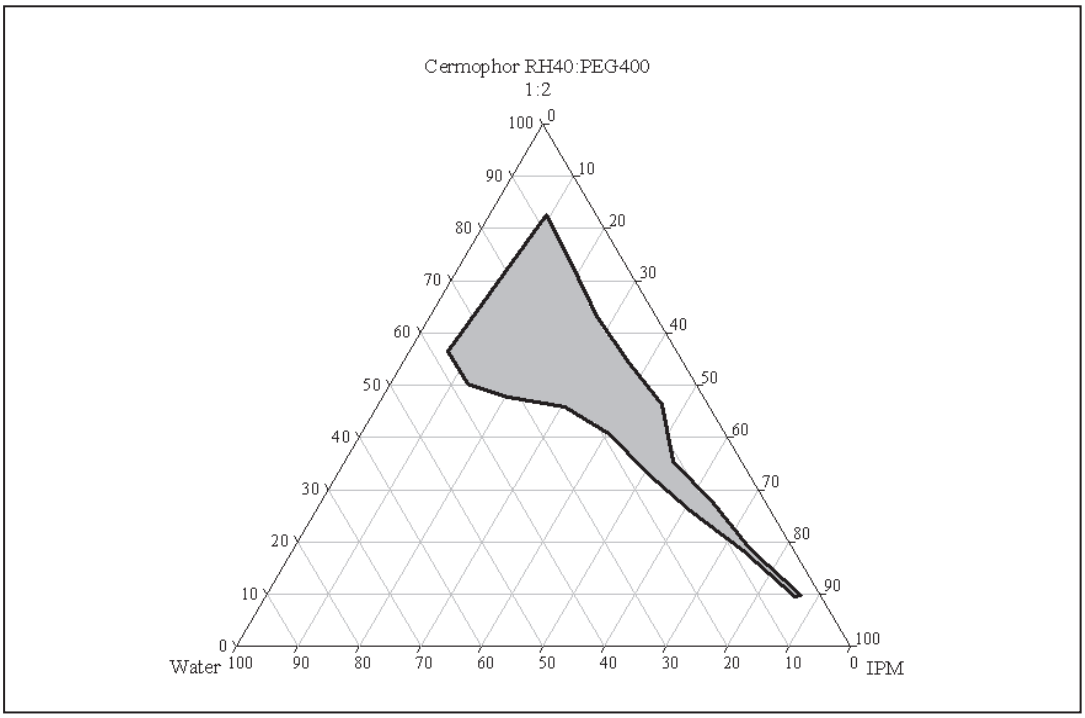

Figure 39 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: PEG400 ratio 1:2

