Preparation of Nicotinamide Microemulsions and Formulation Development for Cosmetic Application

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A Thesis Submitted in Fulfillment of the Requirements for the Degree
of Master of Sciences Program in Cosmetic Sciences (International Program)
Prince of Songkla University
2011

Thesis Title	Preparation of nicotinamide microemulsions and formulation development for cosmetic application
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ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere gratitude for the encouragement I received while completing this research and my degree.

First and foremost I would like to especially thank my advisor, Assist. Prof. Dr. Prapaporn Boonme, for her amazing research mentorship and for her friendship. She has been my guide and my influence and I could not have asked for a more dedicated, intelligent and selfless individual to lead my academic pursuits.

I would like to sincerely thank my co-advisor, Assist. Prof. Dr. Sarunyoo Songkro, for her kindness in supporting me the materials in the *in vitro* release study and providing valuable suggestions for improvement in this research through examining the thesis patiently.

I would like to thank Assoc. Prof. Wibul Wongpoowarak for his mathematic discussion.

I am grateful to Assoc. Prof. Dr. Varaporn Junyaprasert and Assoc. Prof. Nattha Kaewnopparat for being member of my examining committee.

I wish to acknowledge Graduate School and Faculty of Pharmaceutical Sciences, Prince of Songkla University for the fund support during my research.

Thank you to all my friends and colleagues who provided a wonderfully stimulating intellectual environment to work in and around, especially supporting me all materials and lab instruments.

I would like to thank my parents and my brother to the wonderful family. Without their constant emotional, moral, financial support and for everything I would not be where I am today

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project and other person who have not been mentioned here.

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Academic Year	2010

ABSTRACT

This thesis aimed to investigate the phase behavior of systems composed of cosmetic acceptable components and to prepare and characterize nicotinamide-loaded microemulsions as well as microemulsion-based gels. For phase behavior investigation, studied surfactant was oleth-10, the studied oils were silicone oil and soybean oil while the studied cosurfactants were isopropyl alcohol (IPA), propylene glycol (PG) and sorbitan monooleate (Span 80). Among studied systems, water/soybean oil/9:1 oleth-10:IPA system provided the largest microemulsion region. Therefore, two microemulsion formulations were selected from this system to be incorporated with nicotinamide, a skin-lightening agent. The concentrations of nicotinamide, water, soybean oil and surfactant mixture in ME-1 were 3% w/w, 7% w/w, 18% w/w and 72% w/w, respectively. Those in ME-2 were 3% w/w, 7% w/w, 25% w/w and 65% w/w, respectively. The ME-1 and ME-2 had non-significant differences in physicochemical characteristics (i.e., the conductivity, viscosity, pH, and particle size), stability, and *in vitro* release profiles. However, ME-2 was selected for further converting to microemulsion-based gels due to its low concentration of surfactant mixture. The microemulsion-based gels were prepared by adding gelling mixture, i.e., 5% colloidal silica to obtain ME-G1, 5% of 0.5% Carbopol® Ultrez 21 gel to obtain ME-G2 and mixture of 3% of 0.5% Carbopol® Ultrez 21 gel and 2% PEG-40 hydrogenated castor oil to obtain ME-G3. Only ME-G1 was clear gel with suitable viscosity. Therefore, it was compared for the *in vitro* release through cellulose membrane with ME-1, ME-2 and an o/w commercial cream. The rank order of percents cumulative release and release rate of nicotinamide from different formulations was ME-G1 > ME-2 ≥ ME-1 ≥ the o/w commercial cream. Low affinity

between lipophilic colloidal silica and hydrophilic nicotinamide provided high release of nicotinamide from ME-G1. In addition, ME-G1 had aesthetic appearance, suitable viscosity and good stability at 4ºC as well as at room temperature (~30 ± 2ºC) throughout 2 months of storage.

ชื่อวิทยานิพนธ์ การเตรียมนิโคตินามายด์ไมโครอิมัลชันและการพัฒนาตำรับเพื่อใช้ทางเครื่องสำอาง

ผู้เขียน นางสาว ณัฐิญา สุขสวัสดิ์

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ปีการศึกษา 2553

บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาพฤติกรรมวัฏภาคของระบบที่ประกอบไปด้วยส่วนประกอบต่างๆ ที่ได้รับการยอมรับทางเครื่องสำอาง และเพื่อเตรียมและศึกษาสมบัติของไมโครอิมัลชัน และไมโครอิมัลเจลที่ได้ผสมกับนิโคตินามายด์ สำหรับการศึกษาพฤติกรรมวัฏภาค สารลดแรงตึงผิวที่ศึกษา ได้แก่ โอเลท-10 น้ำมันที่นำมาใช้ในการศึกษา ได้แก่ น้ำมันถั่วเหลืองและน้ำมันซิลิโคน ขณะที่สารลดแรงตึงผิวร่วมที่นำมาใช้ในการศึกษา ได้แก่ ไอโซโพรพิลแอลกอฮอล์ (IPA) โพรไพลีนไกลคอล (PG) และซอร์บิเทนโมโนโอลิเอท (Span 80) จากทุกระบบที่ทำการศึกษาพบว่า ระบบที่ประกอบด้วยน้ำ/น้ำมันถั่วเหลือง/โอเลท-10:IPA (9:1) ให้พื้นที่ไมโครอิมัลชันขนาดใหญ่ที่สุด ดังนั้นไมโครอิมัลชันสองตำรับจากระบบนี้จึงถูกเลือกเพื่อนำมาผสมกับนิโคตินามายด์ซึ่งเป็นสารที่ทำให้ผิวขาว ความเข้มข้นของนิโคตินามายด์ น้ำ น้ำมันถั่วเหลือง และของผสมสารลดแรงตึงผิวที่ใช้ในตำรับ ME-1 คือ 3%โดยน้ำหนัก 7%โดยน้ำหนัก 18%โดยน้ำหนัก และ 72%โดยน้ำหนัก ตามลำดับ ความเข้มข้นของสารเหล่านี้ในตำรับ ME-2 คือ 3%โดยน้ำหนัก 7%โดยน้ำหนัก 25%โดยน้ำหนัก และ 65%โดยน้ำหนัก ตามลำดับ ตำรับ ME-1 และ ME-2 ไม่มีความแตกต่างอย่างมีนัยสำคัญในด้านสมบัติทางกายภาพและเคมี ได้แก่ ค่าการนำไฟฟ้า ความหนืด ความเป็นกรดด่าง ขนาดอนุภาค ความคงตัว และ ลักษณะการปลดปล่อย อย่างไรก็ตาม ME-2 ถูกเลือกมาเปลี่ยนเป็นไมโครอิมัลชันเจลต่อไปเนื่องจากการมีปริมาณความเข้มข้นของสารลดแรงตึงผิวน้อยกว่า ไมโครอิมัลชันเจลต่างๆ เตรียมโดยการผสมไมโครอิมัลชันร่วมกับสารก่อเจลต่างๆ ได้แก่ 5%คอลลอยด์ดอลซิลิกาได้เป็นตำรับ ME-G1 5%ของ0.5%คาร์โบพอลอัลเทรต 21เจล ได้เป็นตำรับ ME-G2 และ 3%ของ0.5%คาร์โบพอลอัลเทรต 21เจล กับ 2% พีอีจี-40 ไฮโดรจิเนเทตคัสตรัลออยด์ได้เป็นตำรับ ME-G3 พบว่าตำรับ ME-G1 เท่านั้นที่มีลักษณะเป็นเจลใสและมี

ความหนืดที่เหมาะสม ดังนั้น ME-G1 จึงนำมาศึกษาการปลดปล่อยผ่านเซลลูโลสเมมเบรนเปรียบเทียบกับ ME-1 ME-2 และ ครีมชนิดน้ำมันในน้ำที่มีจำหน่ายทางการค้า ผลจากการเปรียบเทียบจากการปลดปล่อยสะสมและอัตราการปลดปล่อยพบว่า ME-G1 > ME-2 $\geq$ ME-1 $\geq$ ครีมที่มีจำหน่ายทางการค้าชนิดน้ำมันในน้ำ เนื่องจากความสัมพันธ์ระหว่างคอลลอยด์ดอลซิลิกาที่ชอบน้ำมัน และ นิโคตินามายด์ที่ชอบน้ำ มีความดึงดูดต่อกันต่ำจึงทำให้นิโคตินามายด์ถูกปลดปล่อยออกจาก ME-G1 ได้สูง นอกจากนี้ ME-G1 มีลักษณะภายนอกที่สวยงาม มีความหนืดที่เหมาะสม และ มีความคงตัวที่อุณหภูมิ 4ºC และที่อุณหภูมิห้อง (ประมาณ 30 ± 2ºC) ในการศึกษาเป็นระยะเวลา 2 เดือน

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LIST OF ABBREVIATIONS

ºC	degree Celsius (centigrade)
cm^2	square centimeter
cP	centi point
CPP	critical packing parameter
et al.	et alli, and other
g	gram
HLB	hydrophile-lipophile balance
HPLC	high performance liquid chromatography
i.e.	id est, that is
IPA	isopropyl alcohol
ln	logarithm natural
M	molarity
ME	microemulsion
µl	microliter
µm	micrometer
µS	micro siemens
mg	milligram
ml	milliliter
mm	millimeter
mPa	millipascal
ND	not determined
n.d.	no date given
nm	nanometer
o/w	oil in water
%	percent
%O	weight percent of oxygen content
PCS	photon correlation spectroscopy
PG	propylene glycol
pH	negative logarithm of the hydrogen ion concentration

LIST OF ABBREVIATIONS (continued)

PI	polydispersity index
Q_0	initial amounts of drug in the preformed preparations
Q_t,	cumulative amounts of drug release in time
r^2	linear regression coefficient
RPM	revolutions per minute
RSD	relative standard deviation
sec	second
SD	standard deviation
t	time
TEA	triethanolamine
UV	ultra violet
V	hydrophobic portion volume of the interface
v/v	volume by volume
w/o	water in oil
w/w	weight by weight
z-ave	mean particle size

CHAPTER 1

INTRODUCTION

1. Background and Rationale

Skin color originates in the presence of a pigment called melanin. Once melanin is produced, the melanosomes are transferred into the surrounding keratinocytes. Subsequently, degraded melanosomes is left in the strateum corneum. In Asia, light or fade skin is preferable; therefore, skin lightening or whitening products are popular. Among a lot of skin lightening agents, nicotinamide is a widely used and well-known compound. It can inhibit melanosome transfer from melanocytes to keratinocytes. Therefore, it provides safety mechanism since inhibition process occurs after melanogenesis within melanosome and does not affect intrinsic biosynthesis of melanin production. In addition, it can be applied for moisturizing and treatment of acne vulgaris (Hakozaki *et al*., 2002; Soma *et al*., 2005; Solano *et al*., 2006; Kaymak and Onder, 2008). Since nicotinamide is hydrophilic, it is difficult to penetrate into the skin due to the lipid bilayer structure of the stratum corneum (Hakozaki *et al*., 2006; Nicoli *et al*., 2008). To improve the skin penetration of nicotimide, advanced vehicles are necessary. Microemulsions are one of the interesting novel vehicles using in cosmetics because of their advantages especially in skin penetration enhancement. Nowadays, microemulsions have been applied as the vehicles for numerous cosmetic active ingredients such as whitening agents, antioxidants, moisturizers, sunscreens and others in order to increase the product efficiency and respond to the consumers' demand (Boonme, 2007; Boonme, 2009; Boonme *et al*., 2009; Boonme and Songkro, 2010). Therefore, they were used in this study.

Although microemulsions can provide a lot of benefits in cosmetic productions, the risk of skin irritation caused by microemulsions is quite high because microemulsions need high concentrations of a surfactant and a cosurfactant to reduce the interfacial tension and to increase the flexibility of the interfacial film,

respectively. Thus, the selection of amphiphiles in formulations of cosmetic microemulsions needs careful contemplation (Boonme, 2009). In this study, acceptable cosmetic ingredients were used to formulate microemulsions. Oleth-10 is a nonionic surfactant. It has been widely used in topical pharmaceutical formulations and cosmetics primarily as an emulsifying agent for w/o and o/w emulsions (Malcolmson and Lawrence, 1995; Boonme *et al*., 2004; Boonme *et al*., 2006; Junyaprasert *et al*., 2007). Isopropyl alcohol (IPA), propylene glycol (PG) and sorbitan monooleate (Span 80) were used as cosurfactants. IPA is approved by the U.S. FDA and chemindustry lab for food additive uses, solvents, pharmaceuticals, cosmetics and personal care products (Manzall and Valkenburg, 1982). PG is most frequently applied in dermal products (Heuschkel *et al*., 2008). Span 80, a nonionic surfactant, is an approved pharmaceutical excipient. It is widely used in topical product (Wu *et al*., 2001; Kato *et al*., 2008). Silicone oil and soybean oil were selected as oil phases. Silicone oil is a synthetic oil used in cosmetic formulations and industrial applications (Mehta and Somasundaran, 2008). Soybean oil, commonly labeled as vegetable oil, is inexpensive, safe and available used in microemulsion (Darmstadt *et al*., 2002; Polizelli *et al*., 2006). All studied oils, surfactant and cosurfactants are acceptable for cosmetic purposes. Thus, the obtained microemulsions can be incorporated with nicotinamide (cosmetic active ingredient).

In some cases, microemulsion system is unsuitable for topical use due to low viscosity. Thus, adding the thickening agents in the system is recommended (Boonme, 2007; Souto and Boonme, 2009). Microemulsion-based gel can increase the adhesiveness to the skin because of viscous characteristics. Other advantages of the gel formulation include attractiveness, transparent, shiny and non-sticky appearance (Kochansky and Shimanuki, 1999). In the current investigation, three gel bases, colloidal silica, Carbopol® Ultrez 21 and Carbopol® Ultrez 21 plus PEG-40 hydrogenated castor oil, were selected to be incorporated with the nicotinamide microemulsions in order to increase the viscosity of the microemulsion system. Colloidal silica was used as the representative thickener of w/o thickening phase for microemulsions (Spiclin *et al*., 2003). It was reported that colloidal silica at concentration above 3.00% (w/w), achieved a suitable thickening agent for w/o microemulsions (Spiclin *et al*., 2003). Carbopol was selected as the gelling agent in

consequence of the following advantages: high viscosity at low concentration, compatibility with many active components, good bioadhesive properties, temperature stability, and excellent organoleptic characteristics and good tolerability by patients (Romanko *et al*., 2009). In this work, Carbopol® Ultrez 21 polymer was selected for microemulsions because it provides excellent clarity, a non-grainy glossy appearance to gels and suspending properties to a variety of personal care applications in the cosmetic manufacture (Amnuaikit *et al*., 2008). It is generally recognized that PEG-40 hydrogenated castor oil has been used in a wide variety of cosmetics and personal care products. Its functions have extended as an emulsifier, pigment dispersant, solvent, solubilizer, and wetting agent. In addition, it is used to enhance the gelling properties of other surfactants in w/o emulsions. The safety data of PEG-40 hydrogenated castor oil has been assessed by the Cosmetic Ingredient Review (CIR) Expert Panel and permitted by The Food and Drug Administration (FDA).

In this study, microemulsion systems composed of oleth-10 (nonionic surfactant), water, various oils and cosurfactants were investigated the phase behavior. The suitable microemulsion system which had the largest size of microemulsion region was used to develop nicotinamide microemulsions and nicotinamide microemulsion-based gel for cosmetic application. The obtained microemulsions and microemulsion-based gel were evaluated for *in vitro* release through a synthetic membrane compared to a commercial cream. The stability of the products was also determined.

Objectives of the study were:

- to investigate the phase behavior of systems composed of oleth-10, water, various oils and cosurfactants,
- to prepare nicotinamide microemulsions and microemulsion-based gels,
- to study physicochemical properties of nicotinamide microemulsions and microemulsion-based gels,
- to study the *in vitro* release of nicotinamide from microemulsions and microemulsion-based gels compared to a commercial cream, and
- to study stability of nicotinamide microemulsions and microemulsion based gels.

CHAPTER 2

REVIEW OF LITERATURE

1. Skin Color

Skin color occurs due to the presence of a substance called melanin, the pigment in the skin. Melanin is synthesized and stored within melanosome which is produced from melanocytes, the pigment producing cells in the epidermis. Its role is to protect the skin from ultraviolet rays (Radhakrishnan *et al*., 2007; Ebanks *et al*., 2009). Human skin contains two types of melanin, the brownish-black eumelanin and the reddish-yellow pheomelanin. Melanin production in human melanocytes is controlled primarily by the rate-limiting enzyme for melanin synthesis, tyrosinase, which catalyzes the hydroxylation of tyrosine to L-DOPA and the oxidation of L-DOPA to dopaquinone. The subsequent steps in the pathway to melanin formation are thought to play a role in determining what type of melanin is formed (Jablonski, 2004; Ebanks *et al*., 2009) as shown in Figure 1. It illustrates that tyrosinase, the rate limiting enzyme of melanogenesis, catalyzes the hydroxylation of L-tyrosine to DOPA and the oxidation of DOPA to DOPAquinone. If cysteine or glutathione is present, it reacts with DOPAquinone to produce cysteinylDOPA and the benzothiazine derivatives of pheomelanin. As cysteine is diminished, DOPAquinone cyclizes into DOPAchrome. TYRP-2 catalyzes the tautomerization of DOPAchrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA), which is later oxidized to DHICA-melanin subunits. The oxidation of DHICA to eumelanin is thought to be catalyzed by TYRP-1. In the absence of TYRP-2 the carboxylic acid moiety of DOPAchrome is spontaneously lost to form 5,6-dihydroxyindole (DHI). DHICA in conjunction with DHI comprise subunits of eumelanin (Ito, 2003; Ebanks *et al*., 2009)

After the synthesis of melanin, melanosomes with pigment are normally transferred from melanocytes to surrounding keratinocytes. Four processes for melanosome transfer to keratinocytes are proposed, i.e., (A) cytophagocytosis, (B) exocytosis, (C) fusion, and (D) membrane vesicles as shown in Figure 2. Afterwards,

they become aggregated and surrounded by a membrane in a melanosome complex as explained in Figure 3 (Jablonski, 2004; Bossche *et al*., 2006).

Intensity of skin coloration is determined by many factors as follows:

- the total number of melanosomes in the keratinocytes and melanocytes, and their degree of dispersion,
- the rate of melanin production (melanogenesis),
- the degree of melanization of melanosomes,
- the rate of transport and type of incorporation of melanosomes into keratinocytes,
- the degradation of melanosomes within the keratinocytes, and
- a person's chronological age since the number of metabolically active melanocytes decreases over time (Halaban *et al*., 1988; Jablonski, 2004).

For Asian human, light or fade skin is preferable; therefore skin lightening products are popular. The lightenting agents that are currently used achieve depigmentation by one or more mechanisms (Seiberg *et al*., 2000) as demonstrated in Figure 4. Numerous scientific publications have presented data on the skin lightening properties of cosmetic ingredients such as arbutin, kojic acid, vitamin C, vitamin B3, azelaic acid, alpha hydroxyacids and hydroquinone (Farris, 2005; Solano *et al*., 2006; Radhakrishnan *et al*., 2007; Boonme *et al*., 2009). All of the ingredients are commonly found in skin whitening products. Most skin lightening ingredients work by inhibiting the activity of tyrosinase, resulting in lightening skin, such as arbutin, kojic acid, azelaic acid and hydroquinone (Boonme *et al*., 2009). Unlike many other skin lightening agents, nicotinamide, the active ingredient in this study, acts by different mechanism.

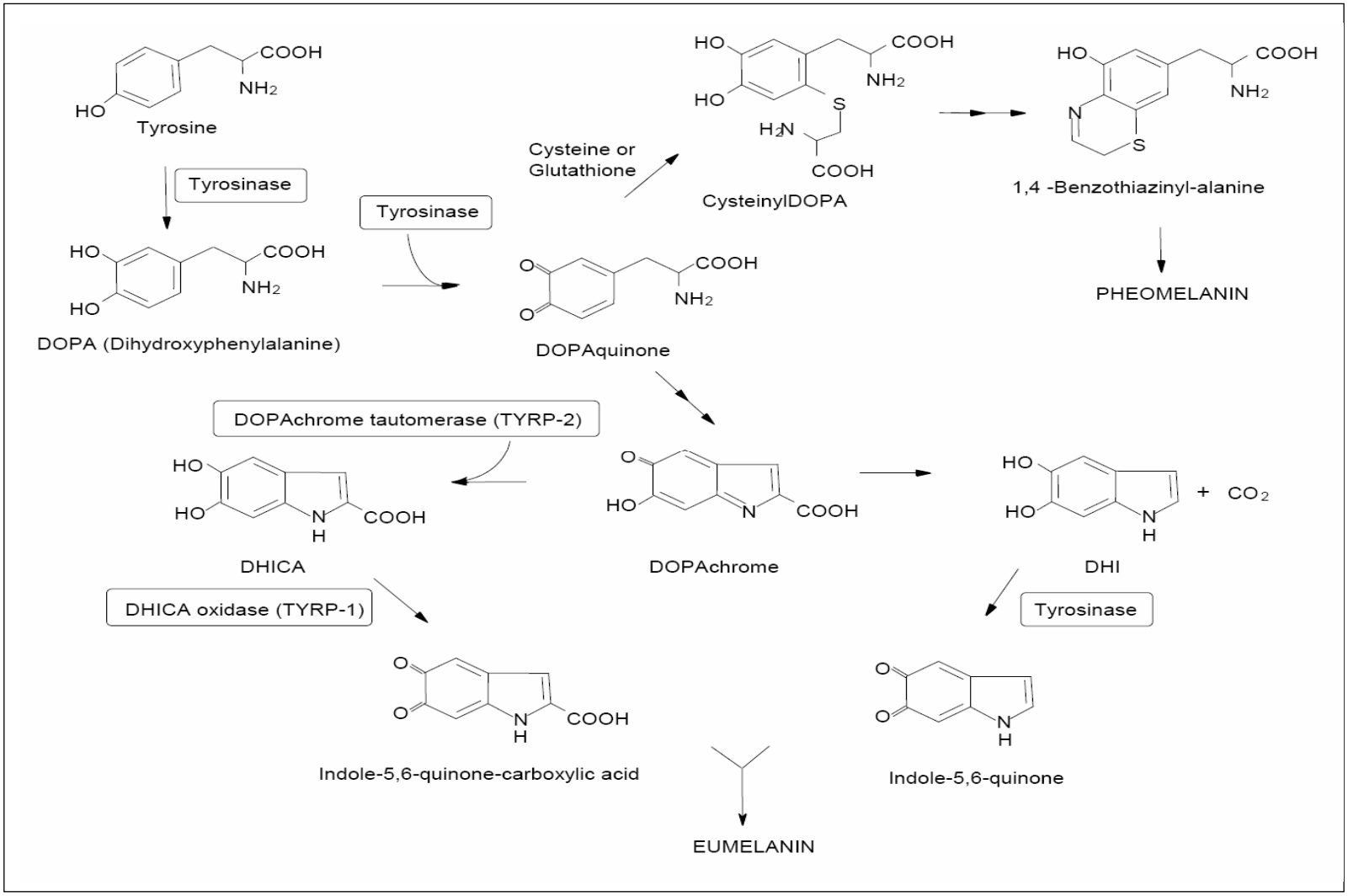


Figure 1. The pathways of melanogenesis within epidermal melanosomes (Ebanks *et al*., 2009).