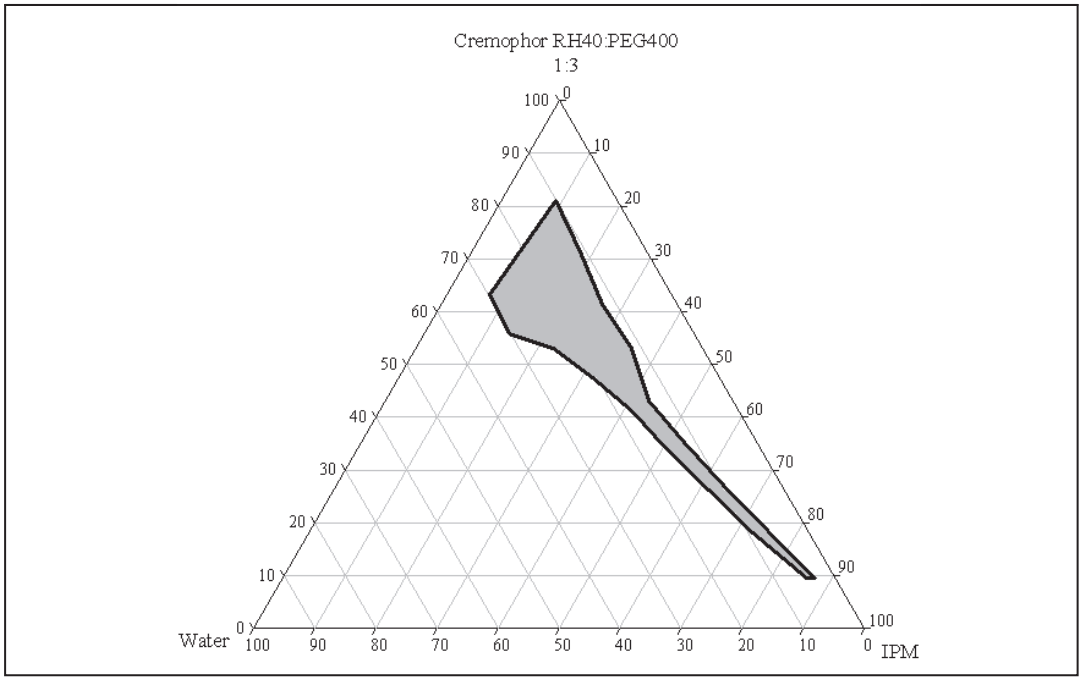

Figure 40 Pseudo-ternary phase diagram of microemulsion system consisted of IPM, Water, Cremophor®RH40: PEG400 ratio 1:3

APPENDIX C

Characterization of microemulsion formulation before 2.5% ketoprofen loaded

Table 7 Viscosity of microemulsion formulation before 2.5% ketoprofen loaded

Formulation	Viscosity (cP)				
	1	2	3	mean	SD
A1	254.66	253.01	253.01	253.56	0.95
A2	81.03	81.03	81.03	81.03	0.00
A3	310.89	307.58	307.58	308.68	1.91
A4	949.39	954.39	951.05	951.61	2.55
A5	1119.53	1119.53	1119.53	1119.53	0.00
A6	1137.72	1136.07	1139.06	1137.62	1.50
B1	110.8	110.8	110.8	110.80	0.00
B2	190.17	188.52	188.52	189.07	0.95
B3	949.39	954.39	951.05	951.61	2.55
B4	1155.91	1160.87	1159.22	1158.67	2.53
C1	385.3	383.65	383.65	384.20	0.95
C2	234.82	234.82	234.82	234.82	0.00
C3	279.47	276.16	277.82	277.82	1.66
C4	484.52	482.87	484.52	483.97	0.95
C5	949.39	954.39	951.05	951.61	2.55
C6	1066.8	1066.8	1068.46	1067.35	0.96

Table 8 Droplet size of microemulsion formulation before 2.5% ketoprofen loaded

Formulation	Droplet size (nm)				
	1	2	3	mean	SD
A1	74.76	75.68	83.15	77.86	4.60
A2	258.00	259.50	255.30	257.60	2.13
A3	104.5	121.9	91.69	106.03	15.16
A4	170.9	170.3	175.1	172.10	2.62
A5	167.5	176.0	169.1	170.87	4.52
A6	105.9	104.0	107.3	105.73	1.66
B1	44.81	45.69	46.51	45.67	0.85
B2	195.2	214.4	203.2	204.27	9.64
B3	170.9	170.3	175.1	172.10	2.62
B4	231.9	263.0	265.0	253.30	18.56
C1	465.1	436.9	501.9	467.97	32.59
C2	287.0	183.7	230.4	233.70	51.73
C3	263.3	214.6	199.3	225.73	33.42
C4	199.7	199.4	213.7	204.27	8.17
C5	170.9	170.3	175.1	172.10	2.62
C6	404.8	375.1	403.8	394.57	16.87

Table 9 Conductivity of microemulsion formulation before 2.5% ketoprofen loaded

Formulation	Conductivity (μS/cm)				
	1	2	3	mean	SD
A1	1.7	1.7	1.7	1.70	0.00
A2	1.8	1.7	1.7	1.73	0.06
A3	9.7	9.7	9.7	9.70	0.00
A4	16.5	16.4	16.5	16.47	0.06
A5	24.0	24.0	24.0	24.00	0.00
A6	34.6	34.4	34.5	34.50	0.10
B1	0.2	0.2	0.2	0.20	0.00
B2	13.2	13.2	13.3	13.23	0.06
B3	20.7	20.9	21.0	20.87	0.15
B4	37.2	37.4	37.2	37.27	0.12
C1	6.7	6.9	6.8	6.80	0.10
C2	8.9	8.8	9.0	8.90	0.10
C3	8.8	8.8	8.7	8.77	0.06
C4	16.5	16.4	16.5	16.47	0.06
C5	20.7	20.9	21.0	20.87	0.15
C6	18.6	18.6	18.6	18.60	0.00

Table 10 pH of microemulsion formulation before 2.5% ketoprofen loaded

Formulation	pH				
	1	2	3	mean	SD
A1	8.46	8.48	8.49	8.48	0.02
A2	8.09	8.06	8.09	8.08	0.02
A3	7.79	7.79	7.78	7.79	0.01
A4	7.38	7.34	7.36	7.36	0.02
A5	7.17	7.14	7.13	7.15	0.02
A6	6.86	6.84	6.82	6.84	0.02
B1	7.81	7.80	7.83	7.81	0.02
B2	7.78	7.79	7.79	7.79	0.01
B3	7.38	7.34	7.36	7.36	0.02
B4	7.11	7.07	7.05	7.08	0.03
C1	7.75	7.77	7.78	7.77	0.02
C2	7.65	7.62	7.61	7.63	0.02
C3	7.50	7.49	7.47	7.49	0.02
C4	7.42	7.45	7.45	7.44	0.02
C5	7.38	7.34	7.36	7.36	0.02
C6	7.30	7.33	7.29	7.31	0.02

Characterization of microemulsion formulation after 2.5% ketoprofen loaded

Table 11 Viscosity of 2.5% ketoprofen loaded microemulsion formulation

Formulation	Viscosity (cP)				
	1	2	3	mean	SD
A1	229.86	229.86	228.21	229.31	0.95
A2	224.90	221.59	223.25	223.25	1.66
A3	358.85	363.81	360.5	361.05	2.53
A4	737.54	722.65	735.88	732.02	8.16
A5	911.17	914.48	917.79	914.48	3.31
A6	793.76	792.11	790.45	792.11	1.66
B1	132.29	132.29	130.64	131.74	0.95
B2	122.37	120.72	119.06	120.72	1.66
B3	737.54	722.65	735.88	732.02	8.16
B4	883.06	897.94	896.29	892.43	8.16
C1	277.82	282.78	281.12	280.57	2.52
C2	286.08	282.78	281.12	283.33	2.52
C3	262.93	261.28	274.51	266.24	7.21
C4	426.65	423.34	434.91	428.30	5.96
C5	737.54	722.65	735.88	732.02	8.16
C6	945.9	954.17	957.47	952.51	5.96

Table 12 Droplet size of 2.5% ketoprofen loaded microemulsion formulation

Formulation	Droplet size (nm)				
	1	2	3	mean	SD
A1	91.55	93.72	98.93	94.73	3.79
A2	198.7	217.0	202.7	206.13	9.62
A3	206.9	225.2	190.3	207.47	17.46
A4	182.3	171.1	175.8	176.40	5.62
A5	163.8	140.8	152.2	152.27	11.50
A6	97.94	95.71	102.4	98.68	3.41
B1	53.05	46.11	52.03	50.40	3.75
B2	180.9	206.3	195.8	194.33	12.76
B3	182.3	171.1	175.8	176.40	5.62
B4	270.8	273.5	283.3	275.87	6.58
C1	284.4	280.4	292.3	285.70	6.06
C2	362.3	387.1	393.7	381.03	16.56
C3	166.0	141.0	194.9	167.30	26.97
C4	150.6	125.7	119.1	131.80	16.61
C5	182.3	171.1	175.8	176.40	5.62
C6	239.7	227.0	221.3	229.33	9.42