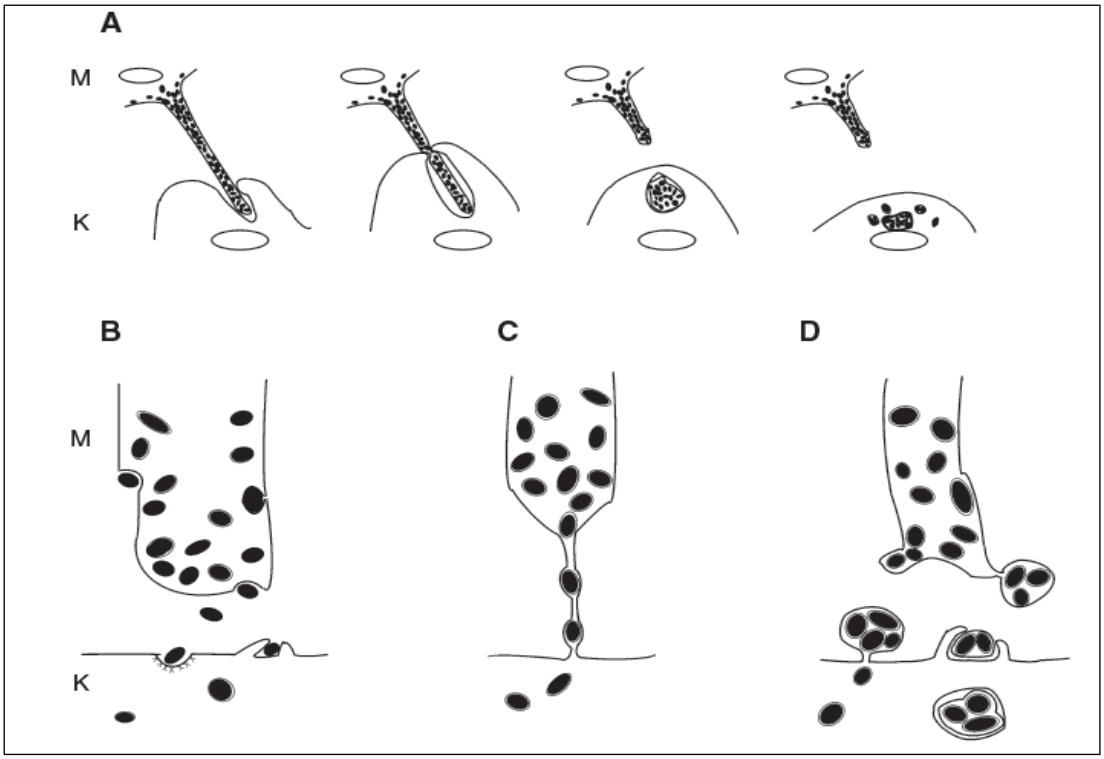


Figure 2. Different modes of melanin transfer. (A) Cytophagocytosis: a melanocytic dendrite is pinched off and phagocytosed, leading to a phagolysosome from which melanin granules disperse throughout the cytoplasm. (B) Exocytosis: melanin is externalized by fusion of the melanosomal membrane with the plasma membrane and is then taken up by endocytosis or phagocytosis. (C) Fusion: plasma membranes of both cells merge creating a channel which allows passage of melanosomes. (D) Membrane vesicles: melanosomes are shed in vesicles which either fuse with the keratinocyte plasma membrane or are ingested by phagocytosis. M: melanocyte, K: keratinocyte (Bossche *et al*., 2006).

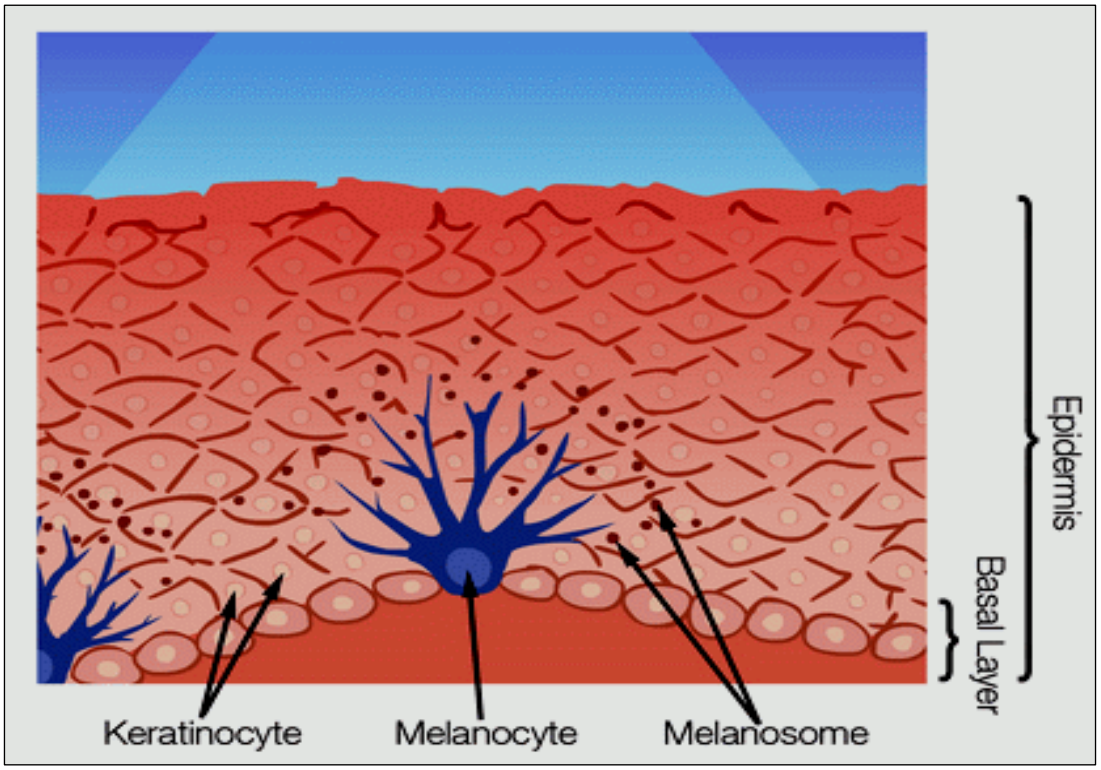


Figure 3. A schematic diagram of the epidermis. The melanocyte resides in the basal layer and supplies melanin (packaged within melanosomes) to surround keratinocytes (http://photoprotection.clinuvel.com).

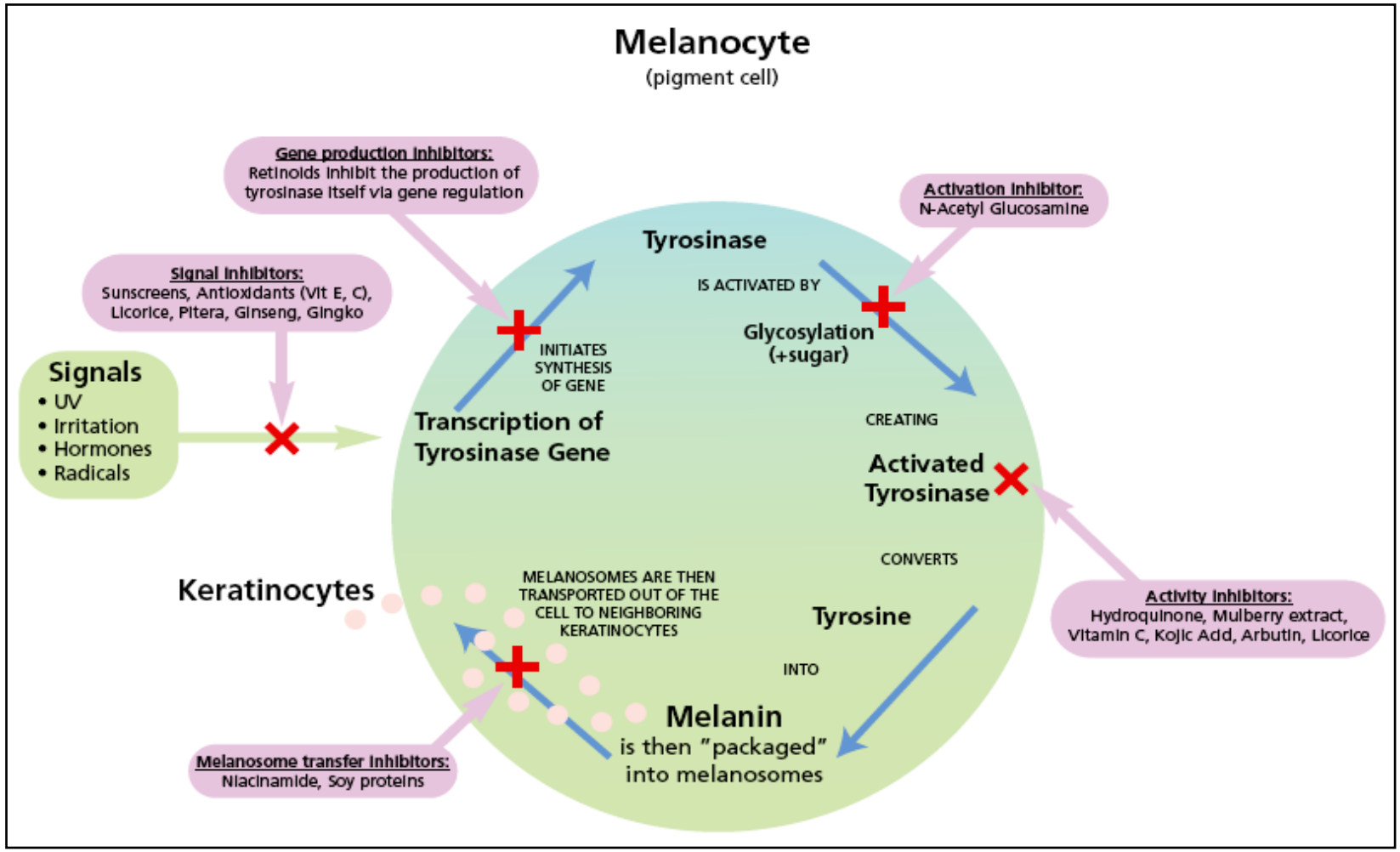


Figure 4. The different depigmentation mechanisms of many lightening agents. Symbol X's indicate where the cycle may be interrupted and at which point actives intervene (http://www.pgbeautygroomingscience.com).

2. Nicotinamide

Nicotinamide, which is also called niacinamide, is one of the two principal forms of vitamin B3. It is also called vitamin B3. Another form of vitamin B3 is nicotinic acid (niacin). Vitamin B3 is commercially available in two forms: nicotinic acid and nicotinamide. Two related forms occur naturally with niacin being the most prevalent in plants and niacinamide in animals (Salvador and Chisvert, 2007). The chemical structures of nicotinic acid and nicotinamide are illustrated in Figure 5**.** In addition, their physicochemical properties are exhibited in Table 1. Nicotinamide is the amide form of niacin and more water soluble. Nicotinamide plays an important role in human as a precursor of two important coenzyme - nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) which are used to generate energy inside the cells. These coenzymes are involved in many intracellular oxidation-reduction reactions. In contrast to its related structure, nicotinic acid, nicotinamide has no effects on blood pressure, pulse or body temperature (Lieberman, 1997; Hakozaki *et al*., 2002; Otte *et al*., 2005; Ebanks *et al*., 2009).

For the stability, nicotinamide is resistant to heat, air, and oxidants, but it is hydrolysed in strong acids and alkaline solutions (Draelos, 2000; Leskova *et al*., 2006). Nicotinamide melts at 129˚C and the decomposition happens by volatilization at 254˚C when there is the total mass loss in the thermogravimetry/derivative thermogravimetry (TG/DTG) curves (Moreschi *et al*., 2009). It is assumed to be the most stable water-soluble vitamin. Stability of nicotinamide remained constant during the storage at 20, 30 and 37°C for 12 months (Albala-Hurtado *et al*., 2000). The nutritional value of nicotinamide is well recognized. Its utility as a topical agent to provide skin-care benefits is also being elucidated based on recently published studies. Nicotinamide was considered to be safe for using in cosmetics. Benefits of topical nicotinamide on the skin have been reported (Bissett *et al*., 2004; Bissett *et al*., 2005; Otte *et al*., 2005; Salvador and Chisvert, 2007).

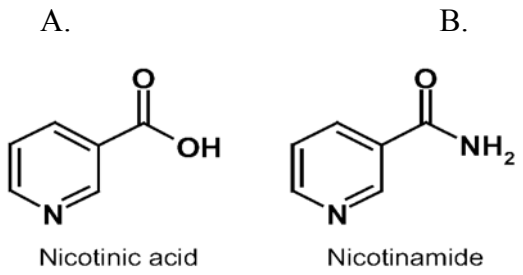


Figure 5. Chemical structures of two related forms of vitamin B3; niacin (A) and niacinamide (B) (Gille *et al*., 2008).

Table 1. Physicochemical properties of vitamin B3 (Xu and Trissel, 2003; Cosmetic Ingredient Review Expert Panel, 2005; Bissett, 2009)

Physicochemical properties	**Forms of vitamin B3**	
	Nicotinic acid (niacin)	**Nicotinamide (niacinamide)**
Molecular Formular	$C_6H_5NO_2$	$C_6H_6N_2O$
Chemical Name	pyridine-3-carboxylic acid	3-pyridinecarboxamide
Molecular Weight	123.1	122.1
Appearance	White crystals or crystalline powder	White crystalline powder or colorless crystals
Solubility	16.7 mg/ml	1g/ml
pK_a	4.85	-
% Use	0.01-0.1%	2-5%
Uses in cosmetics	Similar to nicotinamide but is used in few cosmetic products	Whitening agent, Anti-acne, Anti-aging, Reduced TEWL, Reduced skin yellowing, Reduced skin pore size and improved texture, Reduced facial red blotchiness

In 2005, Otte *et al*. reviewed about biologic actions of nicotinamide as a cosmetic agent. The review article has revealed that nicotinamide as a component of NADH has a higher reducing power than well-known antioxidants like vitamin C and vitamin E. NADH acts directly as an operating antioxidant and can effectively protect the cell and in particular its membrane from destruction by free radicals. In addition, topical nicotinamide as a precursor of NADH and NADPH, which has been observed to be depleted with increasing age, can command the reported broad spectrum of activity through local correction of homeostatic balance of these two nucleotide coenzymes. Nicotinamide increases biosynthesis of ceramides, which upon degradation produce sphingosine. The anti-inflammatory of nicotinamide is widespread use of topically treatment in anti-acne. This may occur by means of inhibition of neutrophil chemotaxis and secretion of inflammatory cytokines, suppression of lymphocyte transformation as well as inhibition of mast cell histamine release and blockade of histamine receptors. The topical application of nicotinamide in addition to sunscreen formulations is caused by its possible skin lightening effects, stimulation of DNA-repair, suppression of UV photocarcinogenesis and further anti-aging effects.

Furthermore, the depigmenting effect of nicotinamide seems to be rather based on the suppression of pigment spreading than direct inhibition of melanin production. Unlike many other skin lightening agents, nicotinamide acts by different mechanism. Attractively, nicotinamide can act as an effective skin lightening compound by inhibiting melanosome transfer from melanocytes to keratinocytes (Soma *et al*., 2005; Solano *et al*., 2006; Kaymak and Onder, 2008). This safety mechanism which occurs after the process of melanogenesis within melanosomes does not affect intrinsic biosynthesis of melanin production. From *in vivo* study, niacinamide gave 35–68% inhibition of melanosome transfer in the coculture cell line model and reduced cutaneous pigmentation in the a pigmented reconstructed epidermis (PREP) model (Hakozaki *et al*., 2002). In the *in vitro* trials, the effects of niacinamide on melanogenesis in cultured melanocytes was investigated. It was found that nicotinamide did not affect on amount of synthesized melanin and had no inhibitory effect on melanocyte tyrosinase activity in co-cultures of melanocytes and keratinocytes (Hakozaki *et al*., 2002). It is concluded that the mechanism of

nicotinamide is a significant inhibition of melanosome transfer. Thus, the depigmenting effect of nicotinamide seems to be rather based on the suppression of pigment spreading than the direct inhibition of melanin production. In a split-face double-blind trial, 18 female subjects with multiple types of brown hyperpigmentation (senile lentigines, melasma or freckles) applied a 5% nicotinamide o/w emulsion for 8 weeks twice daily. After 4 weeks of treatment, the side of the face receiving nicotinamide showed a significant ($p < 0.05$) decrease in total hyperpigmented area in comparison with the side receiving only vehicle, as demonstrated in Figure 6.

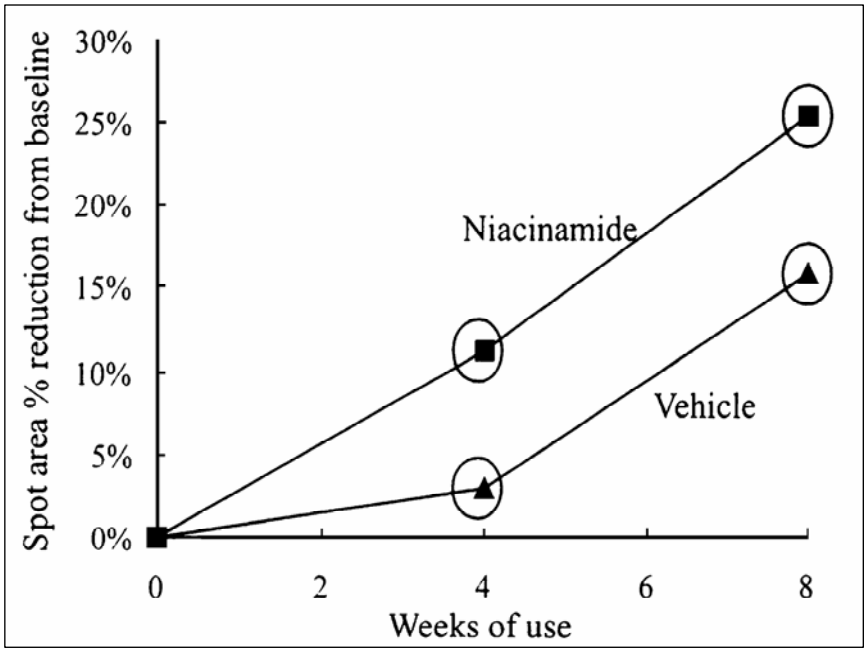


Figure 6. Percentage reduction of area of hyperpigmentation from baseline for nicotinamide 5% o/w emulsion and vehicle-treated sides of the face (Otte *et al.*, 2005).

2.1 Side Effects of Nicotinamide

According to "final report of the safety assessment of niacinamide", Cosmetic Ingredient Review Expert Panel reviewed that niacinamide, evaluated in an *in vitro* test to predict ocular irritation, was not an acute ocular hazard. Animal testing of niacinamide in rabbits in actual formulations produced mostly non-irritant reactions, with only some marginally irritating responses. Skin irritation tests of up to 2.5% niacinamide in rabbits produced only marginal irritation. Skin sensitization tests of niacinamide at 5% during induction and 20% during challenge were negative in guinea pigs. Neither cosmetic ingredient was mutagenic in Ames tests, with or without metabolic activation. Niacinamide and niacin at 2 mg/ml were negative in a chromosome aberration test in Chinese hamster ovary cells, but did produce large structural chromosome aberrations at 3 mg/ml. Niacinamide induced sister chromatid exchanges in Chinese hamster ovary cells, but niacin did not. Under certain circumstances, niacinamide can cause an increase in unscheduled DNA synthesis in human lymphocytes treated with UV or a nitrosoguanidine compound. Niacinamide itself was not carcinogenic when administered (1%) in the drinking water of mice. No data on the carcinogenic effect of niacin were available. Niacinamide can moderate the induction of tumors by established carcinogens. Niacinamide in combination with streptozotocin (a nitrosourea compound) or with heliotrine (a pyrrolizidine alkaloid), produced pancreatic islet tumors. On the other hand, niacinamide reduced the renal adenomas produced by streptozotocin and intestinal and bladder tumors induced by a preparation of bracken fern. Niacinamide evaluated in the *in vitro* test systems did affect development, but niacinamide reduced the reproductive/developmental toxicity of 2-aminonicotinamide-amino-1,3,4-thiadiazole hydrochloride and urethane. Clinical testing of niacinamide produced no stinging sensation at concentrations up to 10%. The tests provided no irritation at concentrations up to 5%. However, certain formulations were marginal to slight ocular irritants.

2.2 Difficult of Topical Delivery of Nicotinamide

Since nicotinamide is hydrophilic, it is difficult to permeate into the stratum corneum, the main barrier of the skin (lipid bilayer structure) (Kai *et al*., 1990; Hakozaki *et al*., 2006). In 1990, Kai *et al* studied the effects of alcohol treatment on skin barrier function of hairless mouse using nicotinamide as a model penetrant. The results showed that when an aqueous nicotinamide solution was applied to untreated hairless mouse skin the average apparent steady state flux was 0.022 μg/cm^2 h. In contrast, when ethanol or propanol was used in the pretreatment solution, the flux of nicotinamide (1.02 or 2.00 μg/cm^2 h) enhanced significantly. Recent report indicated that the presence of nicotinamide significantly decreased the transdermal flux of ethyl paraben when applied to the skin at the constant concentration of 0.08%. Especially, ethyl paraben flux showed 22.4±4.4 μg cm^{-2} h^{-1} in the absence of nicotinamide, while decreased to 10.3±1.8 μg cm^{-2} h^{-1} and to 2.1±0.5 μg cm^{-2} h^{-1} in the presence of nicotinamide at 3.5 and 20% respectively. When the saturated solutions of parabens in the presence of nicotinamide 20% (w/v) were applied to the skin, a ten-fold reduction of the permeability coefficient was measured with respect to saturated solutions of parabens alone, due to the decrease of the partition parameter. It could be seen that nicotinamide at the concentration of 20% modified the partitioning between the stratum corneum and the vehicle either by modifying the polarity of the vehicle or by forming a complex more hydrophilic than the paraben alone (Nicoli *et al*., 2008).

To overcome the stratum corneum barrier, several technigues have been employed to enhance the skin penetration of nicotinamide. For example, it was reported that high-frequency ultrasound (5MHz) could enhance the skin penetration of nicotinamide, resulting in the reduction of facial hyperpigmentation. Although the specific enhancement mechanism by 5MHz ultrasound radiation remains to be elucidated, the obtained data suggest that 5MHz ultrasound system is safe and effective (Hakozaki *et al*., 2006). Ridout *et al*. (1991) studied the effects of five zwitterionic surfactants in the *in vitro* test on skin barrier function of hairless mouse skin. The results suggested that all surfactants decreased skin barrier function to some extent. The degree of nicotinamide penetration enhancement induced was correlated with the ratio of the surfactant pretreatment concentration to the surfactant critical

micelle concentration, suggesting that solubilization of stratum corneum lipids may be an important mechanism in explaining the effects observed. In addition, advanced vehicles are also facilitative the skin permeation of drug delivery (Kogan and Garti, 2006). Amongst the alternatives, novel formulation aiming to enhance skin penetration is the preparation of microemulsions.