Table 13 Conductivity of 2.5% ketoprofen loaded microemulsion formulation

Formulation	Conductivity (μS/cm)				
	1	2	3	mean	SD
A1	0.4	0.4	0.4	0.40	0.00
A2	2.5	2.5	2.5	2.50	0.00
A3	8.9	8.8	8.8	8.83	0.06
A4	18.8	18.7	18.7	18.73	0.06
A5	23.3	23.3	23.3	23.30	0.00
A6	33.1	33.1	33.1	33.10	0.00
B1	0.6	0.6	0.6	0.60	0.00
B2	15.5	15.5	15.5	15.50	0.00
B3	18.8	18.7	18.7	18.73	0.06
B4	35.5	35.5	35.5	35.50	0.00
C1	9.2	9.2	9.2	9.20	0.00
C2	7.8	7.9	7.9	7.87	0.06
C3	8.9	8.9	8.8	8.87	0.06
C4	14.1	14.0	14.0	14.03	0.06
C5	18.8	18.7	18.7	18.73	0.06
C6	20.9	21.0	21.0	20.97	0.06

Table 14 pH of 2.5% ketoprofen loaded microemulsion formulation

Formulation	pH				
	1	2	3	mean	SD
A1	6.76	6.77	6.75	6.76	0.01
A2	6.73	6.71	6.7	6.71	0.02
A3	6.57	6.55	6.54	6.55	0.02
A4	6.37	6.34	6.34	6.35	0.02
A5	6.17	6.17	6.16	6.17	0.01
A6	6.10	6.08	6.08	6.09	0.01
B1	6.77	6.77	6.77	6.77	0.00
B2	6.71	6.69	6.69	6.70	0.01
B3	6.37	6.34	6.34	6.35	0.02
B4	6.26	6.26	6.23	6.25	0.02
C1	6.64	6.65	6.66	6.65	0.01
C2	6.59	6.58	6.58	6.58	0.01
C3	6.53	6.53	6.53	6.53	0.00
C4	6.44	6.43	6.44	6.44	0.01
C5	6.37	6.34	6.34	6.35	0.02
C6	6.03	6.05	6.05	6.04	0.01

Table 15Loading capacity of microemulsion formulation

Formulation	Loading capacity (%w/w)				
	1	2	3	mean	SD
A1	18.75	18.91	19.05	18.90	0.15
A2	18.14	17.97	18.41	18.18	0.22
A3	17.10	17.14	17.33	17.19	0.12
A4	16.41	16.67	16.81	16.63	0.20
A5	15.67	15.70	15.85	15.74	0.10
A6	9.07	9.13	9.21	9.14	0.07
B1	20.89	21.11	21.25	21.08	0.18
B2	20.28	20.44	20.62	20.45	0.17
B3	16.41	16.67	16.81	16.63	0.20
B4	11.42	11.40	11.65	11.49	0.14
C1	20.19	20.89	20.45	20.51	0.35
C2	20.36	20.60	20.69	20.55	0.17
C3	17.68	17.21	17.77	17.55	0.30
C4	17.20	17.44	17.49	17.38	0.16
C5	16.41	16.67	16.81	16.63	0.20
C6	13.91	13.87	13.90	13.89	0.02
Water	0.03	0.03	0.03	0.03	0.00
IPM	1.89	1.90	1.92	1.90	0.01
Cremophor:PEG 1:1	29.42	29.52	29.69	29.54	0.14

Table 16 Drug content assay of 2.5% ketoprofen loaded microemulsion formulation

Formulation	% Drug content				
	1	2	3	mean	SD
A1	98.83	98.83	99.47	99.04	0.37
A2	98.72	99.00	99.08	98.94	0.19
A3	101.32	100.67	100.78	100.92	0.35
A4	97.77	98.54	98.63	98.32	0.47
A5	102.41	101.54	101.59	101.84	0.49
A6	99.25	99.89	99.33	99.49	0.35
B1	97.74	96.85	96.91	97.17	0.49
B2	98.77	98.99	99.09	98.95	0.16
B3	97.77	98.54	98.63	98.32	0.47
B4	102.01	101.95	102.20	102.05	0.13
C1	98.22	98.40	98.86	98.49	0.33
C2	98.38	98.35	98.47	98.40	0.06
C3	100.58	100.76	100.83	100.72	0.13
C4	97.10	97.23	97.22	97.18	0.07
C5	97.77	98.54	98.63	98.32	0.47
C6	98.35	98.60	98.50	98.48	0.13

APPENDIX D

In vitro shed snake skin permeation

Table 17 The cumulative drug permeated of 2.5% ketoprofen loaded formulation A1

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.0179	0.0427	0.0345	0.0317	0.01
2	0.1557	0.1377	0.1977	0.1637	0.03
4	0.6764	0.6086	0.5614	0.6155	0.06
6	1.4480	1.3530	1.3035	1.3682	0.07
8	2.6391	2.3442	2.6523	2.5452	0.17
10	4.1395	3.7256	3.6060	3.8237	0.28
12	5.4651	4.9911	4.5933	5.0165	0.44

Table 18 The cumulative drug permeated of 2.5% ketoprofen loaded formulation A2

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.3579	0.1332	0.0584	0.1832	0.16
2	0.4322	0.3807	0.2629	0.3586	0.09
4	1.1167	1.1457	1.0508	1.1044	0.05
6	2.3339	2.5137	2.2729	2.3735	0.13
8	3.6831	4.0625	3.7553	3.8336	0.20
10	5.2299	5.5595	5.4462	5.4119	0.17
12	7.4283	7.3828	7.3658	7.3923	0.03

Table 19 The cumulative drug permeated of 2.5% ketoprofen loaded formulation A3

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.2300	0.1145	0.2121	0.1855	0.06
2	0.6207	0.3273	0.4824	0.4768	0.15
4	1.7759	1.1450	1.5638	1.4949	0.32
6	3.3929	2.5481	3.5269	3.1560	0.53
8	4.9185	4.3922	6.3157	5.2088	0.99
10	6.8727	5.8550	8.5678	7.0985	1.37
12	9.0206	8.5086	11.6194	9.7162	1.67

Table 20 The cumulative drug permeated of 2.5% ketoprofen loaded formulation A4

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.0384	0.0113	0.5833	0.2110	0.32
2	0.1694	0.1182	1.4623	0.5833	0.76
4	0.9827	0.7349	3.0703	1.5960	1.28
6	2.4301	1.6905	3.9255	2.6820	1.14
8	4.5883	3.3845	5.5099	4.4942	1.07
10	8.1062	5.5652	6.7858	6.8191	1.27
12	11.0928	8.5147	8.7182	9.4419	1.43

Table 21 The cumulative drug permeated of 2.5% ketoprofen loaded formulation A5

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.1620	0.0223	0.0445	0.0763	0.08
2	0.2486	0.1705	0.2050	0.2081	0.04
4	0.8996	0.9713	1.3410	1.0707	0.24
6	2.2808	2.5241	2.9853	2.5967	0.36
8	3.7828	5.1185	5.2907	4.7307	0.83
10	5.8701	8.2448	7.7418	7.2855	1.25
12	8.7924	13.1109	10.9440	10.9491	2.16

Table 22 The cumulative drug permeated of 2.5% ketoprofen loaded formulation A6

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.4452	0.1587	0.3125	0.3055	0.14
2	0.7121	0.3518	0.3185	0.4608	0.22
4	1.8947	1.2995	1.4186	1.5376	0.31
6	4.0623	3.0681	2.9384	3.3562	0.61
8	6.4694	5.3996	4.6520	5.5070	0.91
10	9.7034	7.9364	6.6944	8.1114	1.51
12	12.7081	10.4609	10.0931	11.0873	1.42

Table 23 The cumulative drug permeated of 2.5% ketoprofen loaded formulation B1

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.2395	0.0611	0.2580	0.1862	0.11
2	0.2877	0.2760	0.2233	0.2623	0.03
4	0.5333	0.7456	0.6555	0.6448	0.11
6	0.7983	1.6211	1.4065	1.2753	0.43
8	1.3338	2.7718	2.3884	2.1647	0.74
10	2.0312	4.1059	3.4806	3.2059	1.06
12	3.4133	5.7775	4.8603	4.6837	1.19