3. Microemulsions

The microemulsion concept was introduced as early as the 1940s by Hoar and Schulman who generated a clear single-phase solution by titrating a milky emulsion with a medium-chain alcohol such as pentanol and hexanol. Afterwards, Schulman and coworkers subsequently coined the term microemulsion in 1959 (Lawrence and Rees, 2000).

Microemulsions are optically transparent liquids containing dispersions of oil and water stabilized by interfacial film with ultralow interfacial tensions of a surfactant, typically in combination with a cosurfactant. The spontaneous system of the miscibility of oil, water and amphiphile (surfactant plus cosurfactant) depends on the overall composition which is systemic specificity. Figure 7 shows the stability of macroemulsions, miniemulsions and microemulsions. The data show that microemulsion is themodynamically stable over long period of time. Thermodynamic stability of the microemulsions has been proposed by Ruckenstein and Chi who considered that the free energy of formation comprises interfacial free energy, interaction energy between droplets and entropy of dispersion. The interaction energy between droplets has been shown to be negligible and the free energy of formation can be zero or even negative if the interfacial tension is of the order of 10^{-2}–10^{-3} mN/m. The stability of the microemulsion can be influenced by addition of salt, other additives, temperature or pressure (Lawrence and Rees, 2000; Paul and Moulik, 2001).

The properties of microemulsions are as follows:

- clear,
- low viscosity (Newtonian fluids),

- thermodynamically stable,
- small droplet size in range of about 10-140 nm, and
- could solubilize both aqueous and oil-soluble compounds.

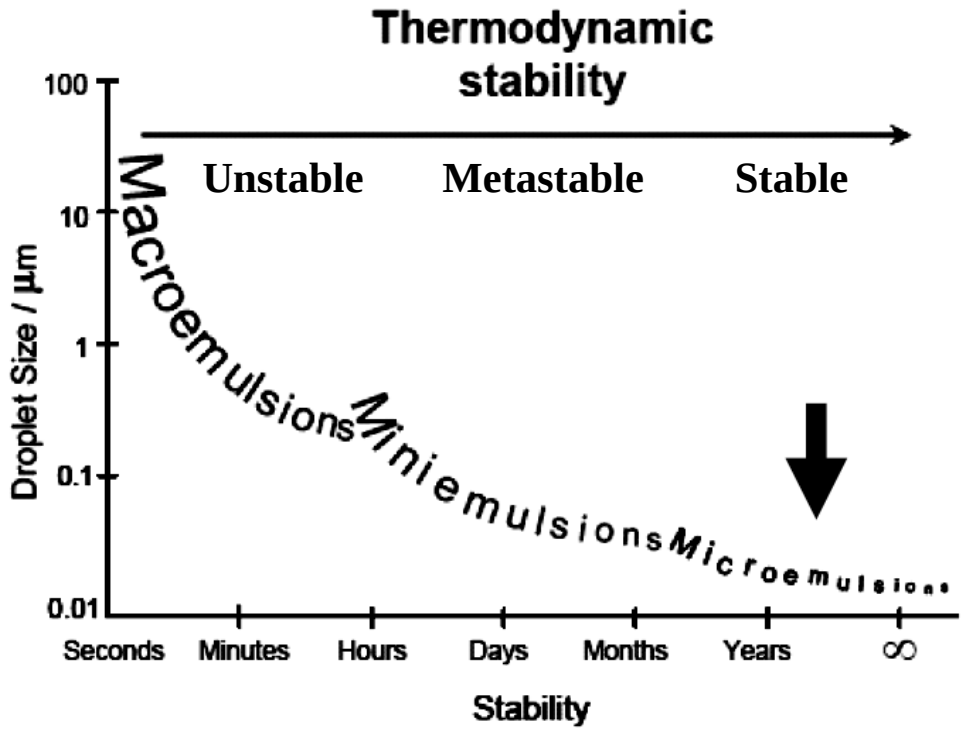


Figure 7. Thermodynamic stability of microemulsion characteristic over long period time (www.mpikg.mpg.de/pdf/KolloidChemie/Scripte/Emulsions-Part 3).

Microemulsions can be classified into three types: oil-in-water (o/w) microemulsions, bicontinuous microemulsions, and water-in-oil (w/o) microemulsions as shown in Figure 8.

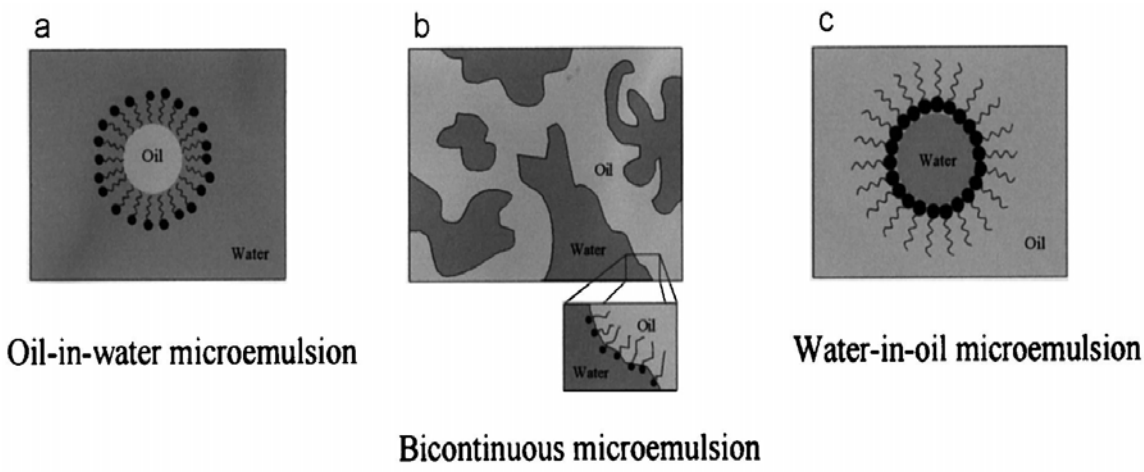


Figure 8. Schematic representation of the three most commonly encountered microemulsion microstructures: (a) oil-in-water, (b) bicontinuous, and (c) water-in-oil microemulsion (Lawrence and Rees, 2000).

The presence of o/w microemulsion droplets is likely to be a feature in microemulsions where the volume fraction of oil is low. In systems where the amounts of water and oil are similar, a bicontinuous microemulsion may result. The presence of w/o microemulsion droplets is likely to be a feature in microemulsions where the volume fraction of water is low (Lawrence and Rees, 2000; Junyaprasert and Boonme, 2002).

In fact, the combination of oil, water, surfactant and cosurfactant does not only offer microemulsions but also provide association structures depending on the chemical nature and concentration of the components as well as temperature and pressure. Microemulsions are merely one of a number of association structures. The other association structures include, regular emulsions, micellar and mesomorphic phases of various constructions such as lamellar, hexagonal, cubic and various gels, and oily dispersions. Generally of a mixing of water, oil and surfactant, the coexistence structure can form spontaneously as displayed in Figure 9.

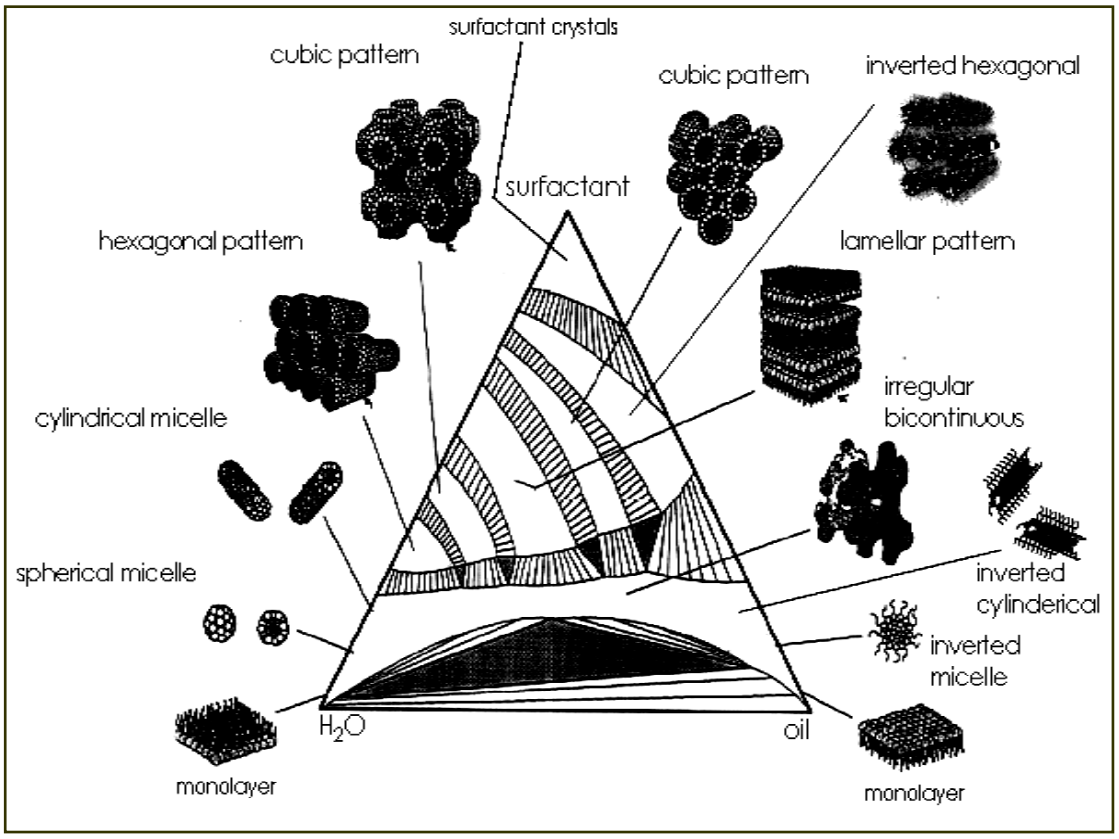


Figure 9. The association structures in phase diagram of a combination of oil, water and surfactant (www.mpikg.mpg.de/pdf/KolloidChemie/Scripte/Emulsions-Part 3).

To find the suitable types and amounts of each component to form microemulsions, the construction of a phase diagram is a useful approach to find the microemulsion regions. A ternary or pseudoternary phase diagram can be constructed by two methods: (i) titrating a mixture of two components with the third component and (ii) preparing a large number of samples of different ratios of components. If all mixtures reach equilibrium rapidly both methods give identical results. In contrast, if all mixtures do not reach equilibrium rapidly, the second method is recommended (Lawrence and Rees, 2000; Boonme *et al*., 2004). Therefore, the second method was used in this study.

Surfactant and cosurfactant are added in a system to reduce the interfacial tension and to increase the flexibility of the interfacial film, respectively. The result of these action provides the thermodynamically stable of microemulsions. The relationship between surfactant and the possible origin of microemulsion can be guide in term of the following factors, i.e., hydrophile-lipophile balance (HLB) and critical packing parameter (CPP).

According to the review of Lawrence and Rees (2000), the HLB is referred to the dispersion tendency of hydrophilic and hydrophobic fragments of the surfactant molecule. It is generally accepted that low HLB (3–6) surfactants are preferred for the formation of w/o microemulsions whereas surfactants with high HLBs (8–18) are favoured for the formation of o/w microemulsions. It can be noted that real amphiphiles may be designed to be soluble or insoluble in water by adjusting the magnitude balance between hydrophobicity and hydrophilicity. In other hand, using CPP to predict the type of association structures that are probably to form in the surfactant systems can be calculated using the following equation:

$$CPP = v/a_o\, l_c$$

The *CPP* is assigned to the ratio of the alkyl chain volume (v) to the area occupied at the interface by the polar head group (a_o) and the critical alkyl chain length (l_c). It is a predictive parameter of the type of association structures that are likely to form in the surfactant systems.

Recent published reports have informed about some mechanisms of microemulsions in skin permeation enhancement (Kogan and Garti, 2006; Boonme, 2007) as follows:

- Microemulsion formulations have a potency of solubility a large amount of active ingredient as a result of increasing skin permeation.
- The thermodynamic activity of the active ingredient in the microemulsions can be modified to favor partitioning into the stratum corneum.
- The surfactants in the microemulsions may reduce the diffusional barrier of the stratum corneum.
- Microemulsions have small molecules as nano sized droplets in range of about 10-140 nm can bring the ability of the absorption through skin by themselves.
- Microemulsions can act as active ingredient reservoirs where loaded active ingredient is released from the inner phase to the outer phase and then to the skin
- A percutaneous absorption of active ingredient can arise from the hydration effect of the stratum corneum if the water content in the microemulsions is high enough.

The fundamental observation being microemulsions, the isotropic nature of microemulsions including optical clarity appearance is widely used. Generally, the viscosity values of microemulsions are low. The flow pattern of microemulsions is Newtonian behavior. Therefore, viscosity and rheological studies can be used to determine microemulsions. There are two major types of flow behavior, i.e., Newtonian and non-Newtonian flows. Non-Newtonian flow can be classified into three minor types, i.e., plastic, pseudoplastic and dilatants as illustrated in Figure 10.

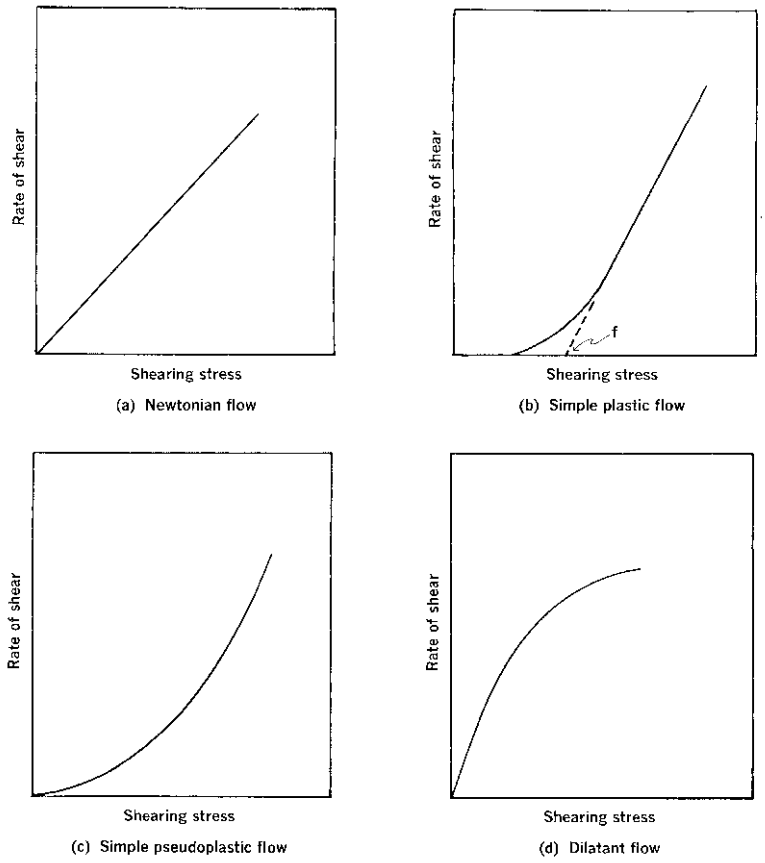


Figure 10. Viscosity types of flow behavior; Newtonian (a), plastic (b), pseudoplastic (c) and dilatant (d) flows (Sinko and Martin, 2006).

- Newtonian Flow

  Newtonian behavior in Figure 10 shows that the relationship between shear stress and shear rate is a straight line. Fluids with Newtonian flow will display the constant viscosity as the shear rate is varied. Newtonian is frequently observed in water and gas.

- Plastic Flow

  This type of flow behavior is a solid under static conditions. As seen in Figure 10, the flow require a finite yield stress before it begins to flow (the plot of shear stress against shear strain does not pass through the origin). A certain amount of force must be applied to the fluid before any flow is induced. This force is called the "yield value". Below the yield value, the material behaves essentially as an elastic solid**.** In addition, the phenomenon known as thixotropy (gel-sol-gel transformation) by a breakdown of structure with agitation and followed by reformation of rigid structure was exhibited in plastic flow such as bentonite magma and petrolatum

- Psuedoplastic Flow

  This type of flow behavior is sometimes called shear-thinning. Fluids with psuedoplastic flow will display a decreasing viscosity with an increasing shear rate. Probably the most of pharmaceutical products such as liquid dispersions of methylcellulose, tragacanth, sodium alginate exhibit psuedoplastic flow. Some psuedoplastics show thixotropy due to a breakdown of structure with shear.

- Dilatant Flow

  This type of flow behavior is sometimes called shear-thickening. Increasing viscosity with an increase in shear rate characterizes the dilatant fluid. Dilatant is frequently observed in fluids containing high levels of deflocculated solids, such as pastes, printing inks and paints.

3.1 Effect of surfactants on the permeation

It is well-known that anionic, cationic, nonionic and zwitterionic surfactants were used to stabilize microemulsion. Walters *et al*. (1993) reviewed the effects of the surfactants on skin penetration.

Anionic surfactants can penetrate and interact strongly with skin, producing large alterations in barrier properties. For example, the alkyl sulphates can penetrate and destroy the integrity of the stratum corneum within hours of application. Sodium decyl and dodecyl sulphates have been shown to enhance the permeation rates of several compounds, including chloramphenicol, naloxone and naproxen. The action of these surfactants on skin appears to be linked to their ability to interact with and bind to epidermal proteins. It has been postulated that the hydrophobic interaction of the alkyl chains with the substrate leaves the negative end group of the surfactant exposed, creating additional anionic sites in the membrane.

Cationic surfactants have not been widely studied as enhancers of skin penetration due to their irritancy. They are more irritating than anionic surfactants. They have, however, demonstrated a tendency for enhancement activity in the cases where they have been evaluated. Furthermore, some cationic surfactants, such as long-chain alkyl amines, are widely used in cosmetic formulations with no apparent untoward effect. This has stimulated the evaluation of this group as potential enhancers of permeation mediated by an ion-pair carrier mechanism. There are also indicated that the long-chain amines can enhance skin permeation by a mechanism unrelated to their carrier properties.

Nonionic surfactant is well-known for the least potential for irritancy. Polyoxyethylene alkyl ethers and esters have been shown to be more effective enhancers of permeation than the polysorbates. In most cases, the magnitude of enhancement is dependent on surfactant structure, both the hydrophobic alkyl chain and the hydrophilic.

Zwitterionic surfactants are not mentioned to the details in the review.

3.2 Microemulsions in cosmetics

Based on published researches and patents, nowadays, microemulsions are considered as acceptable vehicles used in cosmetics including personal care, hair care and many kinds of skin care products. In view of the development of cosmetic products, microemulsions can be employed as the vehicles for numerous cosmetic active ingredients such as whitening agents, antioxidants, moisturizers, sunscreens and others in order to increase the product efficiency and respond to the consumers' demand. This is due to the prominent advantages of microemulsions such as good appearance, thermodynamic stability, huge solubilization capacity and ease of preparation. It is believed that microemulsion formulation can provide skin penetration enhancement in many cosmetic applications (Paul and Moulik, 2001; Boonme, 2007; Boonme, 2009; Boonme *et al*., 2009). Especially, when the overall compositions being used are safe for human; the use of microemulsions becomes widely spread. To overcome the irritative skin problem of microemulsions, all compositions of microemulsions prepared in the study must be acceptable cosmetic ingredients.

Cosmetic formulations for skin care products using commercial nonionic surfactants and oils generally employed in cosmetics are also investigated. Tokuoka *et al.* (1995) studied the solubilization of several systems consisting of water, surfactant and synthetic perfumes (viz. d-limonene, *a*-ionone, benzyl accetate, linalol, eugenol and *a*-hexylcinnamaldehyde), clarifying (a) the influence of fragrance structure on the phase regions in a water/nonionic surfactant systems, (b) the distribution coefficient between micelles and the bulk phase, and (c) the partition between dissolved and solubilized perfume components on their volatility. In this regard, the phase equilibria in water, lecithin, soybean oil and vanillin have been studied. Soybean oil is saturated or unsaturated long-chain fatty acid triglycerides. Soybean oil, commonly labeled as vegetable oil, is inexpensive, safe and available used in microemulsion (Darmstadt *et al*., 2002; Polizelli *et al*., 2006). For hair care products, an amino-functional polyorganosiloxane (nonionic surfactant), an acid and/or a metal salt (solubilization of fragrance) and silicone oils can be achieved in microemulsions. Silicone oil, linear polydimethylsiloxane, is synthetic oil used in cosmetic formulations and industrial applications (Mehta and Somasundaran, 2008).

Prommetta *et al*.(1996) reported that microemulsion system of silicone oil using tween 20 as a surfactant and 1- pentanol as a cosurfactant. The result showed that 1-4% w/w of silicone oil could form microemulsions which had good physical stabity. The properties of silicone 350 oil are described in Tables 2. Warisnoicharoen *et al*. (2000) studied the effect of oil type on phase behavior of o/w microemulsions prepared using nonionic surfactants. The nonionic surfactants examined, namely, polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$), polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$ or Oleth-10), *N*,*N*-dimethyldodecylamine-*N*-oxide (DDAO) and *N*,*N*-dimethyloleylamine-*N*-oxide (DOAO), were used in their studies. Migylol 812, ethyl butyrate, ethyl caprylate, ethyl oleate, tributryin and soybean oil were used as oil phase in systems. All of oils and surfactants are currently used in a range of pharmaceutical and cosmetic formulations (Paul and Moulik, 2001). Due to their safety, soybean oil, silicone oil and polyoxyethylene-10-oleyl ether (nonionic surfactant) were mainly selected in this study.

In this work, acceptable cosmetic ingredients were employed to formulate microemulsions. Oleth-10 is a nonionic surfactant. The hydrophile-lipophile balance (HLB) value of oleth-10 is 12.5. Its other well-known names are $C_{18:1}E_{10}$, Brij 96, Brij 97, Sympatens-AO/100 and polyoxyethylene-10-oleyl ether. It has been widely used in topical pharmaceutical formulations and cosmetics primarily as an emulsifying agent for w/o and o/w emulsions. It has also been employed in several microemulsion formulations (Malcolmson and Lawrence, 1995; Boonme *et al*., 2004; Boonme *et al*., 2006; Junyaprasert *et al*., 2007). Isopropyl alcohol (IPA), propylene glycol (PG) and sorbitan monooleate (Span 80) were used as cosurfactants. IPA is odorless liquid. It is considerably less toxic than ethylene glycol. Furthermore, it has been used as a food additive. IPA can dissolve a wide range of nonpolar compounds. It is approved by the U.S. FDA and chemindustry lab for food additive uses, solvents, pharmaceuticals, cosmetics and personal care products (Manzall and Valkenburg, 1982). PG is a colorless, viscous. The oral toxicity of PG is low. It is most frequently applied in dermal products (Heuschkel *et al*., 2008). Span 80, a nonionic surfactant with HLB values of 4.3, is an approved pharmaceutical excipient. It has been used in oral preparations since it is safe and potentially low toxic. In addition, it is wildly used in topical products (Wu *et al*., 2001; Kato *et al*., 2008). All

studied surfactant and cosurfactants are acceptable for cosmetic purposes. The properties of all amphiphiles are described in Tables 3 and 4, respectively.

Table 2. The properties of silicone oil used in the study (Somasundaran *et al*., 2006; Mehta and Somasundaran, 2008; Patravale and Mandawgade, 2008; http://www.newdruginfo.com/pharmacopeia/usp28/v28230/usp28nf23s0_m26510)

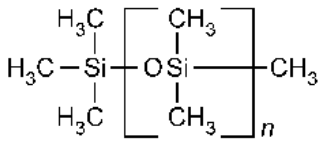
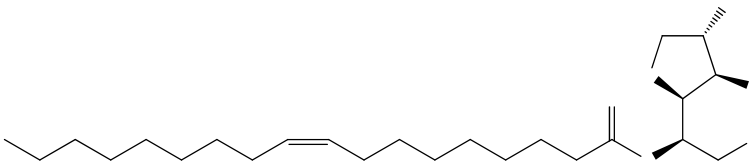
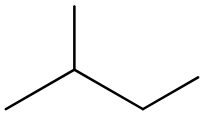
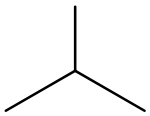
	Silicone oil
Chemical Structure	
Chemical Name	Polydimethylsiloxane, Dimethicone
Viscosity	350 centistokes
Appearance	Colorless viscous liquid
Physical properties	Water repellency, heat stability, and high resistance to chemical and UV attack
Use	Personal care, cosmetics, drug delivery, textile softening and printing ink formulations

Table 3. The properties of the surfactant used in the study (Sigma Material Safety Data, n.d.)

	Oleth-10
Chemical Structure	$C_{18}H_{35}$-O-$(CH_2CH_2)_{10}H$
Chemical Name	Polyoxyethylene-10-oleyl ether
Formula	$C_{18:1}E_{10}$
Molecular Weight	estimated as 710
Appearance	Pale yellowish liquid
Use	Nonionic surfactant

Table 4. The properties of cosurfactants used in the study (Manzall and Valkenburg, 1982; Wu *et al*., 2001; Heuschkel *et al*., 2008; Kato *et al*., 2008)

	Span 80
Molecular Structure	
Chemical Name	Sorbitan monooleate
Formula	$C_{24}H_{44}O_6$
Molecular Weight	estimated as 428.6
Appearance	Oily liquid
Use	Cosurfactant
	Propylene Glycol (PG)
Molecular Structure	
Chemical Name	1,2-propanediol
Formula	$C_3H_8O_2$
Molecular Weight	estimated as 76.1
Appearance	Colorless viscous liquid
Use	Cosurfactant
	Isopropyl alcohol (IPA)
Molecular Structure	
Chemical Name	1,2-propanediol
Formula	C_3H_7OH
Molecular Weight	estimated as 60.1
Appearance	Colorless liquid
Use	Cosurfactant

OH

4. Gel Formulation

Generally, microemulsion system exhibits low viscosity which is unsuitable for topical use in some cases, thus adding the thickening agents in the system is recommended (Boonme, 2007; Souto and Boonme, 2009). Advantages of the gel formulation are attractiveness, transparent, shiny, and non-sticky appearance (Kochansky and Shimanuki, 1999).

In the current investigation, the gel base was selected to be incorporated with the nicotinamide microemulsion in order to increase the viscosity of the microemulsion system. Colloidal silica was used as the representative thickener of w/o thickening phase for microemulsions based on a previous report (Spiclin *et al*., 2003). It was also reported that colloidal silica at concentration above 3.00% (w/w), achieved a suitable thickening agent for w/o microemulsions (Spiclin *et al*., 2003). In 2009, Romanko *et al*. revealed that as a gelling agent, carbopol has several advantages such as high viscosity at low concentration, compatibility with many active components, good bioadhesive properties, temperature stability, and excellent organoleptic characteristics and good tolerability by patients.

In this work, Carbopol® Ultrez 21 polymer, acrylates / C10-30 alkyl acrylate crosspolymer, was selected as the representative thickener of o/w thickening phase for microemulsions because it provides excellent clarity, a non-grainy glossy appearance to gels and good stability for a long period of time. Carbopol® Ultrez 21 polymer is a hydrophobically modified crosslinked polyacrylate polymer and is designed to efficiently impart thickening, stabilizing, and suspending properties to a variety of personal care applications in the cosmetic manufacture (Amnuaikit *et al*., 2008; http://www.lubrizol.com/PersonalCare/Products/Carbopol/CarbopolUltrez21.html). It is generally recognized that PEG-40 hydrogenated castor oil has been used in a wide variety of cosmetics and personal care products. Its functions have extended as an emulsifier, pigment dispersant, solvent, solubilizer, and wetting agent. In addition, it is used to enhance the gelling properties of other surfactants in w/o emulsions. The safety data of PEG-40 hydrogenated castor oil has been assessed by the Cosmetic Ingredient Review (CIR) Expert Panel and permitted by The Food and Drug Administration (FDA).

CHAPTER 3

MATERIALS AND METHODS

MATERIALS

1. Acetonitrile (Lab Scan, Thailand)
2. Brilliant blue (Winner's, Thailand)
3. Carbopol® Ultrez 21 (Namsiang International Co., Ltd., BKK, Thailand)
4. Cellulose acetate membrane (Spectra/ Por®3 Dialysis Membrane, MWCO 3,500 Dalton, USA)
5. di-Sodium hydrogen orthophosphate (Ajax Finechem, Australia)
6. Isopropyl alcohol (AnalaR®, VRW International Ltd, England)
7. Methanol, analytical grade (RCI Labscan, Thailand)
8. Nicotinamide (Fluka, Switzerland)
9. PEG-40 hydrogenated castor oil (BASF, Ludwigshafen, USA)
10. Polyoxyethylene-10-oleyl ether (Kolb Distribution Ltd., Switzerland)
11. Potassium dihydrogen phosphate (Ajax Finechem, Australia)
12. Propylene glycol (S.Tong Chemicals Co., Ltd, Thailand)
13. Silica, fumed, colloidal silica (Sigma-Aldrich, USA)
14. Silicone 350 oil (P.C. Drug Center Co., Ltd, Thailand)
15. Soybean oil (Thai Vegetable Oil Public Company, Thailand)
16. Sodium chloride (S.Tong Chemicals Co., Ltd, Thailand)
17. Span 80 (S.Tong Chemicals Co., Ltd, Thailand)
18. Sterile water for injection B.P. (Thai Otsuka Pharmaceutical Co., Ltd, Thailand)
19. Triethanolamine (P.C. Drug Center Co., Ltd, Thailand)
20. Triethylamine (Unilab, Australia)

EQUIPMENTS

The equipments used in this study were listed in Table 5.

Table 5. Instrumental equipment used in this study

Instrument	**Model**	**Company**
Conductivity meter	CM-115	Kyoto Electronic, Japan
Electrical balance*	AB54-S	Mettler toledo, Switzerland
Electrical balance**	AB135-S	Mettler toledo, Switzerland
Filtration membranes	Membrane Disc Nylon 47mm	S P Filters, China
High performance liquid chromatography (HPLC) systen	Agilent 1100 series - Pump - UV detector - Degasser - Autosampler	Palo Alto, USA
Hot air oven	DIN 12880-KI	Memmert, Germany
Hot plate	Single plate ring-cooker-18715	E.G.O, Germany
Magnetic stirrer	MR 3000D	Heidolph, Germany
Modified Franz diffusion cells	57-6 M	Hanson®, USA
Photon correlation spectroscopy (PCS)	Series 7032 Multi-8-Correlator	Malvern, USA
pH meter	S20-K	Mettler toledo, Switzerland

Table 5. Instrumental equipment used in this study (continued)

Instrument	**Model**	**Company**
Syringe filter	NYLON Syringe	Vertical Chromatography Co., Ltd., Thailand
Viscometer	DV-III ultra	Brookfield Engineering Laboratories Inc, U.S.A
Vortex mixer	VORTEX-2 GENIE	Scientific, USA

* For construction of phase diagram

** For chemical analysis process

METHODS

1. Construction of Pseudoternary Phase Diagram

Construction of pseudoternary phase diagram is process as the key for seeking for suitable system which provide the largest microemulsion region. In this study, acceptable cosmetic ingredients composed of oleth-10 (nonionic surfactant), water, various oils and cosurfactants were used to form microemulsions. The comparisons of different oils, i.e., silicone oil and soybean oil as well as different cosurfactants, i.e., isopropyl alcohol (IPA), propylene glycol (PG) and sorbitan monooleate (Span 80) were considered. The pseudoternary phase diagram of each system was constructed by preparing a large number of individual tubes.

1.1 Construction of Pseudoternary Phase Diagram by Preparing a Large Number of Individual Tubes

In each system, oleth-10 or the mixtures of oleth-10 and cosurfactant at fixed weight ratios were mixed with oil at the weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 as displayed in Table 6. Calculated amount of purified water was added at 10% increment to obtain water concentrations in the range of 0-90% by weight. The mixtures were heated at 80°C for 5 minutes in water bath for viscosity reduction and returning to room temperature and then vigorously mixing with a vortex mixer (Boonme *et al*., 2006). The samples were stored at room temperature for at least 24 hours in order to reach equilibrium before further investigation. The obtained samples were then classified as microemulsions when they appeared as clear isotropic liquids, as emulsions when they appeared as milky or turbid liquids, as gels when they did not show a change in the meniscus after tilting to an angle of 90° and as unstable dispersions when they separated to two clear-liquid layers (Malcolmson and Lawrence, 1995; Boonme *et al*., 2004). The microemulsion region of each system was constructed on a triangular graph as pseudoternary phase diagram using SigmaPlot software. A cut-and-weigh method was used to determine the percentage of the total area of the phase diagram covered by the microemulsions (Junyaprasert *et al*., 2007).

Table 6. The weight ratios of surfactant: cosurfactant and various oil

Oil	**Weight ratios of surfactant mixture**			
	Surfactant*	**Cosurfactant***		
	Oleth-10	PG	IPA	Span 80
Silicone oil	1	1		
	2	1		
	3	1		
Soybean oil	1	0		
	1	1		
	2	1		
	5	1		
	8	1		
Soybean oil	1		0	
	1		1	
	5		1	
	7		1	
	8		1	
	9		1	
Soybean oil	1			0
	1			1
	5			1

* Only the weight ratios of surfactant:cosurfactant were shown in the results of this study (data not shown in other ratios).

2. Preparation and Characterization of Nicotinamide-Loaded Microemulsions

2.1 Preparation of Nicotinamide Microemulsion Formulations

After all of the pseudoternary phase diagrams were constructed, the system of soybean oil/water/9:1 oleth-10:IPA was selected for further studies since it provided the largest microemulsion region. Two formulations of this system were prepared. The first formulation composed of 10% w/w water, 18% w/w oil phase and 72% w/w surfactant mixture. It was a representative in middle concentration of surfactant mixture and designated as ME-1. The second formulation composed of 10% w/w water, 25% w/w oil phase and 65% w/w surfactant mixture. It was a representative of low concentration of surfactant mixture and designated as ME-2. Nicotinamide at concentration of 3%, the same concentration in the commercial cream, was incorporated in the formulations by substituting part of 10% w/w water phase. The 3% nicotinamide loaded microemulsion formulations were stored at room temperature at least 24 hours to achieve equilibrium before further investigation. The compositions of microemulsion formulations are described in Table 7.

Table 7. Compositions of nicotinamide loaded microemulsion formulations

Formulation	**Composition (%w/w)**				
	Oil phase	Surfactant mixture		Water phase	Active ingredient
	Soybean oil	Oleth-10	IPA	Sterile water	Nicotinamide
ME-1	18	64.8	7.2	7	3
ME-2	25	58.5	6.5	7	3

2.2 Investigation of Physicochemical Properties

2.2.1 Characterization of Microemulsion Types

Dilution test with brilliant blue aqueous solution was used to characterize microemulsion type. The o/w microemulsions were expected to be miscible with brilliant blue aqueous solution. In contrast, the w/o microemulsions

were expected to be immiscible with brilliant blue aqueous solution. The measurements were performed in triplicate.

To confirm the dilution test, conductivity measurement was carried out. The samples was measured using a CM-115 Kyoto Electronic conductivity meter (Kyoto, Japan) with a cell constant of 0.1074 cm^{-1} at 25°C. The measurements were performed in triplicate.

2.2.2 Viscosity and Flow Measurement

Viscosity and flow property of the samples were measured using a Brookfield DV-III Ultra Rheometer (Brookfield Engineering Laboratories Inc, U.S.A.). Brookfield Rheocalc operating software (version3.1-1) controlled the rheometer. The sample was measured using a LV spindle No.SC4-31 at five different speeds. In addition, a commercial cream was measured using a LV spindle No.3 at five different speeds. The determinations were performed at 32°C, equal to the temperature of human skin. The measurements were performed in triplicate.

2.2.3 pH Measurement

The pH of the formulations was determined by digital pH meter (Mettler toledo, Switzerland). The measurements were performed at 25°C in triplicate.

2.2.4 Particle Size Analysis

Analysis of the particle size was performed by photon correlation spectroscopy (PCS) (Malvern Instruments, USA). PCS yields the mean particle size (z-ave) and the polydispersity index (PI) which is a measure of the width of the size distribution. The z-ave and PI values were obtained by averaging of 10 measurements at an angle of 90° in 10 mm diameter cells at 25°C.

2.2.5 Stability Study

The stabilities of the prepared formulations were investigated by storing the formulations at three temperatures (4°C, room temperature (~30 ± 2°C) and 60°C) for two months (Spiclin *et al*., 2003). Physical changes of formulations

such as phase separation and clarity were observed. Afterwards, only physical stable formulations were further investigated for the chemical stability study. The chemical changes of formulation were evaluated by determining the amount of non-degraded active ingredient using HPLC method. The 100 µl of each formulation was dissolved with IPA and adjusted the volume in a 10-ml volumetric flask. The 100 µl of this solution was diluted with 80:20 IPA:methanol in a 10-ml volumetric flask. Afterwards, the obtained solution was further diluted with the same solvent in a 10-ml volumetric flask and then filtered with syringe filter before analysis.

2.2.6 Quantitative Determination of Nicotinamide

The high-pressure liquid chromatographic technique was modified from *Stability-indicating HPLC methods for drug analysis* used for analysis of nicotinamide (Xu and Trissel, 2003).

Chromatographic Conditions and Instrumental Setting:

Analytical column	: reversed phase Chrompack® Intersil ODS column (5 µm particle size, 150 × 4.6 nm, Guard-Pak C_{18})
Mobile phase	: 0.1% triethylamine in 0.067 M monobasic potassium phosphate buffer (pH 6.7) and acetonitrile (100:4, v/v)
Detector wavelength	: 260 nm
Flow rate	: 1.0 ml/minute
Injection volume	: 20 µl
HPLC system	: Agilent 1100 series Pumping systems, Algilent 1100 series UV-Visible detector, Agilent 1100 series Autosampler

The nicotinamide concentrations of the samples were quantified from the standard curve by plotting the peak area of nicotinamide against the nicotinamide concentration.

2.2.6.1 Preparation of Standard Solution

Five hundred milligrams of nicotinamide were accurately weighed into a 100-ml volumetric flask and dissolved with isotonic phosphate buffer pH 7.4 to volume. A series of working standard nicotinamide solution was prepared by pipetting 1.0 ml of standard nicotinamide stock solution to a 10-ml volumetric flask and dissolving with isotonic phosphate buffer pH 7.4 to volume. The concentration of this solution was about 500 µg/ml. This solution was further diluted with the same solvent to desired concentrations in rang of 2.5, 5, 10, 20 and 40 µg/ml.

2.2.6.2 Validation of HPLC Method

Specificity

Under the chromatographic conditions selected, the peak of nicotinamide (active ingredient) must separate from the peak of other ingredients in the formulations and receptor fluid incubated with cellulose membrane. Chromatogram of the standard solution of nicotinamide was compared with chromatogram of the vehicle.

Linearity

Nicotinamide standard solutions in the concentration range of 2.5, 5, 10, 20 and 40 µg/ml were prepared and analyzed for three consecutive days (three sets). Each of standard solution was injected three times. Linear regression analysis of the means peak area versus their concentrations was performed. The linear regression coefficient (r^2) $\geq$ 0.99 is the acceptable criteria.

Accuracy

In each of the three chosen days, three sets of standard solutions in the concentrations 5, 10 and 20 µg/ml were prepared and analyzed three times by HPLC. Percent analytical recovery of each sample was calculated by comparing amounts of drug found and amount of drug added. The percent recovery should be 98-102% (Ermer and Miller, 2005).

Precision

The intra-day and inter-day precision was evaluated.

The intra-day precision

Three sets of three standard solutions (5, 10 and 20 μg/ml) of nicotinamide were analyzed by HPLC within one day. Each concentration was determined in 5 replicates. The standard deviation and percent relative standard deviation of peak area were calculated. The percentage of relative standard deviation (%RSD) should not be more than 2.0%.

The inter-day precision

Three sets of five standard solution of nicotinamide were analyzed by HPLC on different days for three consecutive days. Each concentration was determined in 5 replicates. The data used to calculate standard deviation and percent relative standard deviation of peak area. The percentage of relative standard deviation (%RSD) should not be more than 2.0%.

Limit of Detection (LOD) and Quantification (LOQ)

Diluted nicotinamide standard solutions were analyzed, at decreasing concentrations, in the range of 1.00 to 0.10 μg/ml. To assess the limit of detection, the quantification limit was settled as the lower concentration which provided responses with adequate linearity and precision (RSD < 2.0%). The detection and quantification limits were also calculated by the standard deviation and the slope of the calibration curve and the values were compared with those obtained by the response of the diluted solutions (ICH, 1996). LOD and LOQ were calculated using the following equations:

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ = the standard deviation of the response

S = the slope of the calibration curve

3. Preparation of Microemulsion-Based Gels

Two gelling agents, colloidal silica and Carbopol® Ultrez 21 were selected as the thickening agents in the current study.

3.1 Preparation of Thickening Phases

In the preparations of thickening mixtures, colloidal silica and PEG-40 hydrogenated castor oil were used in their original forms while Carbopol® Ultrez 21 was used in the form of 0.5% gel. The 0.5% Carbopol® Ultrez 21 gel was prepared by dispersing Carbopol® Ultrez 21 at 0.5% w/w in water under magnetic stirring. The pH values were subsequently adjusted to 5.5-8 with TEA (Amnuaikit *et al*., 2008).

Three microemulsion-base gels designed as ME-G1, ME-G2 and ME-G3, were prepared. The compositions of microemulsion-base gels as listed in Table 8 were studied.

Table 8. Composition of microemulsion-based gels

Composition / **Formulation**	**Content (by weight)**		
	ME-G1	**ME-G2**	**ME-G3**
Nicotinamide microemulsion (w/o ME)	95	95	95
Thickening Phase			
- Colloidal silica	5	-	-
- 0.5% Carbopol® Ultrez 21	-	5	3
- PEG-40 hydrogenated castor oil	-	-	2

3.2 Investigation of Physicochemical Properties

The obtained microemulsion-based gels were investigated for their physicochemical properties such as viscosity and flow behavior as well as pH by the identical methods as previously mentioned in sections 2.2.2 and 2.2.3, respectively. In addition, their stability was studied according to the method described in section 2.2.5.

4. *In Vitro* Release Study

The release of nicotinamide from prepared formulations (microemulsions, and microemulsion based gels) was investigated using modified Franz diffusion cells (Hanson Model 57-6 M, Hanson Research Corporation, CA, USA) as seen in Figure 11 and a cellulose acetate membrane with an effective diffusion area of 1.77 cm^2. The experiment was also performed with a selected commercial cream. The membrane was cut into 3x3 cm^2 pieces and the obtained pieces were boiled in distilled water to remove the wax. The cleaned membrane pieces were soaked in distilled water, stored in a cool place and used within a week. The hydrated membrane piece was mounted between the donor and receptor compartment of the diffusion cell. The receptor compartment was filled with 11 ml of degassed isotonic phosphate buffer pH 7.4. The diffusion cells were connected to a circulating water bath thermostated at 37°C, giving the membrane surface temperature of 32°C. The diffusion cells were stirred continuously at the speed of 200 rpm using magnetic bar. Then, the membrane was equilibrated for 30 minute. Approximately 1 g of each formulation was placed onto the membrane in the donor compartment, which had a diameter of 1.5 cm (1.77 cm^2 diffusion area). Parafilm was used to wrap the donor compartment as a security of the membrane and prevention of water evaporation. At defined time intervals (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours), 0.5 ml of samples were taken from the receptor compartment and immediately replaced with an equal volume of fresh receptor medium. The withdrawn samples were analyzed for nicotinamide concentration by HPLC method as previously described in section 2.2.6. For each formulation, the experiment was performed in triplicate.

The cumulative amount of nicotinamide released through the membrane into the receptor fluid was calculated using the following equation:

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i$$

Where C_t is the drug concentration of the receptor fluid at each sampling time, C_i is the drug concentration of the i^{th} sample, and V_r and V_s are the volumes of the receptor fluid and the sampling volume, respectively. Nicotinamide release kinetics were analyzed by zero order, first order and Higuchi's model as shown in Table 9, which were applied considering the amounts of drug released from 30 minutes to 24 hours.

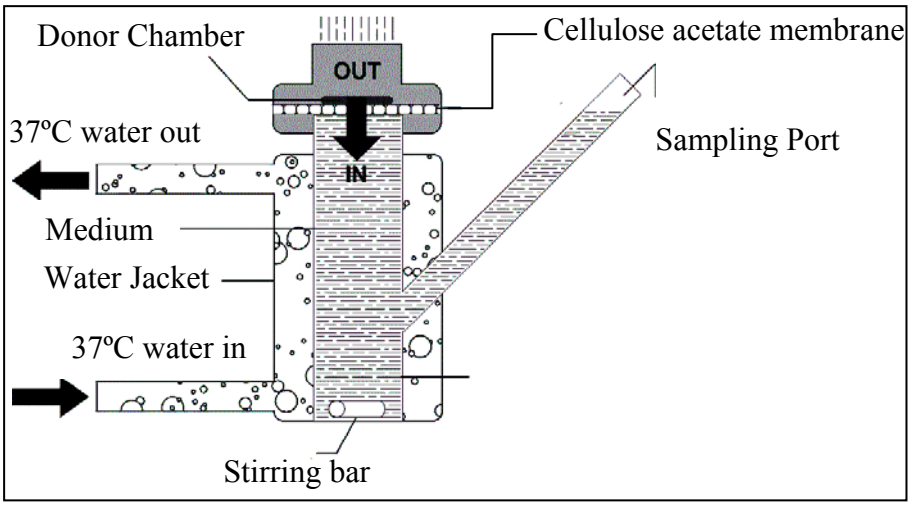


Figure 11. Modified franz diffusion cell (modified from http://www.ses-analysesysteme.de/SES_Franz_Cell_dt.htm)

Table 9. Kinetics of drug release

Model	Equation
Zero order	$Q_t = Q_0 - k_0 t$
First order	$\ln Q_t = \ln Q_0 - k_f t$
Higuchi	$Q_t = k_H t^{1/2}$

Where Q_t, is the cumulative amounts of drug release in time t; Q_0 is initial amounts of drug in the preformed preparations; k_0, k_f, k_H are release rate constants of zero order, first order and Higuchi, respectively (Merchant *et al*., 2006).

5. Data Analysis

Each experiment was replicated at least three times. The data were expressed as means ± standard deviation (SD). The *t*-test was used to compare pH, viscosity and the total cumulative release per unit area of nicotinamide microemulsions. A one-way ANOVA was used to compare the release rate of nicotinamide from difference formulations (microemulsions, microemulsion-based gel and the commercial cream product). A multiple comparison was used to compare different formulations. Paired t-test was used in stability study. A *p*-value of 0.05 was considered to be significant.

CHAPTER 4

RESULTS AND DISCUSSION

1. Pseudoternary Phase Diagram

The pseudoternary phase diagrams of systems consisted of oleth-10, water, oils (silicone oil and soybean oil) and cosurfactants (IPA, PG and Span 80) were constructed to find out the most suitable microemulsion system using preparing a large number of components in the individual tubes method. Considering the size of the microemulsion region on the pseudoternary phase diagrams as shown in Figures 12-15, it could be concluded that soybean oil was more suitable to be used as oil phase for microemulsion formation than silicone oil while IPA was more proper to be used as cosurfactant for microemulsion formation than PG and Span 80.

Figure 12 exhibits the regions of association structures in the pseudoternary phase diagrams of the systems of water and silicone oil at different weight ratios of oleth-10 and PG (1:1, 2:1 and 3:1). The association structures, e.g., emulsions, gels and unstable dispersions were observed but no microemulsions originated in these systems. Penetration of the oil molecule into the hydrocarbon portion of the surfactant interface would have the effect of increasing the hydrophobic portion volume of the interface (v) and subsequently causing an increase in the effective critical packing parameter (CPP) (Malcolmson and Lawrence, 1995). The chemical structure of silicone oil is a linear polydimethylsiloxane based on a backbone of repeating silicone and oxygen atoms, with methyl groups attached to the silica along its length, resulting in large molecular volume. Thus, the molecular volume of silicone oil might be too large to penetrate into the hydrocarbon portion of the surfactant interface; therefore no microemulsions occurred.

Figure 13 shows the regions of association structures in the pseudoternary phase diagrams of the systems of water and soybean oil at different weight ratios of oleth-10 and PG (1:0, 1:1, 2:1, 5:1 and 8:1). The results suggest that at the same oleth-10:PG ratios, soybean oil provided the microemulsion existence while silicone oil

could not. According to their structures, soybean oil has smaller molecular volume than silicone oil. Microemulsions could originate in the cosurfactant-free system (1:0 oleth-10:PG) but no microemulsions occurred at 1:1 oleth-10:PG since there was too low surfactant in the system and excess polyhydroxy compound might partition to and act as aqueous phase. The surfactant/cosurfactant ratios at weight ratios of 4:1, 6:1, 7:1 and 9:1 oleth-10:PG were investigated (data not shown), but the obtained microemulsion region was less than that of at 8:1 oleth-10:PG. The microemulsion region increased when the amount of oleth-10 further increased (from 2:1 to 8:1 oleth-10:PG); however, it could be noted that PG could not increase microemulsion region compared to the cosurfactant-free system.

Figure 14 demonstrates the regions of association structures in the pseudoternary phase diagrams of the systems of water and soybean oil at different weight ratios of oleth-10 and IPA (1:0, 1:1, 5:1, 7:1, 8:1 and 9:1). The results show that microemulsions were found in the cosurfactant-free system (1:0 oleth-10:IPA) but no microemulsions were seen in 1:1 oleth-10:IPA system since there was too low surfactant in the system and excess short chain alcohol might partition to and act as aqueous phase. The microemulsion region increased when the amount of oleth-10 further increased (from 5:1 to 9:1 oleth-10:IPA). The surfactant/cosurfactant ratios were varied as (2:1, 3:1, 4:1, 6:1 and 7:1 w/w) (data not shown), but the obtained microemulsion region was less than that of at 9:1 oleth-10:IPA. The largest microemulsion region was obtained at 9:1 oleth-10:IPA. These results can be explained that as the amount of surfactant in the system is increased, a greater interfacial area is possible and the oil is distributed among a greater number of micelles. Small amount of IPA could increase flexibility of interfacial film, leading to larger microemulsion regions. The weight percent of oxygen content (%O) of IPA (C_3H_7OH) is lower than that of PG ($C_3H_8O_2$). This descriptor of cosurfactants was reported to affect to the microemulsion formation (Alany *et al*., 2000; Boonme *et al*., 2004).

Figure 15 presents the regions of association structures in the pseudoternary phase diagrams of the systems of water and soybean oil at different weight ratios of oleth-10 and Span 80 (1:0, 1:1 and 5:1). The pseudoternary phase diagrams of the other systems were checked at the weight ratios of oleth-10 and Span 80 (2:1, 3:1, 4:1,

6:1, 7:1, 8:1 and 9:1) (data not shown), these microemulsion regions were less than the microemulsion region at 5:1 oleth-10:Span 80. The hydrophile-lipophile balance (HLB) values of oleth-10 and Span 80 are 12.4 and 4.3, respectively (Malcolmson and Lawrence, 1995; Kato *et al*., 2008). The results show that the suitable amount of Span 80 (5:1 oleth-10:Span 80) could enlarge area of microemulsion existence due to proper obtained HLB. However, Span 80 could not increase a capability of water solubility in the microemulsion system because of its lipophilicity.

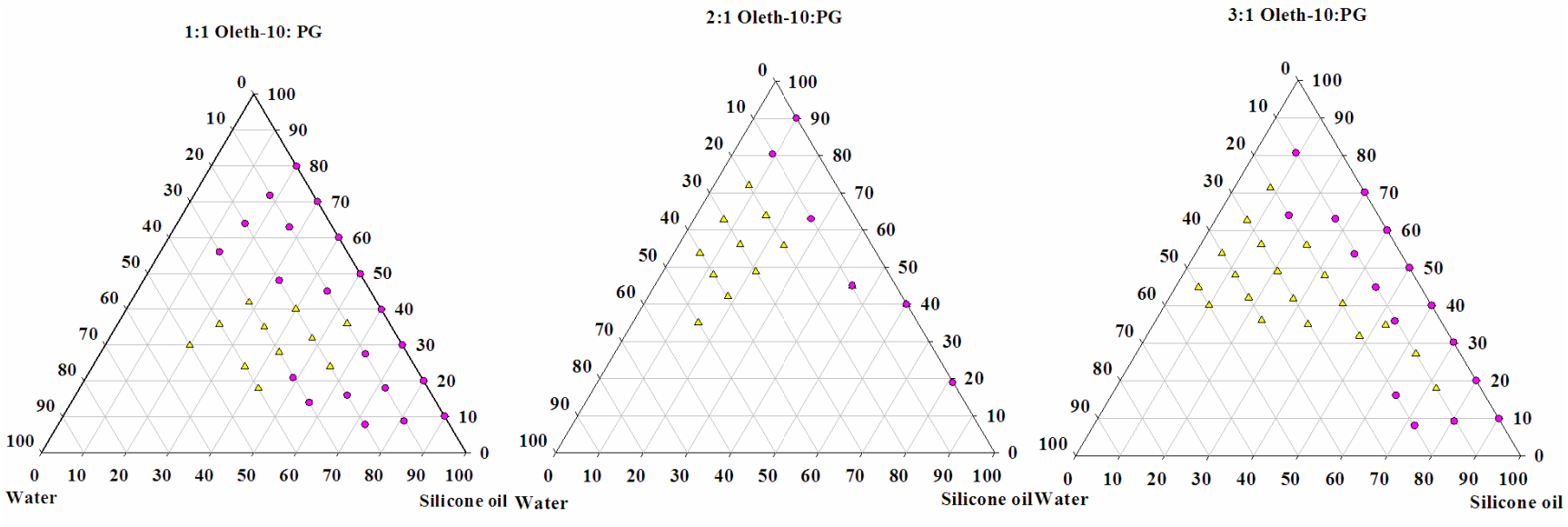


Figure 12. Pseudoternary phase diagrams of the systems of water and silicone oil at different ratios of oleth-10 and PG (1:1, 2:1 and 3:1); ●: emulsion, ▸: gel, white area: unstable dispersion.

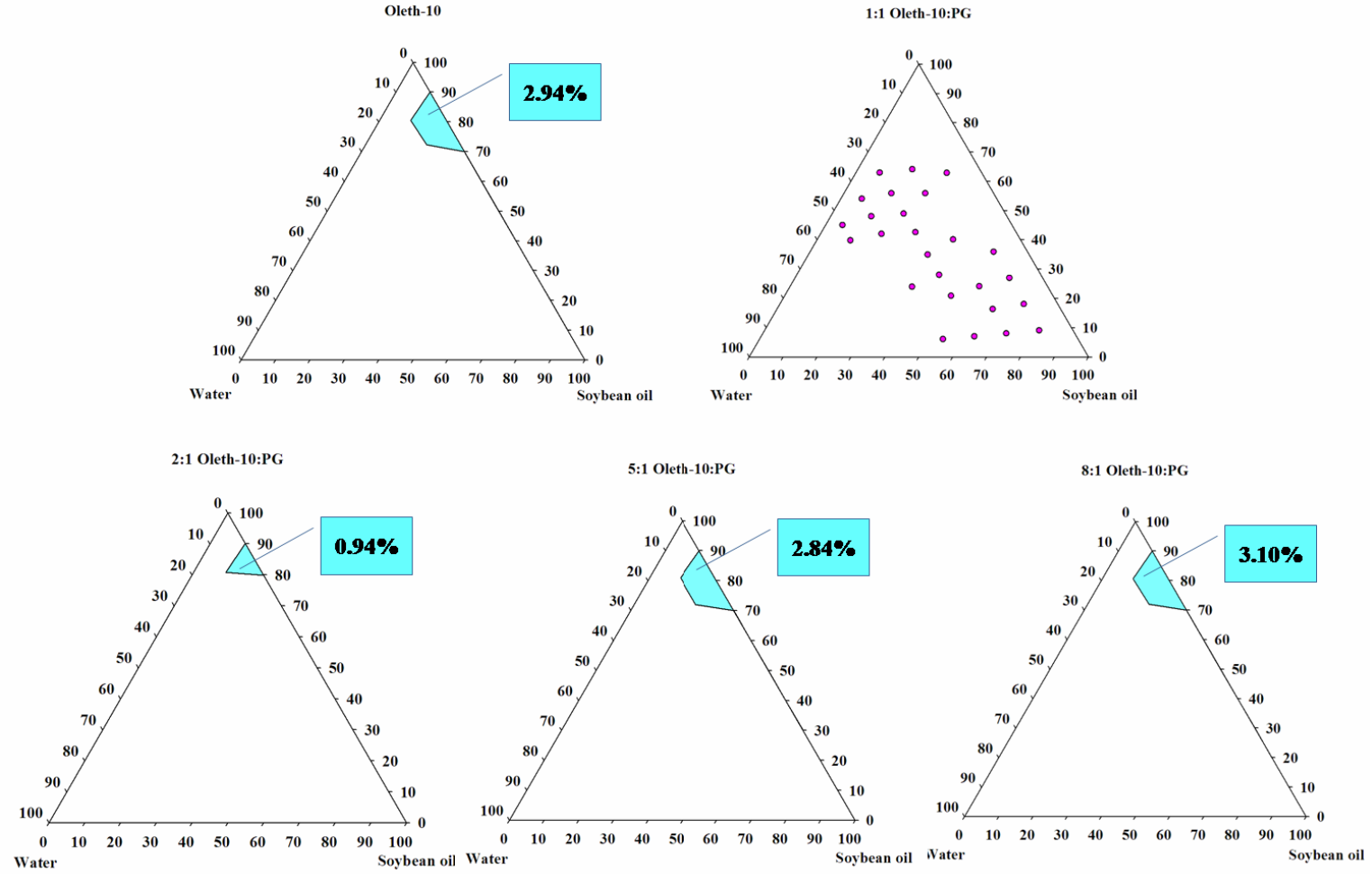


Figure 13. Pseudoternary phase diagrams of the systems of water and soybean oil at different ratios of oleth-10 and PG (1:0, 1:1, 2:1, 5:1 and 8:1); •: emulsion, white area: unstable dispersion, shaded area: microemulsion.

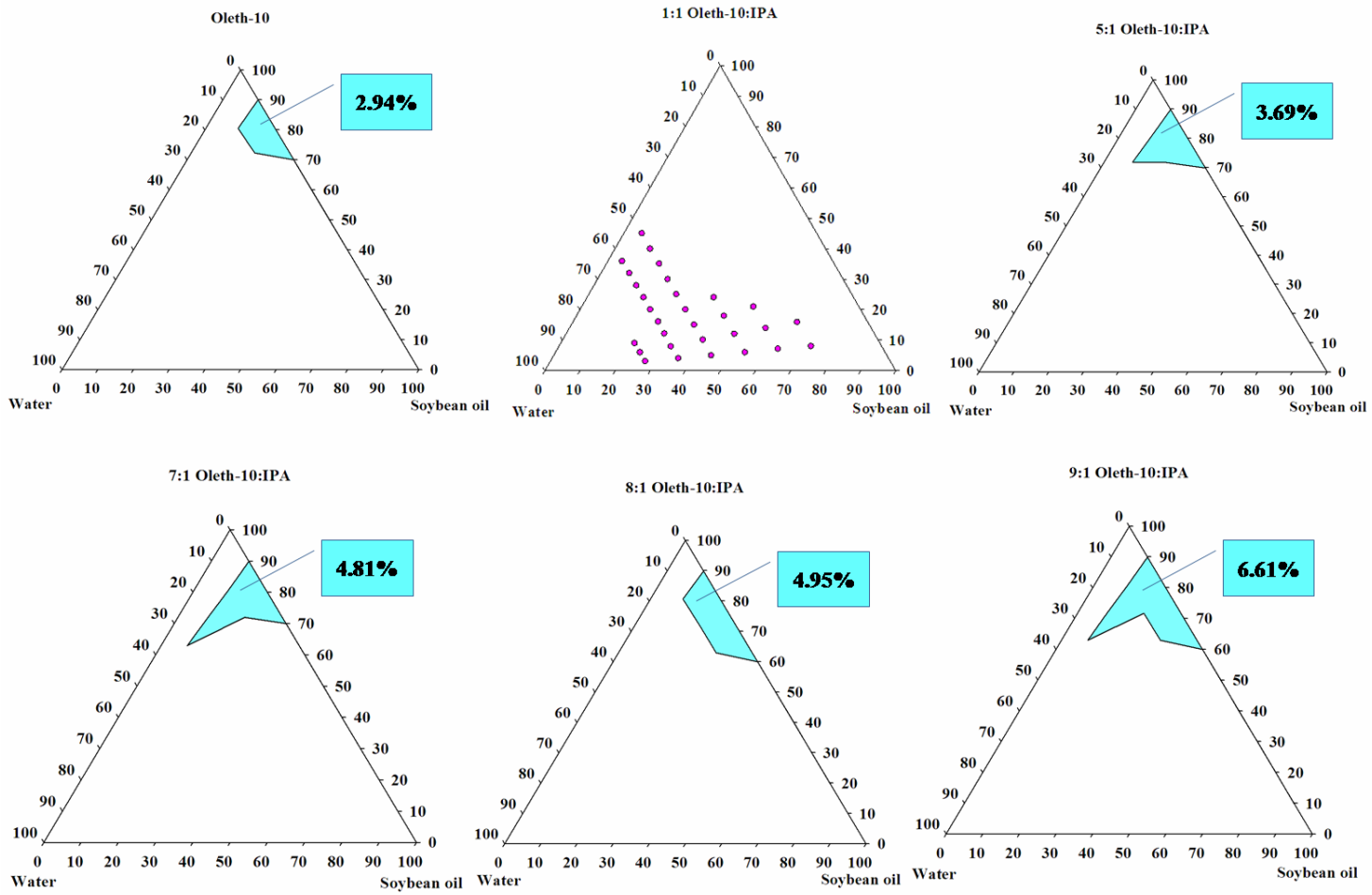


Figure 14. Pseudoternary phase diagrams of the systems of water and soybean oil at different ratios of oleth-10 and IPA (1:0, 1:1, 5:1, 7:1, 8:1 and 9:1); ●: emulsion, white area: unstable dispersion, shaded area: microemulsion.

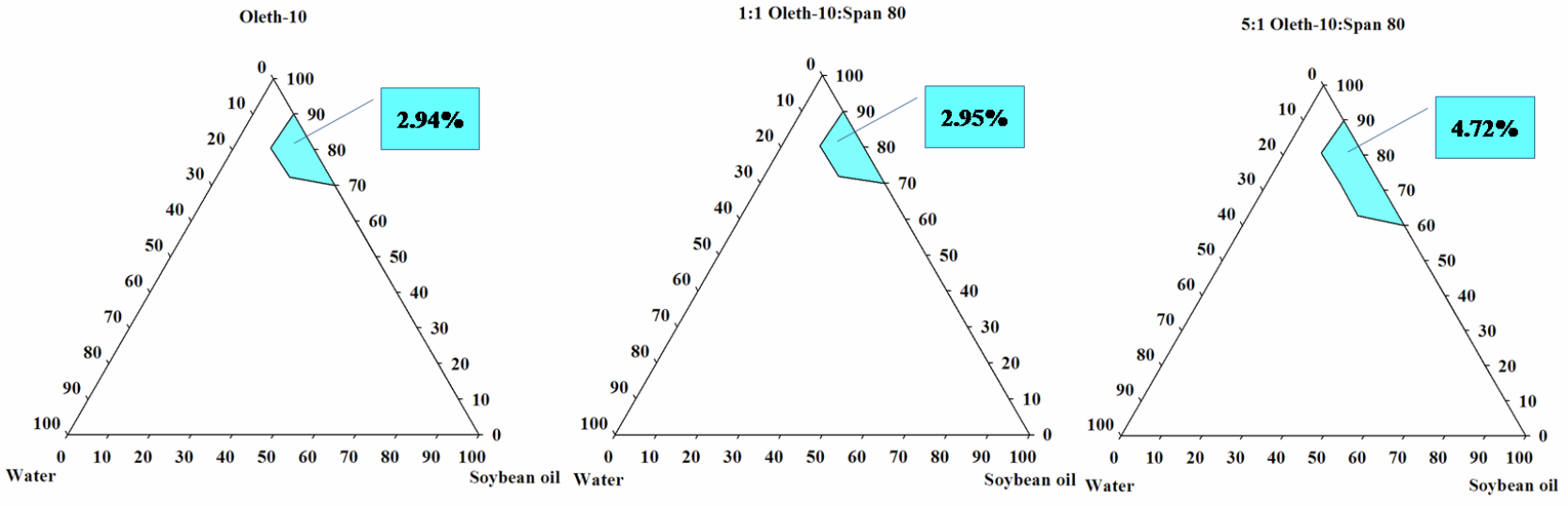


Figure 15. Pseudoternary phase diagrams of the systems of water and soybean oil at different ratios of oleth-10 and Span 80 (1:0, 1:1 and 5:1); white area: unstable dispersion, shaded area: microemulsion.

2. HPLC Validation of Nicotinamide

In the present study, nicotinamide was loaded as an active ingredient in the formulations, the amount of niacinamide in the stability and *in vitro* release studies was quantified by HPLC method (Xu and Trissel, 2003).

2.1 Results of HPLC Validation

Specificity

Under the studied chromatographic conditions, the peak of nicotinamide was found to be separated from the peaks of other ingredients in standard solution and formulation as well as interference peaks from synthetic membrane. The chromatograms of standard solution, formulation released through synthetic membrane into the receptor fluid and microemulsion-based gel are illustrated in Figures 16 and 17, respectively. The retention time of nicotinamide was identically at about 4.5 minutes in standard solution, receptor fluid and microemulsion-based gel.

Linearity

The calibration curve of niacinamide solutions between 2.5 and 40 μg/ml was in the linearity range of the acceptable criteria with linear regression coefficient (r^2) of 0.999 as displayed in Figure 18 and Table 10.

Accuracy

The accuracy of the average predicted concentration for each day was obtained between 99.75 and 101.74% which was in acceptable range (see Table 10). The percent recovery should be 98-102% (Ermer and Miller, 2005).

Precision

Table 10 shows good coefficients of relative standard deviation, which was less than 2%, both intra-day and inter-day. At each concentration, the intra-day relative standard deviation varies between 0.36 and 1.17%. The inter-day precision of the method varies relative standard deviation between 1.25 and 1.73%.

LOD and LOQ

The detection and quantification limits were also calculated by the standard deviation and the slope of the calibration curve and the values were compared with those obtained by the response of the diluted solutions. The obtained detection and quantification limits were 0.0489 ± 0.022 µg/ml and 0.069 ± 0.030 µg/ml, respectively (see Table 10).

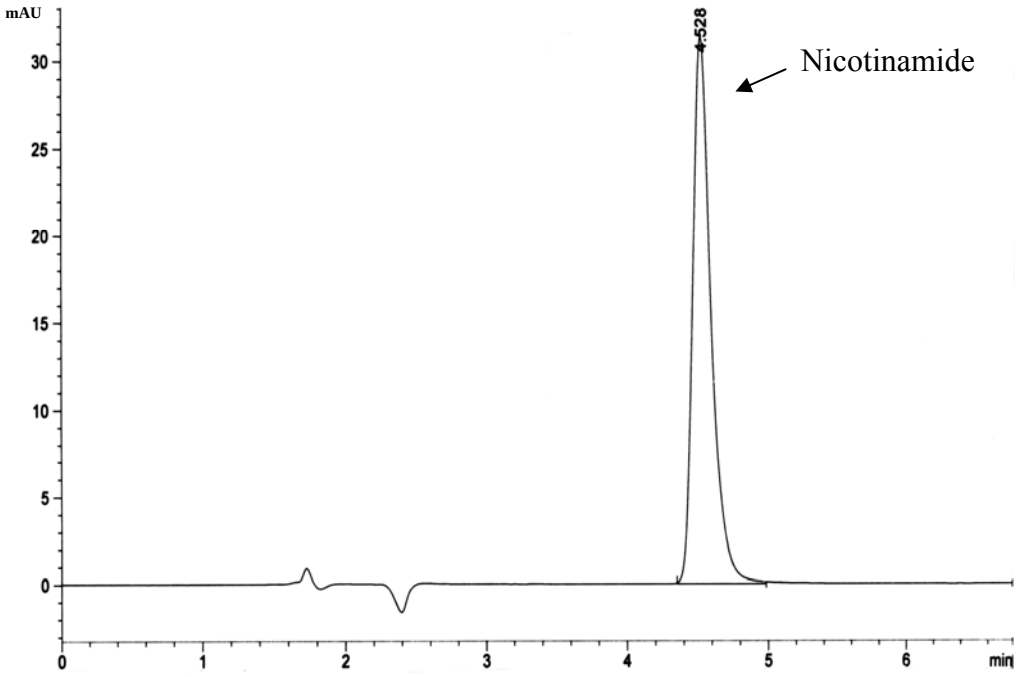
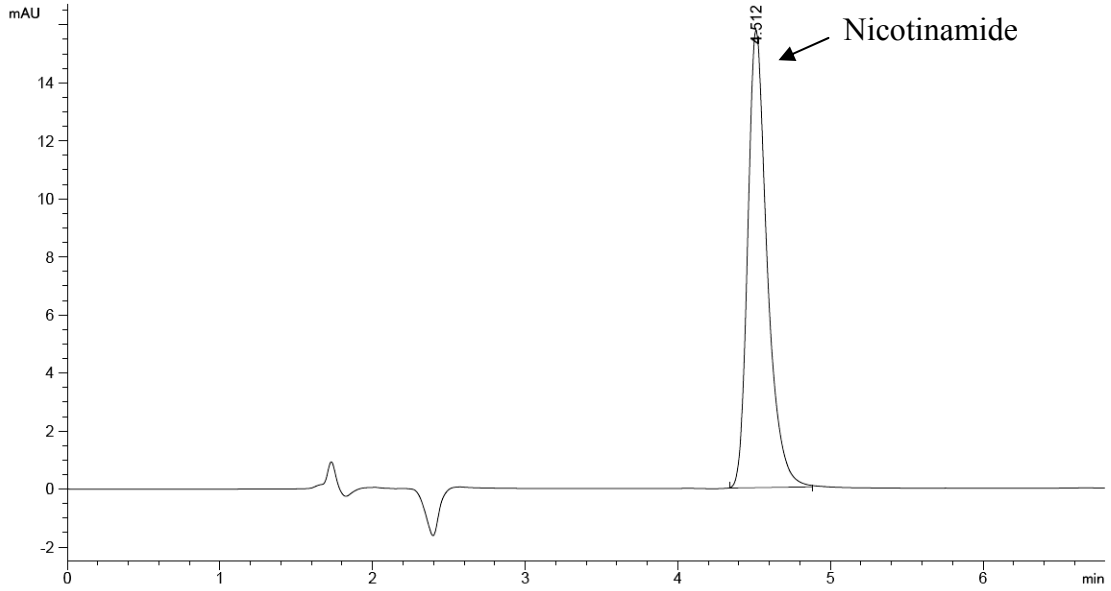
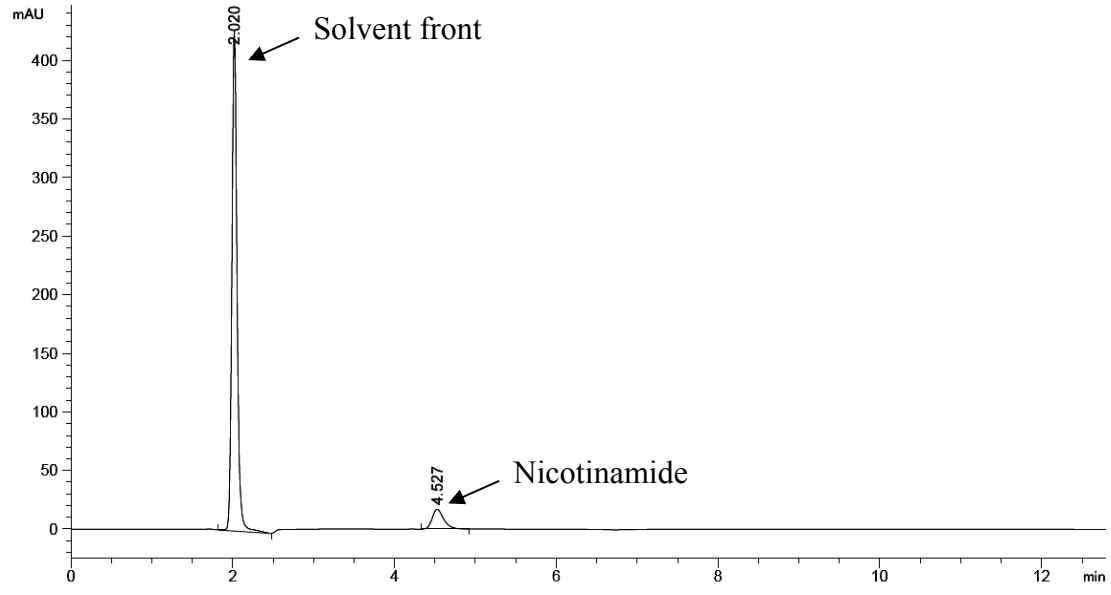


Figure 16. HPLC chromatogram of nicotinamide in standard solution (isotonic phosphate buffer pH 7.4).



(A)



(B)

Figure 17. HPLC chromatograms of nicotinamide released through synthetic membrane into the receptor fluid (A) and in the microemulsion-based gel (B).

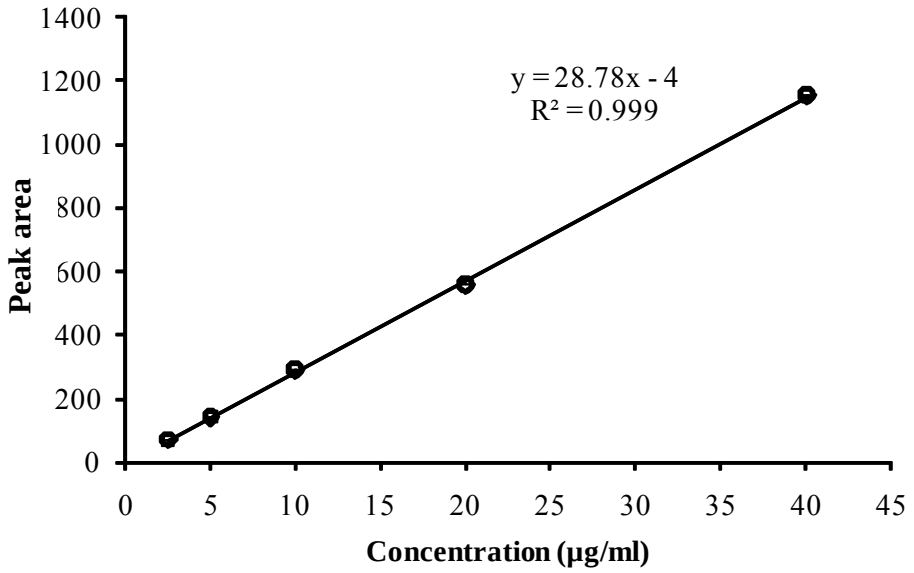


Figure 18. A standard calibration curve of nicotinamide. The plotted data are means ± SD (n = 3).

Table 10. Linearity, intra-day and inter-day, accuracy, LOD and LOQ of nicotinamide from HPLC assay

Drug	Nicotinamide
Linearity (r^2)	0.999
Intra-day (%RSD)	0.36 - 1.17
Inter-day (%RSD)	1.25 - 1.73
Accuracy (%recovery)	99.75 - 101.74
LOD (µg/ml)	0.049 ± 0.022
LOQ (µg/ml)	0.069 ± 0.030

3. Formulation and Characteristics of Nicotinamide-Loaded Microemulsions

3.1 Preparation of Nicotinamide Microemulsion Formulations

Among studied systems, the system of water/soybean oil/oleth-10:IPA (9:1) provided the largest microemulsion region; therefore, this system was used for further study. Two formulations at the concentration of water (10% w/w), oil phase 18, 25% w/w and surfactant mixture 72, 65% w/w were selected as the medium position (ME-1) and low surfactant position (ME-2) in the microemulsion region, respectively as shown in Figure 19. Nicotinamide at concentration of 3% w/w was incorporated in the selected formulations as previously described in Table 6. The obtained formulations were yellowish clear liquid with low viscosity.

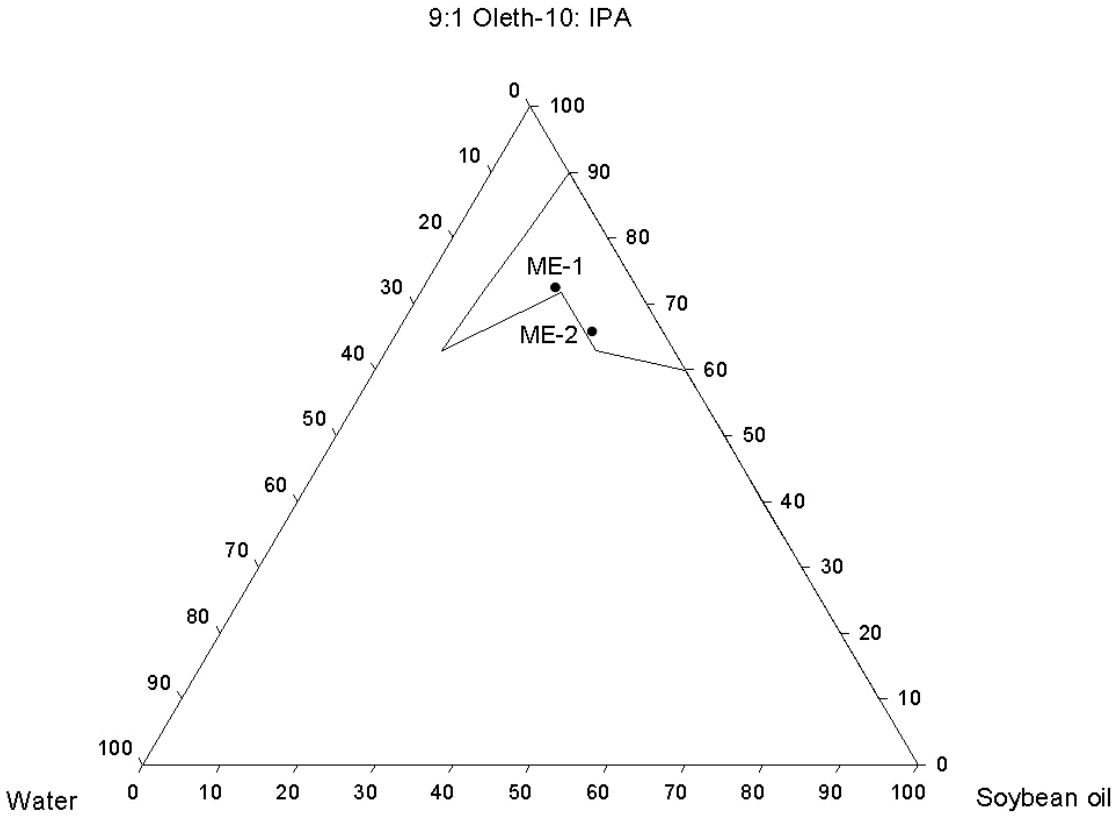
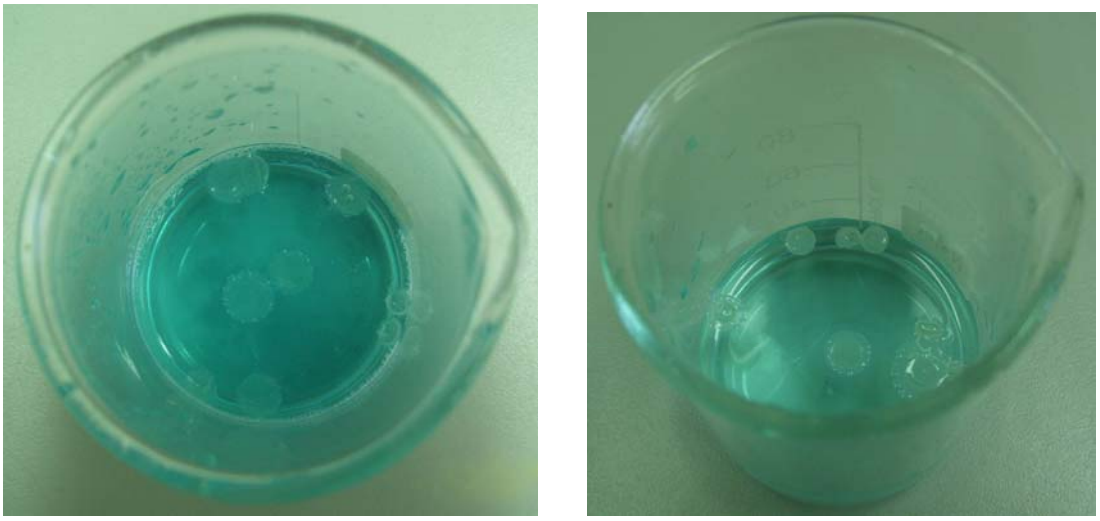


Figure 19 Microemulsion region in system of water/soybean oil/9:1 oleth-10:IPA and selected formulations in this study, i.e., ME-1 and ME-2.

3.2 Physicochemical Properties of Nicotinamide Microemulsion Formulations

3.2.1 Microemulsion Types

The type of microemulsion was assessed by dropping the formulations into the brilliant blue aqueous solution. The results showed that all formulations of nicotinamide-loaded microemulsion were immiscible with the solution as illustrated in Figure 20. It is clearly indicated that the type of two formulations was w/o microemulsions. In order to confirm the dilution test, the conductivity measurements of formulations were carried out. The results indicate that conductivity values of ME-1 (10.45 ± 0.18 μS/cm) and ME-2 (10.47 ± 0.25 μS/cm) were low. The obtained conductivity data were not significantly different because of the same concentration of water in both formulations.



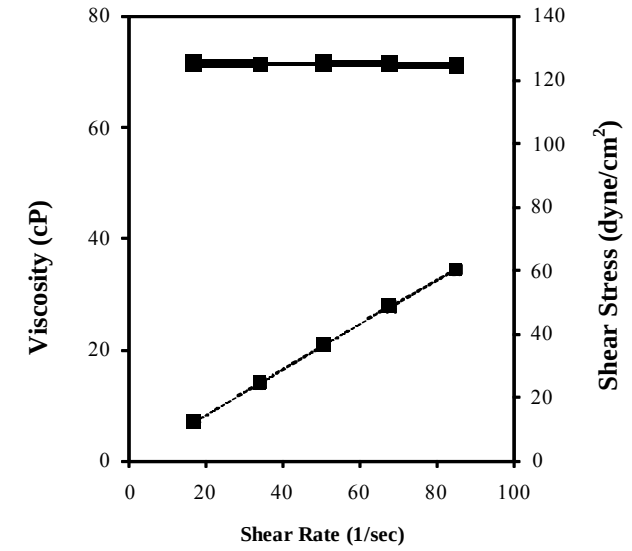
(a) (b)

Figure 20. Dilution test with brilliant blue aqueous solution of both (a) ME-1 and (b) ME-2.

3.2.2 Viscosity and Flow

Figure 21 demonstrates viscosity and flow property of ME-1 and ME-2 formulations using a LV spindle at five different speeds at 32°C. It was found that ME-1 and ME-2 exhibited Newtonian behavior since their viscosity values were not changed by increasing the shear rates.

(A)



(B)

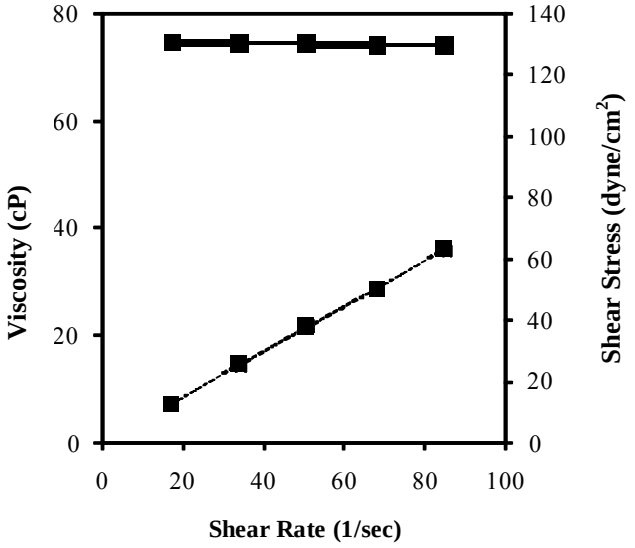


Figure 21. Rheograms of ME-1 (A) and ME-2 (B). ○ represents viscosity (cP) and □ represents shear stress (dyne/cm^2).

3.2.3 pH

. The results showed that the pH values of ME-1 and ME-2 formulations were 7.25 ± 0.005 and 7.21 ± 0.005, respectively. It is generally recognized that the formulations are acceptable for topical application when their pH values are in the range of 5-8 (Saraf *et al*., 2010).

3.2.4 Particle Size

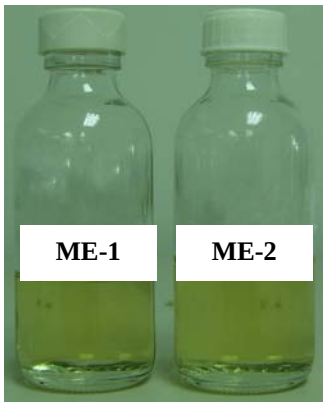
The mean droplet sizes of ME-1 and ME-2 were in the range of 64.5 nm and 79.7 nm, respectively. Both formulations showed different polydispersity index (PI), 0.902 for ME-1 and 0.766 for ME-2. According to the PI, ME-1 had wider size distribution than that of ME-2. It can be concluded that size distribution of ME-2 is optimal because of its narrower size distribution. (Yongyut *et al*., 2009).

3.2.5 Stability

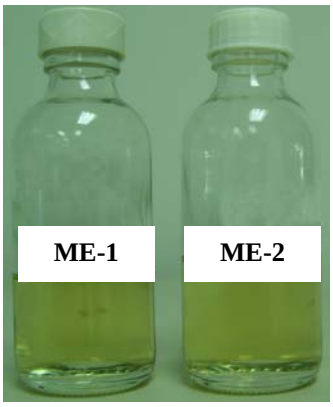
It was found that both formulations (ME-1 and ME-2) remained clear with no phase separation over the period of two months of storage at 4°C and room temperature(~30 ± 2°C). However, at high temperature of 60°C, the color of stored formulations appeared darker (Figure 22). The color change was possible due to the decomposition of hydrocarbons of oil. In order to confirm this assumption, oleth-10, soybean oil, and nicotinamide solution were stored at 60°C for 2 days. Indeed, only the color of soybean oil was darker. It can be noted that high temperature at 60°C affected the physical stabilities of both formulations. Thus, only formulations stored at 4°C and room temperature were selected to investigate for the chemical stability. The results showed that the content nicotinamide of ME-1 was found to be 100.95% at 4°C and 98.59% at room temperature. In addition, the content nicotinamide of ME-2 was found to be 99.92% at 4°C and 99.87% at room temperature as described in Table 10. It can be concluded that both formulations had good chemical stability.

(1)

Before



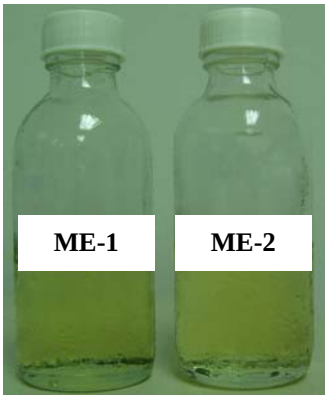
After



(2)

Before

After



(3)

Before

After

Figure 22. Physical appearance of nicotinamide microemulsion formulations: ME-1 (left), ME-2 (right) before and after two months of storage at 4°C (1), room temperature (2) and 60°C (3).

Table 11. Stability of nicotinamide microemulsions kept at three storage temperatures for 2 months

Storage temperature		Microemulsion	
		ME-1	**ME-2**
4°C	**Physical changes**	No changes	No changes
	Nicotinamide content*	100.95 ± 5.29%	99.92 ± 0.31%
Room temperature (~30 ± 2°C)	**Physical changes**	No changes	No changes
	Nicotinamide content*	98.59 ± 3.08%	99.87 ± 1.56%
60°C	**Physical changes**	brownish clear liquid	brownish clear liquid
	Nicotinamide content*	ND	ND

* Calculated based on 100% w/w of freshly prepared samples (n = 3).

ND = not determined

3.2.6 Release Behavior

The cumulative amount of nicotinamide-loaded microemulsion released through the cellulose acetate membrane into the receptor fluid was plotted with time or square root of time in order to characterize the release kinetics of nicotinamide from the microemulsions.

The plotting of the *in vitro* release data to the zero order, first order and Higuchi models are shown in Figures 23, 24 and 25, respectively. The release profiles of both ME-1 and ME-2 were not significantly different all of the three models due to the similar influence of concentration gradient.

The obtained correlation coefficients are summarized in Table 12. It was found that the release profiles of both ME-1 and ME-2 were fitted with Higuchi model rather than zero order and first order model (coefficient of determination > 0.96). The mechanism of release of a hydrophilic compound from w/o microemulsions is likely to be dependent on diffusion or partition process (Kapoor and Chauhan, 2008).The linear relationship between cumulative amount of drug released versus square root of time suggested that the transport of drug was controlled

by diffusion through microemulsions (Kapoor and Chauhan, 2008). This result suggests that the synthetic membrane does not provide any significant effects in the release of the drug from the formulations. The formulations themselves controlled the release of the active substance. Nicotinamide was solubilised in the internal droplets of the formulations which could diffuse into the aqueous receptor fluid.

To select the best formulation, a comparative study of both microemulsion formulations, such as physicochemical stabilities and *in vitro* release study was considered. The results revealed that the obtained data were not significantly different for both studied formulation, nevertheless, ME-2 formulation was chosen due to its lower surfactant concentration.

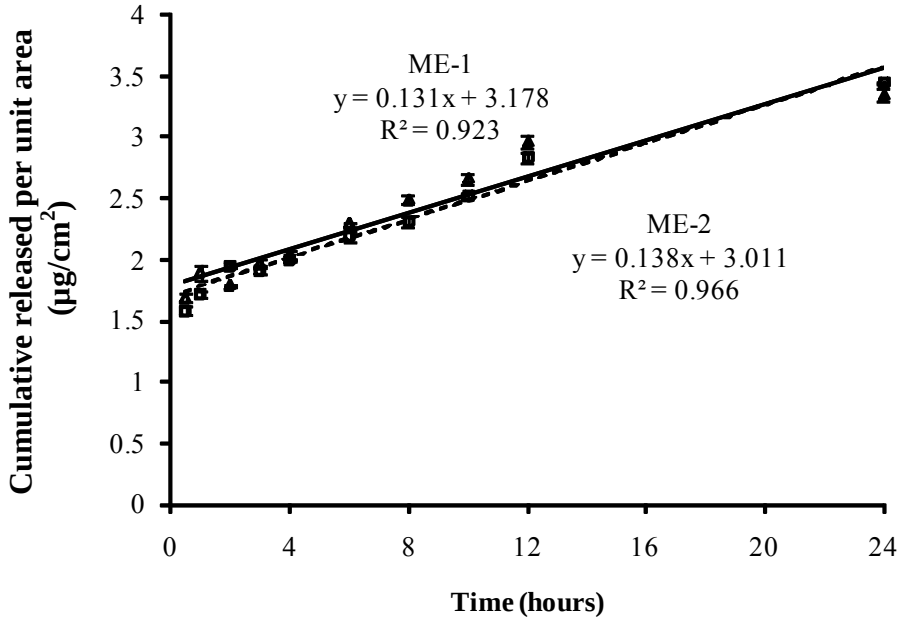


Figure 23. *In vitro* release profiles of nicotinamide from ME-1 (Δ) and ME-2 (□): cumulative amount of drug released versus time. Each point represents mean ± SD, n = 3.

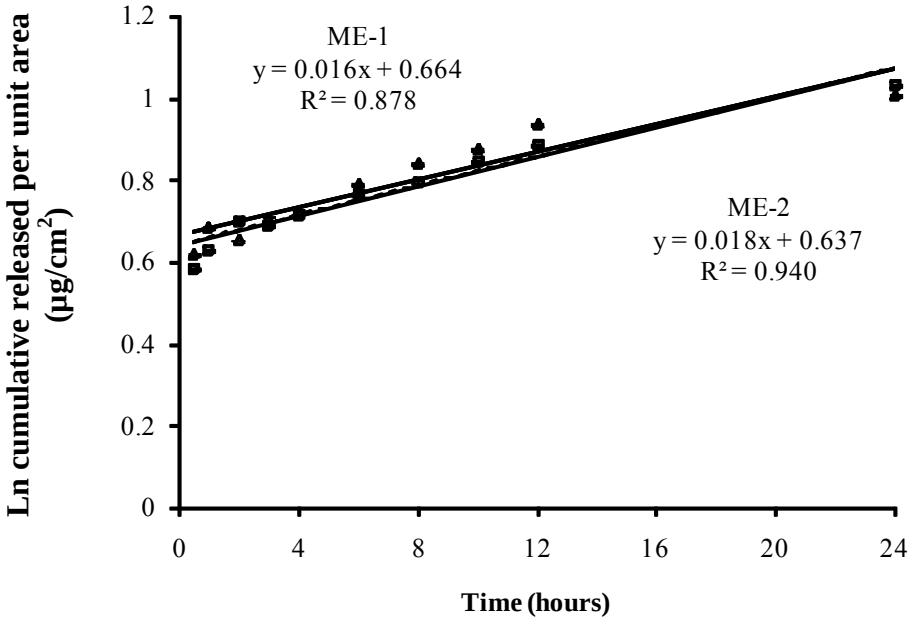


Figure 24. *In vitro* release profiles of nicotinamide from ME-1 (Δ) and ME-2 (□): ln cumulative amount of drug released versus time. Each point represents mean ± SD, n = 3.

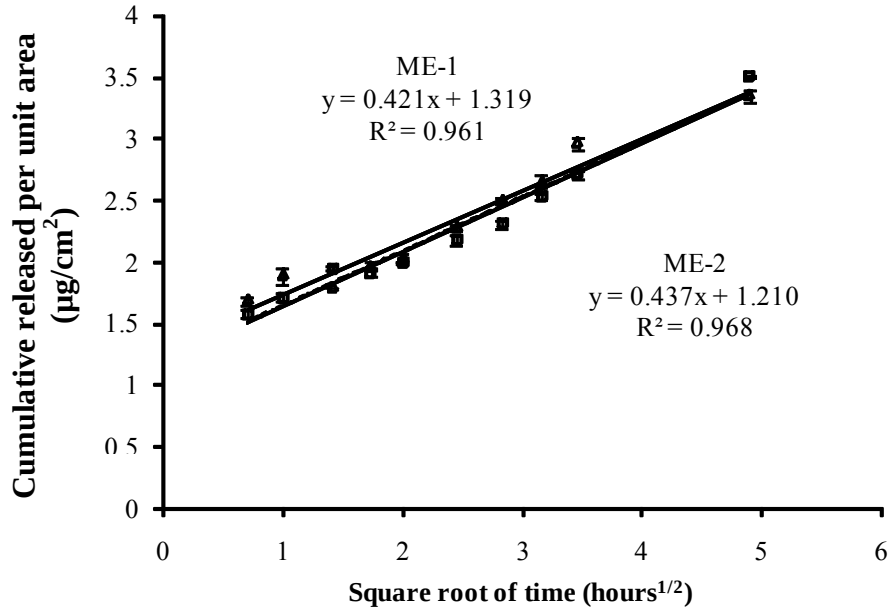


Figure 25. *In vitro* release profiles of nicotinamide from ME-1 (Δ) and ME-2 (□): cumulative amount of drug released versus square root of time. Each point represents mean ± SD, n = 3.

Table 12. Coefficient of determination of different mathematical models for 3% nicotinamide microemulsions

Formulations	Zero order model		First order model		Higuchi model	
	R^2	k_0 (µg/ml.h)	R^2	k_1 (h^{-1})	R^2	k (µg/ml.$h^{1/2}$)
ME-1	0.923	0.073	0.878	0.016	0.961	0.421
ME-2	0.966	0.078	0.940	0.018	0.968	0.437

k_0, k_1 and k are release rate constant of zero order, first order and Higuchi model, respectively.

4. Microemulsion Based Gels

Two gelling agents, colloidal silica and Carbopol® Ultrez 21 prepared for three thickening phases were added to ME-2 formulation. The optical appearance of microemulsion based gels is demonstrated in Figure 26. It was observed that the rank order of clarity of the prepared gels was ME-G1 > ME-G3 > ME-G2. It was found that using colloidal silica in ME-G1 provided a transparent gel. The result can be explained that during the formation of the product, H^+ ions of water attached to some of the small particles of colloidal silica, resulting in the hydrophilic surface and capability of hydrogen bonding. This structure could be formed into a three-dimensional network through the branched interaction of hydrogen bonding of surface hydroxyls' silica (Raghavan, and Khan, 1997; Kamibayashi *et al*., 2005). Unfortunately, ME-G2 was turbid in appearance. It could be explained that microemulsion was changed (see Figure 14, page 49) when excess water was added into the formulation. This excess water came from the addition of 0.5% Carbopol® Ultrez 21 gel in the formulation. Expectably, adding PEG-40 hydrogenated castor oil into ME-G3 had slightly achieved to stabilize formulation, resulting in the reduction of turbid appearance. However, with the addition of PEG-40 hydrogenated castor oil, the appearance of ME-G3 became hazy.

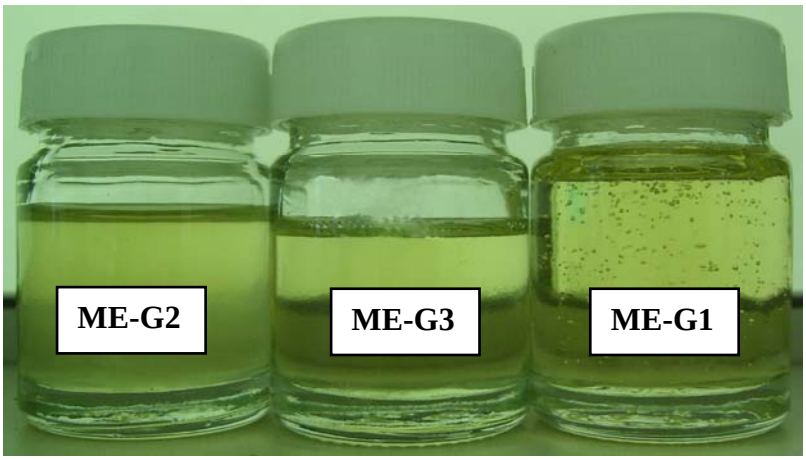


Figure 26. Optical appearance of three microemulsion based gels; ME-G2, ME-G3 and ME-G1.

4.1 Physicochemical Properties of Microemulsion-Based Gels

The details of physicochemical characteristics of the prepared gels compared to ME-2, the initial formulation, are summarized in Table 13.

Table 13. Physicochemical characteristics of microemulsion-based gels and their microemulsion counterpart (ME-2)

Formulation	Viscosity (cPa) at 20 RPM**	pH***	Appearance
ME-G1	1051 ± 1.830*	6.94 ± 0.02	yellowish clear gel
ME-G2	90.50 ± 0.069*	7.10 ± 0.01	yellowish turbid gel
ME-G3	120.89 ± 0.069*	6.96 ± 0.01	yellowish hazy gel
ME-2	74.10 ± 0.000*	7.21 ± 0.01	yellowish clear liquid

* test significant difference using a one-way ANOVA ($p < 0.05$)

** Reported as mean ± SD (three replications) at 32°C.

*** Reported as mean ± SD (three replications) at 25°C.

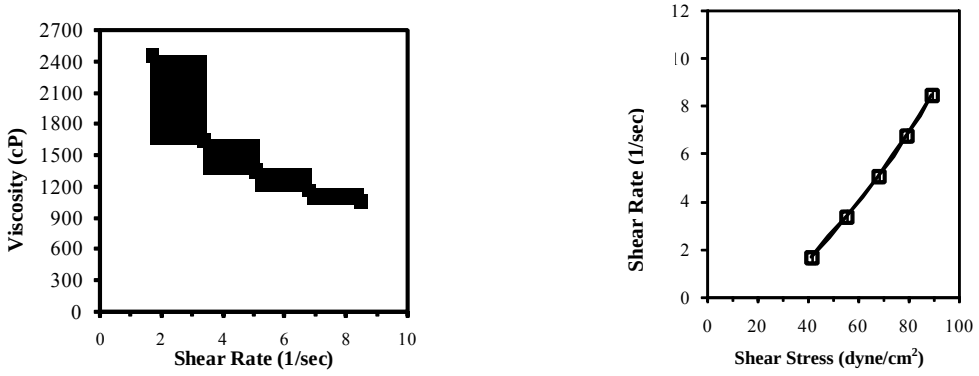
4.1.1 pH

As seen in Table 13, the results showed that all formulations had pH values in the range of 6.9-7.2. This range is acceptable for topical application. It is well known that normal skin pH is between 5 and 6.5 in healthy people, and it varies among the different areas of skin (http://www.probiotic-lab.com/skinph.html).

It is generally recognized that the formulations are acceptable for topical application when their pH values are in the range of 5-8 (Saraf *et al*., 2010).

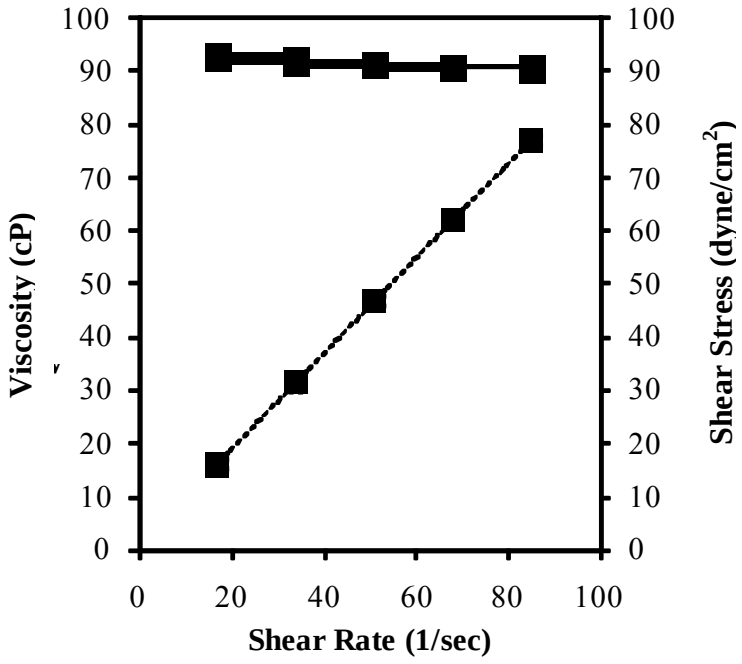
4.1.2 Viscosity and Flow

The addition of gelling agents in ME-2 resulted in the increase of viscosity values in all formulations. All formulations exhibited Newtonian flow, except ME-G1. It was noted that the flow behavior of ME-G1, which contained colloidal silica, had been transformed from Newtonian flow to plastic flow as seen in Figure 27. The flow curve displayed a decreased viscosity with an increased shear rate. When colloidal silica was added into the w/o ME, the viscosity was significantly increased ($p < 0.05$). It can be explained that the interconnected gel network, which is hydrated by hydrogen bonds of water and colloidal silica, resulted in strong interactions between microemulsion droplets (Kantaria *et al*., 1999; Rozman *et al*., 2009). In the previous report, the formation of gelatin-containing microemulsion-based organogels from w/o microemulsions could be explained in term of a model structure. The common theme linking the available models is that there exists within a macroscopic hydrophobic continuous phase, an interconnected gelatin network which is hydrated and stabilized from direct contact with the oil by a monolayer of surfactant (Kantaria *et al*., 1999). However, the viscosity of ME-G2 and ME-G3 was still low (Table 13) with Newtonian flow behavior (Figure 28). It could be explained that Carbopol® Ultrez 21 could not form the network of the external phase of ME-2.

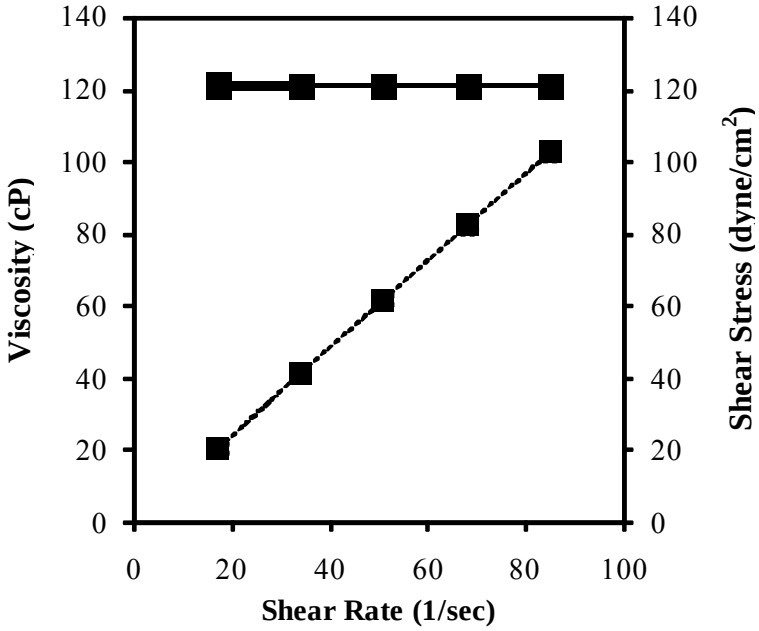


2a 2b

Figure 27. The rheogram of ME-G1. ○ represents viscosity (cP) and □ represents shear stress (dyne/cm^2).



(A)



(B)

Figure 28. Rheograms of two gels: ME-G2 (A) and ME-G3 (B). ○ represents viscosity (cP) and □ represents shear stress (dyne/cm^2).

4.1.3 Stability

The stability testing of the three microemulsion-based gels has revealed that there was no change in physical appearance of all samples over 60 days

storage at 4°C and room temperature (~30 ± 2°C). However, the change in color was observed in the stored formulations at 60°C. The decomposed formulations had brownish color. It can be explained that at 60°C, long chain polymers of hydrogen, oxygen, and carbon became into short-chain hydrocarbons since the hydrocarbons of oil decomposed. This is due to the fact that when compositions used in formulation, e.g., oleth-10, soybean oil, PEG-40 hydrogenated castor oil, and nicotinamide solution were stored at 60°C for 2 days, only the color of soybean oil appeared darken. Although ME-G2 and ME-G3 were physically stable like ME-G1, their appearance was unsatisfactory. Therefore, only ME-G1 was selected to be analysed with HPLC for the chemical stability. The content of nicotinamide was found to be 99.98% at 4°C and 98.18% at room temperature when compared with the initial concentration. It has been reported that microemulsion can be an effective vehicle of certain drug since it can protect the drug from degradation (Kogan and Garti, 2006).

4.1.4. *In vitro* Release

Figure 30 (A, B, C) displays the *in vitro* release profiles of nicotinamide from ME-G1 with three different mathematical models, i.e., zero order model, first order model and Higuchi model. The results showed that the release kinetics of ME-G1 was fitted to zero order. The linear relationship (R^2 = 0.993) between cumulative amounts of drug released versus time suggested that the release of drug from the formulation was increasing in the presence of colloidal silica. It can be concluded that colloidal silica was the main factor affecting on the nicotinamide release.

The results of the percents of nicotinamide released from the prepared formulations (ME-G1, ME-1, ME-2) and a commercial product are shown in Figure 31 and Table 14. It is seen that the percents of nicotinamide released from the ME-G1 were the highest. The rank order of percentage of release was found to be ME-G1 > ME-2 ≥ ME-1 ≥ the commercial cream.

Theoretically, the pH value of the vehicle, the drug solubility in the vehicle and the viscosity of the gel matrix are three important factors to be considered in the evaluation of drug release from the formulation across the

membrane (Arellano *et al*., 1999). The pH value and drug solubility of all formulations were not significantly different. Thus, the viscosity is considered to be the main factor affecting the release of nicotinamide.

The published report has informed that release rate is inversely related to the viscosity of the continuous phase (Santoyo *et al*., 1996). Although a commercial cream is o/w formulation and the hydrophilic nicotinamide locates in outer phase, it provided the lowest percentage of nicotinamide released. It might be caused by the complex partition of nicotinamide among all the compositions, thereby limiting the transferring of nicotinamide through the membrane (Frelichowska *et al*., 2009). Moreover, it could be caused by the effect of its viscosity of all the formulations tested; a commercial cream provided the highest viscosity (6,827.87 ± 549.63 cP). In generally, viscosity is negatively related to release of active substance from formulation as seen in Figure 29 and its penetration through the diffusion barriers (Tas *et al*., 2003). Thus, this high viscosity of formulation resulted in decreasing the release of nicotinamide from the commercial cream.

ME-1 and ME-2 could increase the release of nicotinamide when compared to the commercial cream. This was probably due to the fact that some mechanisms of microemulsions in penetration enhancement (Kogan and Garti, 2006; Boonme, 2007). The possible mechanisms of microemulsions in increasing the nicotinamide release were the thermodynamic process of nicotinamide diffusion across the flexible interfacial surfactant film and partitioning and diffusion into membrane. The release of nicotinamide from ME-1 and ME-2 was similar because they had the identical contents of nicotinamide and water, resulting in the similar concentration gradient. It may be assumed that ME-1 and ME-2 had similarity in the drug solubility due to the identical concentration of water in both formulations. The results suggested that the transport of nicotinamide was controlled by diffuse or partition from water droplets to oil continuous phase of microemulsions before releasing through the cellulose membrane (Kapoor and Chauhan, 2008).

ME-G1 showed the highest release rate (see Table 14, page 71). The results can be explained that the formation of three-dimensional networks of particles, such as branches on a tree, might create the mobility of the network chains in the gel contributing to the loosely feature of the matrix, resulted in nicotinamide

was drived off (Salvado, *et al*., 1996; Gallegos and Franco, 1999; Simovic and Prestidge, 2007). Because the nature structure of this colloidal silica is amorphous, it is composed of submicron-sized spheres, which are 40-60% fused into short chains, very highly branched, 0.1-0.2 microns long (Fumed silica Sigma Productinformation, n.d.). It is obviously seen that the much increase in the release of nicotinamide was found in the presence of colloidal silica. This is due to the loosely connected network of the oxide network (Salvado *et al*., 1996). In addition, hydrophilic nicotinamide could be adsorbed through interchain hydrogen bonding to the silica surface, especially in the oil phase (Frelichowska *et al*., 2009). In fact, the release rate was dependent on the affinity of the solubilized drug in the base, and a greater release of nicotinamide was expected when there was less affinity of the drug for the bases. This less affinity of nicotinamide in the base subsequently increased the release of nicotinamide.

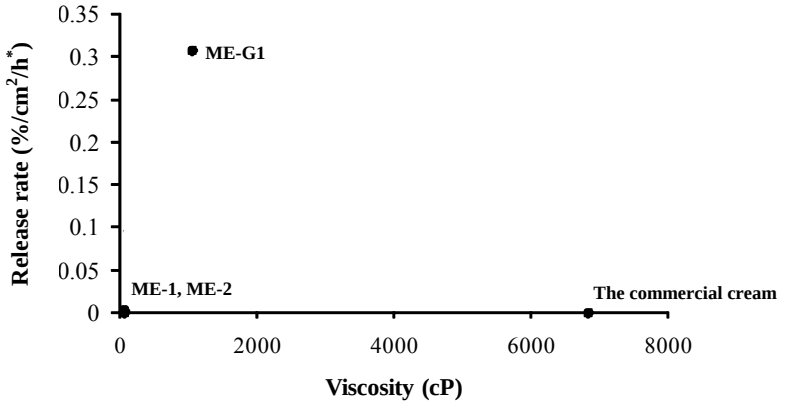
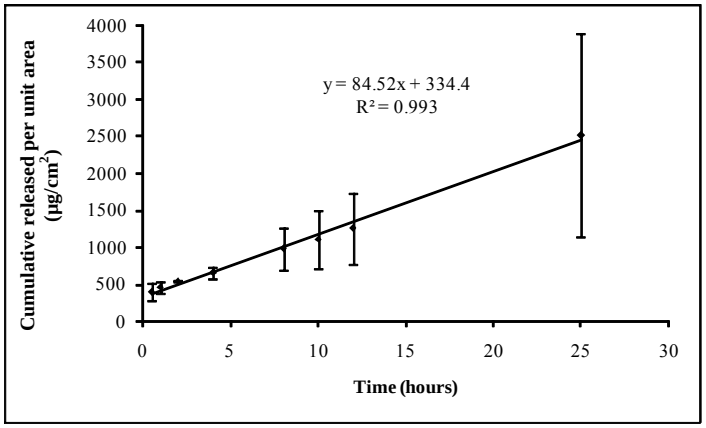
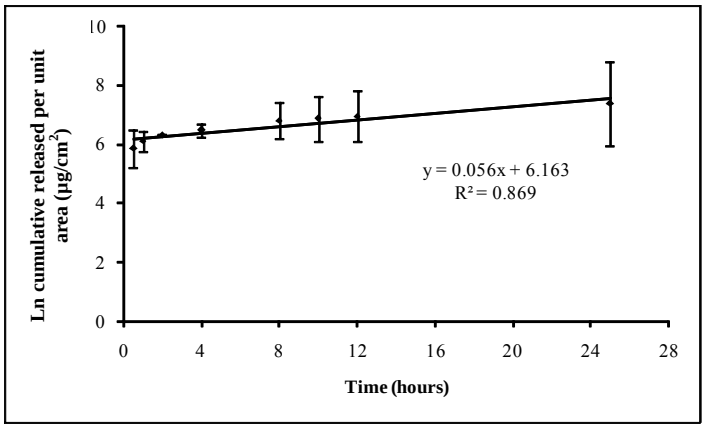


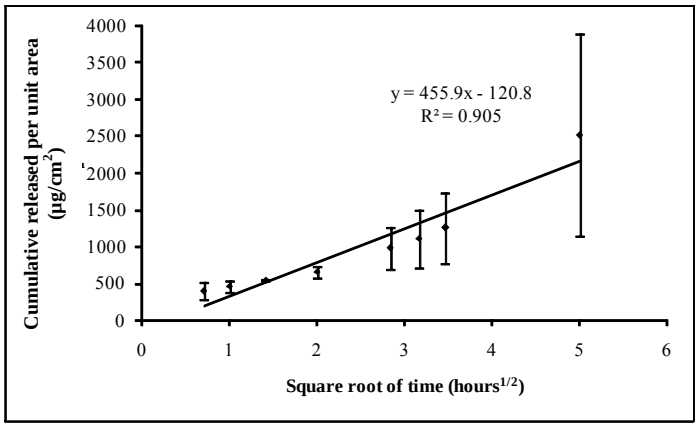
Figure 29. Relationship of release rate and viscosity of compared formulation: ME-G1, ME-2, ME-1, and the commercial cream. The * represents a release rate's unit of each formulation: %/cm^2/h for ME-G1 and %/cm^2/h$^{1/2}$ for ME-1, ME-2, and the commercial cream.



(A)



(B)



(C)

Figure 30. *In vitro* release profiles of nicotinamide from ME-G1 with different mathematical models**:** zero order model (A), first order model (B) and Higuchi model (C).

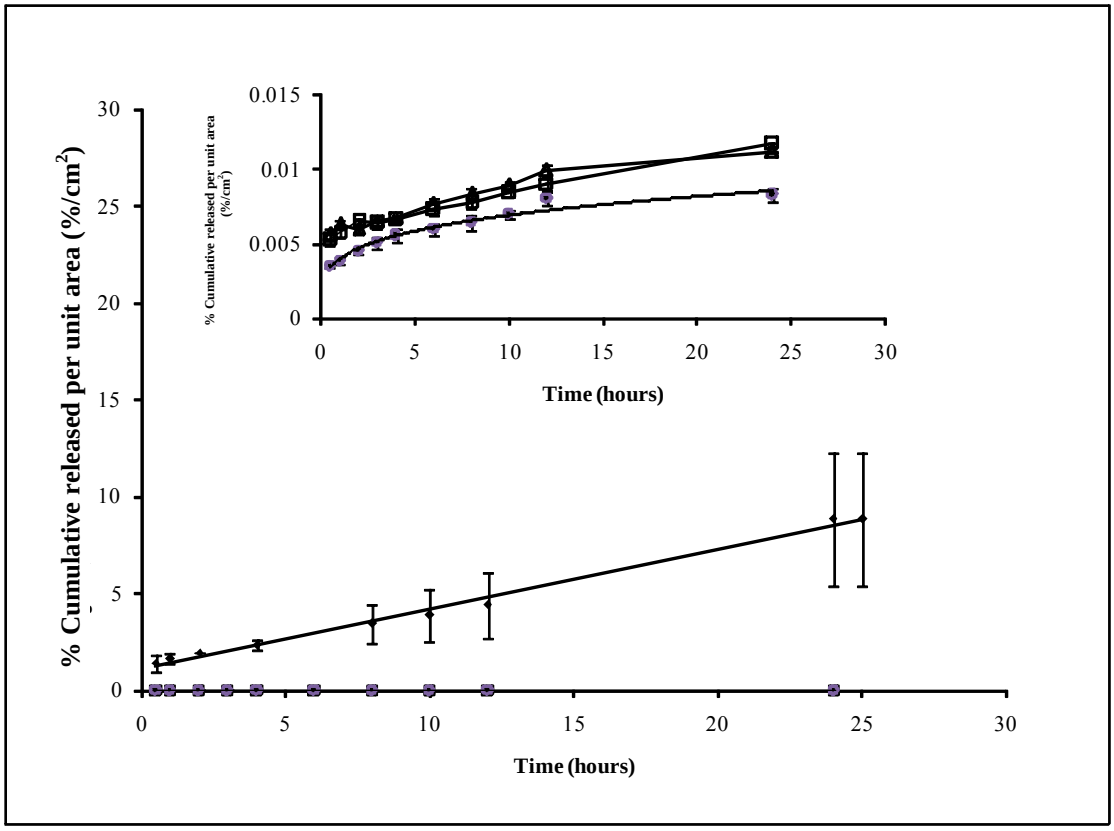


Figure 31. The % cumulative release of nicotinamide from different formulations: ME-G1 (♦), ME-1 (Δ), ME-2 (□) and a commercial cream (●). Each point represents mean ± SD, n = 3.

Table 14. Release parameters of nicotinamide from different formulations

Nicotinamide formulation	**cumulative release at 24 hours (%/cm^2)** **	**release rate**
ME-1	0.0111 ± 0.0001	0.00140 %/cm^2/h$^{1/2}$
ME-2	0.0126 ± 0.0004	0.00145 %/cm^2/h$^{1/2}$
ME-G1	8.8410 ± 3.4136*	0.30716 %/cm^2/h
A commercial cream	0.0083 ± 0.0004	0.00111 %/cm^2/h$^{1/2}$

* test significant difference using a one-way ANOVA ($p < 0.05$)

** Reported as mean ± SD (three replications) at 32°C.

CHAPTER 5

CONCLUSIONS

In this study, the pseudoternary phase diagrams of systems consisted of oleth-10, water, oils (silicone oil and soybean oil) and cosurfactants (IPA, PG and Span 80) were constructed to investigate of their phase behavior. Among studied systems, water/soybean oil/oleth-10:IPA (9:1) provided the largest microemulsion region. The obtained microemulsions were thermodynamically stable and could be further incorporated with cosmetic active ingredients.

Two formulations in the largest microemulsion region system were selected to be incorporated with nicotinamide at concentration of 3% w/w. Both formulations (ME-1 and ME-2) were investigated for their physicochemical properties, i.e., the conductivity values of ME-1 (10.45±0.18 μS/cm) and ME-2 (10.47±0.25 μS/cm), pH values of ME-1 (7.25±0.005) and ME-2 (7.21±0.005), particle size values of ME-1 (64.5 nm, PI = 0.902) and ME-2 (79.7 nm, PI = 0.766). Under the three stability-studied temperatures, only at 4°C and room temperature (~30 ± 2°C), both formulations were still clear with no phase separation over the period of two months. The physical stability data indicated that the samples were stable only at 4°C and room temperature. For the chemical stability study, the content nicotinamide of ME-1 was found to be 100.95% at 4°C and 98.59% at room temperature as well as the content nicotinamide of ME-2 was found to be 99.92% at 4°C and 99.87% at room temperature comparing with initial concentration. When stored at 60°C, the samples were darker; therefore they were not determined for the active content. From the *in vitro* release study, the release profiles of both ME-1 and ME-2 were fitted with Higuchi model. All of the results indicated that these data were not significantly different for both studied formulation due to the similar influence of concentration gradient, nonetheless, ME-2 formulation was chosen as optimal microemulsion because of its lower surfactant concentration.

In preparing microemulsion-based gels, it was observed that the order of clarity in prepared gels was: ME-G1> ME-G3> ME-G2. For physical stability, all samples were stable at 4°C and room temperature (~30 ± 2°C) over the two months of storage. In viscosity study, only ME-G1 was suitable. Therefore, ME-G1 was selected and further investigated for its chemical stabilities using HPLC analysis. The results of chemical stability for the studied period of two months of ME-G1 at 4°C and room temperature exhibited nicotinamide content of 99.98% and 98.18%, respectively.

The *in vitro* release profile of nicotinamide from ME-G1 was fitted to zero order kinetic whereas, the percents of nicotinamide released from ME-1, ME-2, and a commercial cream were fitted to Higuchi model. The rank order of total percent cumulative release and release rate of nicotinamide in different formulations was: ME-G1 > ME-2 ≥ ME-1 ≥ the commercial cream. The addition of colloidal silica as gelling agent in ME-G1 resulted in a high release of nicotinamide. This was due to less affinity of nicotinamide for the bases. Based on screening formulation, it may be concluded that ME-G1 was the most suitable formulation for topical application of nicotinamide since its appearance, viscosity, stability, and *in vitro* release properties were satisfactory.

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APPENDIX

APPENDIX
EXPERIMENTAL DATA

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
1	0.50	4.50	0.00	10.01	89.99	0.00	pale yellowish clear liquid → No change	I
2	0.45	4.03	0.51	9.10	80.69	10.22	pale yellowish clear liquid → No change	I
3	0.40	3.60	1.01	8.06	71.87	20.07	pale yellowish clear liquid → No change	I
4	0.36	3.16	1.50	7.12	62.93	29.94	pale yellowish clear liquid → No change	I
5	0.31	2.71	2.00	6.16	53.98	39.86	turbid gel → No change	-
6	0.26	2.25	2.51	5.13	44.94	49.93	turbid gel → No change	-
7	0.21	1.80	2.99	4.17	35.97	59.85	clear gel → No change	-
8	0.15	1.35	3.51	3.04	26.99	69.98	turbid gel → No change	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
9	0.10	0.90	4.00	2.05	17.97	79.97	white turbid liquid → Two-phase clear liquid	-
10	0.06	0.46	4.50	1.15	9.21	89.63	turbid liquid → Two-phase clear liquid	-
11	1.00	4.00	0.00	20.05	79.95	0.00	pale yellowish clear liquid → No change	I
12	0.91	3.60	0.51	18.07	71.72	10.21	pale yellowish clear liquid → No change	I
13	0.81	3.20	1.01	16.06	63.86	20.07	turbid gel → No change	-
14	0.70	2.81	1.51	13.96	56.06	29.98	turbid gel → No change	-
15	0.61	2.39	2.01	12.16	47.81	40.04	milky gel → No change	-
16	0.51	2.03	2.52	10.06	40.12	49.82	turbid gel → No change	-
17	0.41	1.60	3.01	8.09	31.92	59.98	turbid gel → No change	-
18	0.31	1.20	3.50	6.17	23.95	69.88	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
19	0.21	0.80	4.00	4.16	15.97	79.87	white turbid liquid → Two-phase clear liquid	-
20	0.11	0.41	4.51	2.10	8.16	89.74	turbid liquid → Two-phase clear liquid	-
21	1.50	3.50	0.00	30.03	69.97	0.00	pale yellowish clear liquid → No change	I
22	1.36	3.16	0.50	27.02	62.96	10.02	pale yellowish clear liquid → No change	I
23	1.21	2.81	1.01	24.04	55.90	20.06	turbid liquid → Two-phase yellowish clear liquid	-
24	1.06	2.46	1.50	21.05	48.98	29.97	milky gel → No change	-
25	0.90	2.09	2.00	18.05	41.91	40.04	milky gel → No change	-
26	0.76	1.76	2.51	15.06	35.00	49.94	milky gel → No change	-
27	0.61	1.40	3.00	12.17	27.93	59.89	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
28	0.46	1.06	3.50	9.09	21.06	69.85	milky liquid → Upward creaming	-
29	0.30	0.70	4.00	6.07	14.01	79.92	turbid liquid → Two-phase clear liquid	-
30	0.16	0.35	4.50	3.19	7.00	89.81	turbid liquid → Two-phase clear liquid	-
31	2.01	3.00	0.00	40.07	59.93	0.00	pale yellowish clear liquid → No change	I
32	1.80	2.75	0.51	35.58	54.38	10.04	yellowish turbid liquid → Upward oil	-
33	1.61	2.40	1.00	32.11	47.90	19.99	yellowish turbid liquid → Upward oil	-
34	1.41	2.10	1.51	28.11	41.88	30.02	milky gel → No change	-
35	1.20	1.82	2.01	23.89	36.21	39.91	milky gel → No change	-
36	1.01	1.50	2.50	20.13	29.99	49.88	milky gel → No change	-
37	0.81	1.20	3.00	16.24	23.93	59.83	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
38	0.61	0.90	3.50	12.09	18.03	69.88	milky liquid → Upward creaming	-
39	0.41	0.62	4.01	8.06	12.32	79.62	turbid liquid → Two-phase clear liquid	-
40	0.20	0.30	4.51	4.04	6.02	89.94	turbid liquid → Two-phase clear liquid	-
41	2.51	2.50	0.00	50.05	49.95	0.00	pale yellowish clear liquid → No change	I
42	2.25	2.25	0.50	44.99	45.00	10.01	pale yellowish turbid liquid → Three-phase liquid	-
43	2.01	2.00	1.00	40.14	39.88	19.98	milky gel → No change	-
44	1.75	1.75	1.58	34.47	34.46	31.07	milky gel → No change	-
45	1.51	1.51	2.01	30.12	29.99	39.90	milky gel → No change	-
46	1.26	1.25	2.50	25.20	24.98	49.81	milky liquid → Upward creaming	-
47	1.01	1.00	3.00	20.20	19.99	59.80	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
48	0.75	0.75	3.50	15.07	14.99	69.94	milky liquid → Upward creaming	-
49	0.52	0.50	4.00	10.27	10.02	79.71	turbid liquid → Two-phase clear liquid	-
50	0.26	0.26	4.51	5.23	5.12	89.65	turbid liquid → Two-phase clear liquid	-
51	2.99	2.01	0.00	59.85	40.15	0.00	pale yellowish clear liquid → No change	I
52	2.70	1.80	0.50	53.97	35.96	10.06	pale yellowish turbid liquid → Upward oil	I
53	2.41	1.60	1.02	47.90	31.82	20.28	milky gel → No change	-
54	2.11	1.40	1.51	42.01	27.94	30.06	milky gel → No change	-
55	1.80	1.21	2.01	35.89	24.08	40.03	milky gel → No change	-
56	1.51	1.01	2.51	29.96	20.11	49.94	milky liquid → Upward creaming	-
57	1.20	0.80	3.00	24.00	16.01	60.00	milky liquid → creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
58	0.91	0.60	3.51	18.07	12.00	69.93	white turbid liquid → Two-phase clear liquid	-
59	0.60	0.41	4.01	11.99	8.09	79.93	white turbid liquid → Two-phase c lear liquid	-
60	0.31	0.21	4.51	6.23	4.10	89.68	turbid liquid → Two-phase clear liquid	-
61	3.51	1.49	0.00	70.16	29.84	0.00	pale yellowish clear liquid → No change	I
62	3.16	1.36	0.50	62.95	27.06	9.99	pale yellowish turbid liquid → Upward oil	-
63	2.81	1.20	1.01	55.94	23.98	20.09	milky gel → No change	-
64	2.45	1.05	1.50	48.91	21.04	30.04	milky gel → No change	-
65	2.11	0.91	2.00	41.99	18.10	39.91	milky liquid → Upward creaming	-
66	1.75	0.76	2.51	34.89	15.11	50.00	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
67	1.41	0.60	3.10	27.67	11.75	60.58	milky liquid → Upward creaming	-
68	1.05	0.45	3.51	20.97	9.07	69.96	milky liquid → Upward creaming	-
69	0.70	0.31	4.01	14.05	6.09	79.86	white turbid liquid → Two-phase clear liquid	-
70	0.35	0.17	4.51	6.91	3.34	89.75	turbid liquid → Two-phase clear liquid	-
71	4.00	1.00	0.00	80.03	19.97	0.00	pale yellowish clear liquid → No change	I
72	3.60	0.90	0.51	71.88	17.98	10.14	pale yellowish turbid liquid → Upward oil	-
73	3.21	0.80	1.05	63.50	15.81	20.69	milky gel → No change	-
74	2.81	0.71	1.51	55.92	14.09	29.99	milky liquid → Upward creaming	-
75	2.41	0.60	2.00	48.04	12.05	39.91	milky liquid → Upward creaming	-
76	2.01	0.50	2.51	40.01	9.97	50.02	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	Oil (g)	S+C (g)	Water (g)	Oil (%)	S+C (%)	Water (%)	Observation (Initial → 24 hours)	w/ BBS
77	1.60	0.40	3.01	31.94	7.98	60.07	milky liquid → Upward creaming	-
78	1.21	0.31	3.51	24.04	6.12	69.85	white turbid liquid → Two-phase clear liquid	-
79	0.81	0.20	4.01	16.07	4.02	79.91	white turbid liquid → Two-phase clear liquid	-
80	0.40	0.10	4.49	8.05	2.06	89.89	turbid liquid → Two-phase clear liquid	-
81	4.50	0.51	0.00	89.91	10.09	0.00	pale yellowish clear liquid → No change	I
82	4.05	0.46	0.50	80.86	9.10	10.04	pale yellowish turbid liquid → Upward oil	-
83	3.61	0.40	1.00	72.01	8.00	19.99	milky liquid → Upward creaming	-
84	3.15	0.36	1.51	62.70	7.14	30.16	milky liquid → Upward creaming	-
85	2.70	0.31	2.00	53.90	6.13	39.97	milky liquid → Upward creaming	-
86	2.25	0.26	2.51	44.82	5.14	50.04	milky liquid → Upward creaming	-

Table 15. Optical characteristics of the mixtures of soybean oil, water, and oleth-10:IPA (9:1) at various weight ratios (continued)

No.	**Oil (g)**	**S+C (g)**	**Water (g)**	**Oil (%)**	**S+C (%)**	**Water (%)**	**Observation (Initial → 24 hours)**	**w/ BBS**
87	1.82	0.21	3.00	36.21	4.09	59.70	milky liquid → Upward creaming	-
88	1.36	0.16	3.51	27.03	3.10	69.87	milky liquid → Upward creaming	-
89	0.91	0.11	3.99	18.16	2.29	79.55	white turbid liquid → Two-phase clear liquid	-
90	0.45	0.06	4.50	8.98	1.22	89.80	turbid liquid → Two-phase clear liquid	-

S+C = 9:1 oleth-10:IPA, w/BBS = dilution with brilliant blue aqueous solution, I = immiscible

Table 16. Linearity of three consecutive days (n=3)

Day	Concentration (µg/ml)	Area				SD
		n1	n2	n3	Average	
1	2.50	69.20	69.30	69.30	69.27	0.06
	5.00	139.20	139.20	139.30	139.23	0.06
	10.00	284.50	284.10	284.70	284.43	0.31
	20.00	551.40	551.60	550.80	551.27	0.42
	40.00	1135.90	1135.30	1131.90	1134.37	2.16
2	2.50	71.40	71.20	71.50	71.37	0.15
	5.00	141.40	141.40	140.00	140.93	0.81
	10.00	291.20	290.80	290.30	290.77	0.45
	20.00	565.90	565.70	567.40	566.33	0.93
	40.00	1162.80	1162.50	1162.00	1162.43	0.40
3	2.50	70.20	70.70	70.60	70.50	0.26
	5.00	141.80	142.20	142.00	142.00	0.20
	10.00	292.50	291.80	292.10	292.13	0.35
	20.00	554.90	557.10	557.70	556.57	1.47
	40.00	1160.70	1162.00	1162.50	1161.73	0.93

Table 17. Accuracy of three consecutive days (n=3), three sets of standard solutions were analyzed three times.

Day	Concentration (µg/ml)	Area				SD	Recovery	
		n1	n2	n3	Average		µg/ml	(%)
1	5.00	141.10	140.90	141.20	141.067	0.153	5.04	100.81
	10.00	284.70	284.90	284.50	284.700	0.200	10.03	100.31
	20.00	426.20	426.80	426.80	426.600	0.346	14.96	99.75
2	5.00	140.90	140.90	140.70	140.833	0.115	5.03	100.65
	10.00	285.80	285.70	285.70	285.733	0.058	10.07	100.67
	20.00	427.50	426.80	426.80	427.033	0.404	14.98	99.85
3	5.00	142.00	142.60	142.60	142.400	0.346	5.09	101.74
	10.00	286.30	286.40	285.70	286.133	0.379	10.08	100.81
	20.00	428.10	428.20	426.80	427.700	0.781	15.00	100.00

Table 18. The intra-day precision of three sets of three standard solutions within one day

Conc. (µg/ml)		**Area (n=5)**						%RSD
	No.	n1	n2	n3	n4	n5	Average	
5.00	1	139.20	139.20	139.30	139.10	139.20	139.20	0.36
	Intra-day	141.70	141.40	141.10	141.40	141.40	141.40	
	2	140.20	139.90	140.60	140.30	140.30	140.26	
	Intra-day	141.00	141.90	141.60	141.00	141.40	141.38	
	3	140.80	141.00	141.40	141.00	141.40	141.12	
	Intra-day	141.70	141.70	142.00	142.44	142.70	142.11	
10.00	1	284.50	284.10	284.70	284.30	284.40	284.40	1.17
	Intra-day	291.98	291.1	291.4	292.4	291.1	291.60	
	2	292.40	292.40	291.40	292.40	292.00	292.12	
	Intra-day	307.80	307.40	307.40	307.30	306.60	307.30	
	3	292.40	292.20	292.30	291.90	291.10	291.98	
	Intra-day	298.90	298.6	298.5	298.7	299.10	298.76	
20.00	1	551.40	551.60	550.80	551.20	550.70	551.14	1.09
	Intra-day	565.90	565.70	567.60	551.50	550.90	560.32	
	2	581.50	580.90	580.90	580.70	580.30	580.86	
	Intra-day	607.60	607.90	606.40	606.00	605.70	606.72	
	3	583.90	584.60	584.30	584.30	584.00	584.22	
	Intra-day	604.20	603.60	607.00	599.90	599.30	602.80	

Table 19. The inter-day precision of three consecutive days (n=3)

Day	Concentration (µg/ml)	No.	Area (n=5)					
			n1	n2	n3	n4	n5	Average
1	5	1	139.20	139.20	139.30	139.10	139.20	139.20
		2	140.20	139.90	140.60	140.30	140.30	140.26
		3	140.80	141.00	141.40	141.00	141.40	141.12
	10	1	284.50	284.10	284.70	284.30	284.40	284.40
		2	292.40	292.40	291.40	292.40	292.00	292.12
		3	292.40	292.20	292.30	291.90	291.10	291.98
	20	1	551.40	551.60	550.80	551.20	550.70	551.14
		2	581.50	580.90	580.90	580.70	580.30	580.86
		3	583.90	584.60	584.30	584.30	584.00	584.22
2	5	1	141.40	141.40	140.00	141.60	141.00	141.08
		2	141.70	141.70	141.00	141.47	141.50	141.47
		3	144.60	144.90	144.30	144.80	144.70	144.66
	10	1	291.20	290.80	290.30	292.60	289.70	290.92
		2	307.60	307.20	307.10	307.30	306.70	307.18
		3	298.70	299.50	299.00	298.90	299.10	299.04
	20	1	565.90	565.70	567.40	551.20	550.70	560.18
		2	607.80	607.80	606.30	605.00	605.00	606.38
		3	604.00	603.60	606.90	599.90	599.30	602.74
3	5	1	141.80	142.20	142.00	142.00	142.00	142.00
		2	138.00	138.00	138.00	139.20	139.23	138.49
		3	141.00	141.90	141.60	141.00	141.40	141.38
	10	1	292.50	291.80	292.10	292.60	292.13	292.23
		2	294.00	295.00	294.10	294.30	294.80	294.44
		3	290.00	290.30	290.00	291.80	290.70	290.56
	20	1	554.90	557.10	557.70	556.70	556.57	556.59
		2	602.10	601.60	601.60	601.20	601.76	601.65
		3	561.30	559.40	561.20	559.90	559.30	560.22

Conc. (µg/ml)	Area			Average (n=3)	S.D.	%RSD
	Day 1	Day 2	Day 3			
5	142.40	144.17	140.62	142.40	1.77	1.25
10	289.50	299.05	292.41	293.65	4.89	1.67
20	572.07	589.77	572.82	578.22	10.01	1.73

Table 20. LOD and LOQ of three consecutive days (n=3)

Day	LOD	LOQ
1	0.023	0.028
2	0.062	0.098
3	0.062	0.081

Table 21. Viscosity and release rate of studied formulations

Formulation	viscosity	release rate
A commercial cream	6827.876 ± 549.636	0.00019
ME-1	71.02 ± 0.000	0.0024
ME-2	74.1 ± 0.000	0.00024
ME-G1	1051 ± 1.830	0.30716

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List of Publication and Proceedings

Suksawad, N., Songkro, S., and Boonme, P. (2009). *Phase behavior of systems composed of oleth-10, water, various oils and cosurfactants.* Poster presented at the 35th Congress on Science and Technology of Thailand (STT. 35), Chonburi, Thailand, M_M0003.

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