Table 24 The cumulative drug permeated of 2.5% ketoprofen loaded formulation B2

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.0445	0.0235	0.0078	0.0252	0.02
2	0.1001	0.0626	0.0388	0.0672	0.03
4	0.6185	0.9992	0.5332	0.7170	0.25
6	1.4053	1.2415	1.2645	1.3038	0.09
8	2.1565	1.9092	1.9942	2.0200	0.13
10	3.1028	2.7935	2.9773	2.9579	0.16
12	4.2593	3.8334	4.0604	4.0510	0.21

Table 25 The cumulative drug permeated of 2.5% ketoprofen loaded formulation B3

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.0384	0.0113	0.5833	0.2110	0.32
2	0.1694	0.1182	1.4623	0.5833	0.76
4	0.9827	0.7349	3.0703	1.5960	1.28
6	2.4301	1.6905	3.9255	2.6820	1.14
8	4.5883	3.3845	5.5099	4.4942	1.07
10	8.1062	5.5652	6.7858	6.8191	1.27
12	11.0928	8.5147	8.7182	9.4419	1.43

Table 26 The cumulative drug permeated of 2.5% ketoprofen loaded formulation B4

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.0924	0.1139	0.0739	0.0934	0.02
2	0.3312	0.2696	0.2325	0.2777	0.05
4	2.0022	1.6128	1.6365	1.7505	0.22
6	4.2572	3.9652	4.0988	4.1071	0.15
8	8.0098	6.9764	7.4392	7.4752	0.52
10	10.5951	10.1329	10.7737	10.5006	0.33
12	13.5943	15.1356	14.4585	14.3962	0.77

Table 27 The cumulative drug permeated of 2.5% ketoprofen loaded formulation C1

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.0000	0.0000	0.0202	0.0067	0.01
2	0.0112	0.0179	0.0747	0.0346	0.03
4	0.0984	0.0279	0.1097	0.0787	0.04
6	0.3144	0.1665	0.2555	0.2455	0.07
8	0.5914	0.3700	0.4434	0.4683	0.11
10	1.0176	0.6856	0.7178	0.8070	0.18
12	1.5768	1.0834	1.0793	1.2465	0.29

Table 28 The cumulative drug permeated of 2.5% ketoprofen loaded formulation C2

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.0359	0.0313	0.0552	0.0408	0.01
2	0.1281	0.2880	0.0886	0.1683	0.11
4	0.5633	0.6131	0.4038	0.5268	0.11
6	1.2607	1.2673	0.8933	1.1404	0.21
8	2.0720	2.0201	1.5688	1.8870	0.28
10	3.0855	2.9727	2.3469	2.8017	0.40
12	4.2665	4.0646	3.3054	3.8788	0.51

Table 29 The cumulative drug permeated of 2.5% ketoprofen loaded formulation C3

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.1431	0.0295	0.0924	0.0883	0.06
2	0.2738	0.1339	0.2155	0.2078	0.07
4	0.7359	0.5022	0.6584	0.6322	0.12
6	1.7529	1.2570	1.4753	1.4951	0.25
8	2.9669	2.4725	2.6801	2.7065	0.25
10	4.5556	4.0587	4.0188	4.2110	0.30
12	6.6271	5.3090	5.9018	5.9460	0.66

Table 30 The cumulative drug permeated of 2.5% ketoprofen loaded formulation C4

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.0854	0.0893	0.1240	0.0996	0.02
2	0.2844	0.2320	0.2219	0.2461	0.03
4	1.0379	0.8266	0.7011	0.8552	0.17
6	2.0760	1.8181	1.6054	1.8332	0.24
8	3.4141	3.1022	2.9140	3.1434	0.25
10	5.0659	4.8543	4.6414	4.8539	0.21
12	7.0190	6.5553	6.5899	6.7214	0.26

Table 31 The cumulative drug permeated of 2.5% ketoprofen loaded formulation C5

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.0384	0.0113	0.5833	0.2110	0.32
2	0.1694	0.1182	1.4623	0.5833	0.76
4	0.9827	0.7349	3.0703	1.5960	1.28
6	2.4301	1.6905	3.9255	2.6820	1.14
8	4.5883	3.3845	5.5099	4.4942	1.07
10	8.1062	5.5652	6.7858	6.8191	1.27
12	11.0928	8.5147	8.7182	9.4419	1.43

Table 32 The cumulative drug permeated of 2.5% ketoprofen loaded formulation C6

Time (h)	Cumulative drug permeated (µg/cm^2)				
	1	2	3	mean	SD
1	0.2629	0.1860	0.0605	0.1698	0.10
2	0.5433	0.3213	0.2072	0.3573	0.17
4	1.8111	1.6176	1.3870	1.6052	0.21
6	3.5137	4.1234	3.3243	3.6538	0.42
8	5.5362	7.0997	5.4735	6.0364	0.92
10	8.1743	10.9860	7.9139	9.0248	1.70
12	11.0537	15.9065	11.3617	12.7740	2.72

Table 33 The cumulative drug permeated of 2.5% ketoprofen solution

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.2562	0.2030	0.2056	0.2216	0.03
2	0.3161	0.2439	0.2873	0.2824	0.04
4	0.4001	0.3592	0.3785	0.3793	0.02
6	0.4942	0.5012	0.5467	0.5140	0.03
8	0.6229	0.6167	0.6807	0.6401	0.04
10	0.7116	0.7020	0.7671	0.7269	0.04
12	0.7892	0.7550	0.9245	0.8229	0.09

Table 34 The cumulative drug permeated of 2.5% ketoprofen gel B

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.3070	0.1554	0.1454	0.2026	0.09
2	0.5053	0.2879	0.3502	0.3811	0.11
4	1.1149	1.0581	1.2402	1.1377	0.09
6	2.3682	2.5493	3.0484	2.6553	0.35
8	4.3925	4.8072	5.1934	4.7977	0.40
10	6.6143	7.6862	8.0823	7.4609	0.76
12	9.5938	11.2971	11.7133	10.8681	1.12

Table 35 The cumulative drug permeated of 2.5% ketoprofen gel B

Time (h)	Cumulative drug permeated (μg/cm^2)				
	1	2	3	mean	SD
1	0.1431	0.1138	0.1545	0.1371	0.02
2	0.4201	0.2828	0.4998	0.4009	0.11
4	2.3105	2.2825	1.7793	2.1241	0.30
6	3.7893	3.9759	2.8618	3.5423	0.60
8	4.9843	5.3308	4.3683	4.8945	0.49
10	5.9929	6.4998	5.2135	5.9021	0.65
12	7.5471	7.7587	6.2360	7.1806	0.82

APPENDIX E

Table 36 List of abbreviations

Symbol	Definition
°C	degree Celsius
>	more than
<	less than
%	percent
MW	molecular weight
pK_a	minus logarithm base 10 of K_a, -log K_a
w/w	weight by weight
v/v	volume by volume
cm^2	square centimeter
$inch^2$	square inch
g	gram
nm	nanometer
min	minute
h	hours
PBS	phosphate-buffered saline
pH	The negative logarithm of the hydrogen ion concentration
qs. to	add to
R^2	coefficient of determination
SD	standard deviation
Ave	Average
et al.	and others
etc.	for example, such as
o/w	oil in water
w/o	water in oil
KP	ketoprofen
ME	microemulsion

Table 36 (continue)

Symbol	Definition
mg	milligram
µg	microgram
mL	milliliter
µL	microliter
HPLC	High Performance Liquid Chromatography

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- Narumon Worachun, Praneet Opanasopit, Tanasait Ngawhirunpat "Characterization of Microemulsions composed of Isopropyl Myristate, Cremophor® RH40, Polyethylene Glycol 400 and water." The 3rd Annual Northeast Pharmacy Research Conference 2011, 12-13 February 2011; Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand.