FORMULATION, CHARACTERIZATION AND OPTIMIZATION OF PALM OIL ESTERS BASED NANO-SCALED EMULSIONS FOR TOPICAL DELIVERY OF IBUPROFEN AND THE EVALUATION OF THEIR ANTI-INFLAMMATORY AND ANALGESIC EFFECTS

by

Ghassan Zuhair Abdullah

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PERUMUSAN, PENCIRIAN DAN PENGOPTIMUMAN ESTER MINYAK SAWIT BERDASARKAN EMULSI BERSKALA NANO UNTUK PENGHANTARAN TOPIKAL IBUPROFEN SERTA PENILAIAN KESAN ANTI-INFLAMASI DAN ANALGESIK

oleh

Ghassan Zuhair Abdullah

**Tesis yang diserahkan untuk memenuhi
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LIST OF ABBREVIATIONS

%	Percent
±	Plus minus
η	Viscosity
η_i	Intrinsic viscosity
ζ	Zeta Potential
°C	Degree Celsuis
1/s	1/second
A	Interfacial area
ANOVA	Analysis of variance
β	Beta
BA	Benzoic acid
c	Initial Hind Paw Thickness
C_0	Initial drug concentration
Carb 934	Carbopol 934
Carb 940	Carbopol 940
Carb U10	Carbopol Ultrez 10
CD	Cyclodextrin
CMC	Critical micelles concentration
Cm	Centimeter
cm/sec	Centimeter per second
cm^2	Square centimeter
cm^3	Cubic centimeter
Conc.	Concentration
COX	Cyclooxygenase
c_t	Hind paw thickness at time t
DS	Droplets size
DSC	Differential scanning calorimetry
DW	Distilled water
DLVO theory	Deryaguin, Landau, Verwey and Overbeek theory
dM/ S.dt	Amount of drug that permeates through a unit cross section area of S in unit time of t
*E*r	Enhancement ratio
et al.	And others
F	Yield value
FDA	Food and Drug Administration
G	Formulation
G	Shear rate
GIT	Gastrointestinal tract
Gm	Gram
gm/cm^3	Gram per cubic centimeter
H	Thickness of Membrane
H^+	Hydrogen ion

H-bonding	Hydrogen bonding
HLB	Hydrophilic- lipophilic balance
HPLC	High pressure liquid chromatography
HPMC	Hydroxy-propyl methyl cellulose
Hr	Hour
HSD	Harmonic sample distribution
Jss	Steady state flux
Kg	Kilogram
kHz	Kilo hertz
Kp	Permeability coefficient
L	Limonene
Log *P*	Partition coefficient
M	Molar
M	Menthol
m	Meter
mg	Milligram
mg/kg	Milligram per kilogram
mg/mL	Milligram per milliliter
mL	Milliliter
mL/min	Milliliter per minute
m*M*	Millimolar
mm	Millimeter
mPa	Millipascal
mv	Millivolt
N	Pseudoplasticity index
NaOH	Sodium hydroxide
Ng	Nanogram
nm	Nanometer
NSAID	Non-steroidal anti-inflammatory drug
O/W	Oil in water
OH^-	Hydroxide ion
P	Probability
Pa	Pascal
PB	Phosphate buffer
pKa	Acid dissociation constant
PI	Polydispersity index
POEs	Palm oil esters
Po/w	Amount of drug in the oil / amount of drug in buffer
rpm	Revolution per minute
S	Shear stress
SB	Sodium benzoate
SD	Standard deviation
SEM	Scanning electron microscopy
SEM	Standard error of mean
SANS	Small angle neutron scattering
SAXS	Small angle X-ray scattering

sec	Second
TEM	Transmission electron microscopy
TEWL	Trans-epidermal water loss
µg	Microgram
µL	Microliter
µm	Micrometer
µs	Microsiemens
UV	Ultraviolet
UV-VIS	Ultraviolet-visible
V	Volt
w/o	Water in oil
w/w	Weight by weight

PERUMUSAN, PENCIRIAN DAN PENGOPTIMUMAN ESTER MINYAK SAWIT BERDASARKAN EMULSI BERSKALA NANO UNTUK PENGHANTARAN TOPIKAL IBUPROFEN SERTA PENILAIAN KESAN ANTI-INFLAMASI DAN ANALGESIK

ABSTRAK

tujuan utama kajian ini adalah untuk formulasi ibuprofen sebagai suatu emulsi berskala nano baru, yang terdiri daripada ester minyak sawit tersintesis baru sebagai fasa minyak.

Kajian ini melibatkan pembinaan beberapa gambarrajah fasa pseudo-ternari terdiri daripada air, ester minyak sawit, dan campuran surfaktan bukan ion bagi beberapa nilai HLB. Beberapa formula atau rumusan dipilih bagi mengukur kelarutan ibuprofen. Di samping itu, kesan ibuprofen terhadap pelbagai sifat formula yang dipilih turut dinilai. Sifat reologi, potensi zeta, saiz titisan dan ciri struktur formula juga dikaji.

Satu formula dengan komposisi asas ester minyak sawit: fasa akues: surfaktan (100% Tween 80, HLB 15) pada nisbah 25:37:38 dipilih untuk pengubahsuaian selanjutnya dari segi sifat pengawetan aliran dan penelapan. Beberapa emulsi berskala nano dalam pelbagai kepekatan ibuprofen dan penampan fosfat pada tiga nilai pH yang berbeza ; 4.0, 6.0 dan 7.4 dihasilkan dan kesan bagi setiap parameter di atas turut dinilai.

Asid benzoik, natrium benzoat serta metil dan propil paraben diuji sebagai pengawet. Tiga resin Carbopol® iaitu 934, 940 dan Ultrez 10 digunakan sebagai penyesuai reologi. Sifat pembengkakan dan pembentukan rangkaian-gel Carbopol 940 dinilai dengan menggunakan agen peneutralan yang berbeza (penampan fosfat dan larutan trietanolamina) pada beberapa nilai pH dan kepekatan. Mentol and limonen pada kepekatan berbeza digunakan sebagai penggalak penelapan (permeation promoters) ibuprofen melalui kulit.

Kemampuan beberapa emulsi berskala nano menghantar ibuprofen melalui kulit tikus dinilai secara *in vitro* dengan menggunakan sel resapan Franz. Formulasi-formulasi $C_{S},_{7.4}T,_{0}R$, $C_{S},_{7.4}T,_{0.50}R_{940},M_{10}$ dan $C_{S},_{7.4}T,_{0.50}R_{940},M_{10}$ ditemui sebagai yang terbaik dan kesan farmakodinamiknya dinilai dan dibandingkan dengan sediaan komersial. Aktiviti anti-inflamasi dan analgesik daripada formula tersebut diukur melalui kaedah "carragennan-induced hind paw edema

Sebagai kesimpulan, emulsi berskala nano terdiri daripada 5% w/w ibuprofen sebagai bahan kandungan aktif, ester minyak sawit sebagai fasa minyak, 0.5% w/w Carbopol® 940 sebagai penyesuai reologi yang dineutralkan oleh larutan akues trietanolamina pH 7.4 sebagai fasa luaran, dan 10% w/w mentol atau limonene sebagai penggalak penelapan didapati mempunyai sifat reologi, saiz titisan dan kestabilan yang sesuai.

Formula $C_{S},_{7.4}T,_{0.50}R_{940},M_{10}$ dan $C_{S},_{7.4}T,_{0.50}R_{940},M_{10}$ menunjukkan sifat penelapan *in vitro* yang sangat baik melalui kulit tikus dibandingkan dengan produk sedia ada dalam pasaran (Nurofen® 5% Gel). Sehubungan dengan kesan anti-inflamasi dan analgesik yang amat tinggi apabila dibandingkan dengan produk rujukan, maka kedua-dua formula ini dipilih sebagai produk akhir. Kedua-duanya juga didapati stabil pada suhu yang berbeza sepanjang tempoh pemerhatian.

FORMULATION, CHARACTERIZATION AND OPTIMIZATION OF PALM OIL ESTERS BASED NANO-SCALED EMULSIONS FOR TOPICAL DELIVERY OF IBUPROFEN AND THE EVALUATION OF THEIR ANTI-INFLAMMATORY AND ANALGESIC EFFECTS

ABSTRACT

The main aim of this study stands on formulating ibuprofen as a novel nano-scaled emulsion encompassing newly synthesized palm oil esters as the oil phase.

This study involved the construction of several pseudo-ternary phase diagrams of water, palm oil esters and non-ionic surfactant mixture of several Hydrophilic-Lipophilic Balance (HLB) values. Several promising formulae were chosen, where ibuprofen solubility was measured. Additionally, the effect of ibuprofen on various properties of the selected formulations were also evaluated. Rheological properties, zeta potential, droplets size and structural characteristics of the promising formulae were also studied.

A formula with a basic composition of palm oil esters: aqueous phase: surfactant (100% Tween 80, HLB 15) at a ratio 25:37:38 respectively was selected for further modification in terms of preservation and flow and permeation properties. Several nano-scaled emulsions containing various ibuprofen concentrations and phosphate buffer at three different pH values; 4.0, 6.0 and 7.4 were produced and assessed for their effects

on the above parameters. Benzoic acid, sodium benzoate and methyl and propyl parabens were tested as preservatives. Three Carbopol® resins, namely 934, 940 and Ultrez 10 were later used as rheology modifiers. Carbopol 940 gel-network forming and swelling properties were evaluated using different neutralizing agents (phosphate buffer and triethanolamine solutions) at several pH values and concentrations. Menthol and limonene at two different concentrations were utilized as permeation promoters of ibuprofen through the skin.

The ability of several selected nano-scaled emulsions to deliver ibuprofen through full thickness rat skin was assessed *in vitro* using Franz diffusion cell. Formulations $C_{S,7.4}T_{,0}R$, $C_{S,7.4}T_{,0.50}R_{940},M_{10}$ and $C_{S,7.4}T_{,0.50}R_{940},M_{10}$ were found to be the most promising formulae and their pharmacodynamic effects were evaluated and compared with a reference preparation available commercially. The anti-inflammatory and analgesic activities of these formulae were measured by using carragennan-induced hind paw edema method.

In conclusion, the nano-scaled emulsions comprising 5% w/w ibuprofen as the active ingredient, palm oil esters as the oil phase, 0.5% w/w Carbopol® 940 as the rheology modifier neutralized by triethanolamine aqueous solution pH 7.4 as the external phase, and 10% w/w menthol or limonene as the permeation enhancer were found to have suitable rheological, droplets size and stability properties.

Formulae $C_{S,7.4}T_{,0.50}R_{940},M_{10}$ and $C_{S,7.4}T_{,0.50}R_{940},M_{10}$ showed better *in vitro* permeation properties through full thickness rat skin compared to the marketed product (Nurofen® 5% Gel). As a consequence of their higher anti-inflammatory and analgesic effects compared to that of the reference product, these two formulations were selected as the final products. These formulae were also found stable at different temperatures over the observation period.

CHAPTER ONE

INTRODUCTION

1.1. Introduction

During the last few decades, the treatment of many sicknesses has been achieved by administrating various drugs to humans via different routes which are namely: oral, sublingual, rectal, parental, inhalation, topical delivery, etc. Topical drug delivery has been considered by many researchers to be of extensive importance (Klotz and Schwab, 2005, Lopes *et al.*, 2005, Huang *et al.*, 2008, Zhu *et al.*, 2009). It can be defined as the application of a formulation containing a drug to the skin to directly treat cutaneous disorders (e.g. acne) or to the cutaneous manifestations of a general disease (e.g. psoriasis) with the intention of producing pharmacological or other effects of drugs on the surface of the skin, within the skin or to tissues under the skin.

The topical application of medicines was launched in the ancient history and the nineteen forties witnessed the topical administration of antibiotics and hormones. Generally, any agent whether toxic or beneficial to the human body, is susceptible to be absorbed if it comes into contact with the skin. Topical delivery systems may include drug administration to the skin for various purposes, such as steroid for local effect to treat dermatitis, nicotine patches for systemic effects, cosmetics for surface effect and NSAIDs for effects on deeper tissues like inflamed muscle.

1.2. Advantages and Disadvantages of Topical Drug Delivery Systems

Generally, topical delivery systems are considered versatile pharmaceutical dosage forms and have several applications in pharmacy. These systems include dosage forms, such as gels, ointments, creams, etc. They possess many advantages over conventional dosage forms; nonetheless, they also have several negative drawbacks that limit their usage in some circumstances (Walters and Roberts, 2002a).

1.2.1. Advantages of Topical Drug Delivery Systems

At the first place, topical dosage forms usually avoid liver first pass effect, which is considered as the potential barrier or limiting factor for most orally administered medications. Secondly, topically applied systems are convenient to be used by patients due to the ease of their application. As they function topically, they normally bypass the risks and hassles of intravenous injections (Park *et al.*, 2000). Moreover, topical drug delivery can skip the variation in absorptive conditions of the gastrointestinal tract (GIT), including; diversity of pH values from mouth to colon, enzymes activity, gastric emptying time and so forth. They can even avoid the fluctuation in drug plasma levels of inter- and intra-subject variations. Finally, topical dosage forms are self-applicable to a relatively large body area that can be simply terminated when required.

1.2.2. Disadvantages of Topical Drug Delivery Systems

Apart from all the benefits of topical dosage forms, they also own some disadvantages. First, skin irritations or allergies are quite common problems that are usually caused by drugs and/or excipients leading sometimes to skin dermatitis. Secondly, skin permeation of some drugs is occasionally poor due to the complexity of skin structure in general and stratum corneum, the outermost layer in specific (Huang *et al.*, 2005, Russeau *et al.*, 2009). Drugs absorption can be worsen if the dosage form consists of large droplets. In addition, enzymes available mainly in the epidermis of the skin can denature the applied preparation. Lastly, these dosage forms are more effective locally, i.e. their applications are more to be local than systemic. They can be utilized to produce systemic effect only when very small plasma concentrations of drugs are required to show a pharmacological action.

1.3. Factors Affecting the Absorption of Drugs through Skin

Basically, the rate and extent of drug absorption through the skin are influenced by the physicochemical properties of the drug and dosage form, skin structure and location and skin physiological conditions that most probably affect drug transportation to the systemic circulation (Wiechers, 1989).

1.3.1. Physicochemical Properties of Drug Substances

Many physical and chemical properties are influencing drug absorption through the skin (Park *et al.*, 2000). These characteristics are:

Partition coefficient: preferably, a one to four partition coefficient in octanol-water system is essential for producing a successful topical drug delivery Naik *et al*. (2000).

Drug solubility: higher drugs permeation through the skin is greatly related to their solubilities in the vehicle used in the formulation. Increasing drug solubility causes its permeation to be raised.

Concentration: supersaturated formulations can positively influence drugs permeability. In other word, increasing drugs concentrations beyond the saturated levels leads to an increase in the thermodynamic activity of these drugs in the vehicle. Higher thermodynamic activity is the driving force that can take out greater amounts of drugs from the formulation to across the stratum corneum. However, this technique is mainly limited by the re-crystallization problem that commonly occurs in supersaturated products (Williams, 2003).

Particle size: decreasing particles or droplets size of topically applied preparation causes the effective absorption area to be increased. As a result, the permeation of drugs through the skin becomes higher; therefore, nano- and micro-emulsion systems are of prime interest.

Polymorphism: as mentioned earlier that the permeability of any drug through the skin is highly related to its solubility in the vehicle. Therefore, the most soluble polymorph of any drug should be chosen in producing the final dosage form.

Molecular weight: it was reported by Naik *et al.* (2000) that drugs with a molecular weight less than 400 dalton can easily pass through skin tissues.

1.3.2. Release Properties of Topical Drug Delivery Systems

Emulsion, as a dosage form can accommodate more than one drug because it has external and internal phases. When drug molecules are included only in the internal phase of an emulsion, then drug molecules need to be partitioned from the internal phase to the external phase first before being absorbed by the skin. This process represents the rate limiting step of many topical preparations and it should occur quickly if fast absorption and effect are needed. Moreover, the release of drug from a dosage form may be affected by the composition of drug delivery system. Various excipients used may enhance or retard the absorption of drugs, e.g. the low molecular weight of propylene glycol may reduce the permeation and absorption of drugs, while polyethylene glycols may affect the penetration process oppositely.

1.3.3. Physiological and Pathological Conditions of Skin

Effect of horny reservoir layer: topically applied drugs may bind to the horny layer of the skin that in turn acts as a depot for drugs in therapy.

Lipid film: skin has specific moisture content that is normally controlled by the naturally formed lipid films which act as a protective layer to prevent excessive moisture loss.

Skin hydration: the application of an occlusive cover over the skin leads to an increase in the degree of hydration of the stratum corneum by preventing moisture loss from the skin, which consequently enhances the absorption of drugs.

Skin temperature: increasing the temperature of the skin may produce a chain of events, these are: a raise in drugs thermal energy that augments their diffusion, an increase in drugs solubilities in skin tissues and vasodilatation of the blood vessels. Overall, all these measures may cause the rate of drug permeation through the skin to increase.

Regional variation: the thickness and nature of skin are also variable properties. Various parts of the body demonstrate different thickness and environment. This may also affect drugs penetration, which can be anatomically ordered from least to most permeating as: plantar anterior, fore arm, scalp, ventral thigh, and scrotum and posterior auricular area.

Pathologic injuries of the skin: injured skin generally shows an increase in drugs permeation. The injury majorly affects the first layer of the skin, stratum corneum, which is the main barrier for the penetration of any exterior substance to the body.

Cutaneous drug metabolism: viable epidermis comprises several metabolizing enzymes which may interact with drugs and change them to active or inactive metabolites before reaching the circulation. Both topical bioavailability and pharmacodynamic activity of drugs may change, for example; testosterone is 95% metabolized in the viable epidermis layer (Hadgraft, 2001).

1.4. Penetration Enhancement

Various approaches have been anticipated by many researchers to enhance the passage of drug molecules through the skin by overcoming the normal barriers functions which impede most of the foreign molecules including drugs from penetrating through the skin (Thomas and Finnin, 2004). Many substances are available to enhance chemically drugs permeation through the skin, which can further be increased by physical methods.

1.4.1. Chemical Penetration Enhancers

A reversible damage or alteration in the nature of the stratum corneum is the mechanism of action of most chemical penetration enhancers. Such substances can achieve these changes via increasing the hydration of the stratum corneum and/or changing the lipid and lipoprotein structure. These enhancers include:

Solvents: liquids like water, alcohol, ethanol, propylene glycol, glycerol isopropyl palmitate, etc may enhance the penetration process by swelling the polar pathway of the stratum corneum and/or liquefying the lipids of this membrane.

Surfactants: these compounds are supposed to improve permeation by reversibly disturbing the stratum corneum (Shokri *et al.*, 2001). The commonly used surfactants are:

i. *Anionic surfactants*: these surfactants are potent permeation enhancers, though they can irritate or interact strongly with the skin. Examples include dioctyl sulpho succinate, sodium lauryl sulphate, etc.

ii. *Cationic surfactants*: these surfactants are reported to be more irritating to the skin than the anionic surfactants, thus they are very rarely used for enhancing skin permeation.

iii. Non-ionic surfactants: these are the surfactants with the least potential to cause skin irritation. Examples are Tweens and Spans, which are widely used in topical dosage forms (Black, 1993).

1.4.2. Physical Methods for Enhancing Topical Drug Delivery

Iontophoresis: it is a technique that utilizes an electric current of appropriate polarity to deliver ionic or charged molecules into a skin tissue by the passage of an electrolyte solution containing these ionic molecules (Prausnitz *et al.*, 1996).

Electro-poration: this is a method that relies on causing rapid dissociation of the stratum corneum via the application of transiently high voltage electrical pulse of 250 volts. The rapid dissociation involves structural and conductance changes in the cell membranes causing skin pore size and subsequently absorption to increase (Prausnitz *et al.*, 1996).

Sonophoresis: low frequency ultrasound waves of (25 kHz) are used in this method to improve penetration.

Phonophoresis: in this technique, an ultrasound-coupling agent is placed over the area on the skin to be treated. This area is then massaged with an ultrasound source. The movement of drugs through skin under the ultrasound perturbation is known as phonophoresis.

Vesicular Concept: the technology of various vesicles, like liposomes, niosomes and transferosomes can be employed to enhance drugs penetration as they can reversibly alter the permeability of the cell membranes.

Microfabricated Microneedles Technology: in this technique, silicon micro-needles are prepared to load in various drugs. The introduction of micro-needles into the skin creates conduits for the transfer of the drug through the stratum corneum. This is usually followed by further diffusion of drug into the systemic circulation (Prausnitz, 2004, Teo *et al.*, 2006).

1.5. Semisolid Topical Drug Delivery Systems

Topical medications involved mainly two semi-solid dosage forms that are creams and ointments. Ointment is a water-free topical delivery system utilizing lipophilic vehicles, such as liquid paraffin to dissolve drugs. Conversely, cream is an aqueous system comprising water, oil and surfactant or emulsifier. The emulsifier is used to keep the oil dispersed consistently in the water or vice versa. Therefore, creams can be classified as oil in water (o/w) dispersed systems, where oil represents the internal phase and water is the external phase and inversely water in oil (w/o) dispersed systems. In other words, creams are said to be o/w or w/o emulsions that have a colloidal gel structure (Junginger, 1994). Emulsions, micro-emulsions and nano-emulsions possess many advantages that render them efficient carriers for many drugs required to be topically delivered (Williams, 2007b).

1.5.1. Emulsions

Emulsion is a mixture of at least two immiscible liquids, one of which is dispersed as globules, to constitute the dispersed or internal phase, in the other liquid (the continuous or external phase) that tend to contain these globules. Without the addition of the emulsifying agent as the third component such system turns immediately into two separated phases via various destabilizing processes, like creaming (or sedimentation), flocculation, coalescence or phase inversion (Masmoudi et al., 2005). In other words, the emulsifying agent is crucial for the continuation of a uniform dispersion of these immiscible liquids. Emulsifying agent usually locates itself at the interface formed between these two phases to reduce the interfacial tension normally arises upon mixing the immiscible liquids. The dispersed phase droplets' size ranges between 0.1 to 10 mm. Even though emulsions show several problems with regard to their stability, they have been used extensively to improve the solubility, absorption and bioavailability of poorly water-soluble drugs (Eccleston, 2007).

1.5.2. Micro-Emulsions

Micro-emulsion is a thermodynamically stable system formed spontaneously without an extensive mechanical input during the mixing process. It possesses very small droplets size that usually lies in the range of 20-200 nm (Kwon and Bourne, 1997, Esposito *et al.*, 2003, Cai *et al.*, 2007, Hickey *et al.*, 2010), which renders micro-emulsion to be translucent or transparent in appearance (Lawrence and Rees, 2000). The micro-emulsion free energy is expressed as (γ A) where γ is the interfacial tension that formed upon mixing the two immiscible liquids and A is the overall new interfacial area produced following the formation of the emulsion. A is largely high due to the extreme small droplets size, but γ is nearly zero, as the function of surfactants is to efficiently reduce the interfacial tension, which makes the free energy of the system almost zero.

Such excessive reduction in the interfacial tension is usually achieved by either using two auxiliary surfactants or surfactant-co-surfactant system (Moulik and Paul, 1998). The usage of adjuvant surfactants or surfactant and co-surfactant can create a synergistic effect in reducing the interfacial tension through the formation of a mixed layer at the interface. Consequently, the interfacial tension is further decreased so as to be of negative value. Such combined surfactant systems are used, since single surfactant is mostly insufficient to reduce the interfacial tension to the extent that the system can be formed spontaneously. Some non-ionic surfactants and double alkyl chain surfactant were found to be capable in producing micro-emulsion systems (Biruss and Valenta, 2008).

Micro-emulsion systems have wide pharmaceutical domains due to their superior properties over conventional emulsions. These systems are mainly characterized by three structures; the o/w, the w/o and the bi-continuous micro-emulsions. The first two types represent systems where oil micro-droplets are dispersed in the water external phase and vice versa, respectively. While the bi-continuous system is the one in which both water and oil phases are present as a continuous phase separated by a surfactants rich interfacial layer (Moulik and Paul, 1998). Bi-continuous micro-emulsions are usually associated with systems containing equivalent amounts of oil and water.

Surfactant plays an essential role in determining the type of micro-emulsion formed. Viscous micro-emulsion, such as viscous micro-emulsion with a gel structure can be produced through the usage of non-ionic surfactant mixtures. It was found that many structures can be formed in micro-emulsion systems. Some of these systems mainly showed a combination of hexagonal and cubic crystalline liquid structures (Carlfors *et al.*, 1991, Bolzinger *et al.*, 1998). Non-ionic surfactants are well known to form highly viscous gel o/w micro-emulsions through H-bond formation between the polyoxyethylene chain of the surfactants surrounding the oil droplets (Podlogar *et al.*, 2004). Lecithin also was found to form a highly viscous w/o micro-emulsion system. It has the capability to produce a worm like micelles in which the projected chains from each micelle can attach the chain of other micelles to form a network (Paolino *et al.*, 2002).

1.5.3. Nano-scaled Emulsions

Nano-scaled emulsion is another emulsion system that resembles micro-emulsions and differs from conventional emulsions in having very small size droplets where their diameter lies in the range of 20-500 nm (Sznitowska *et al.*, 2002, Pey *et al.*, 2006). It consists of two immiscible liquids in which one liquid is dispersed as fine droplets in the other one. Nano-scaled emulsions differ from micro-emulsions as they are thermodynamically unstable (Gutiérrez *et al.*, 2008); although they show high kinetic stability due to the very small dispersed droplets that reduce effectively their sedimentation or creaming (Solans *et al.*, 2005). Despite this kinetic stability, nano-scaled emulsions can be destabilized by flocculation and Ostwald ripening phenomena.

The energy preserved inside the system is the major cause of product degradation (Akabori *et al.*, 1978, Welin-Berger and Bergensthl, 2000).

Production of nano-scaled emulsions may require high energy input; particularly, when the concentration of surfactant is low. Two energy methods are reported in the literatures, namely: the high energy and low energy methods. The first method involves the input of high energy to get the system emulsified and to produce droplets in the nano-meter range. Sonication and/or high pressure homogenization can be utilized as an efficient source of energy sufficient to transform and break up the internal phase of the system into droplets of submicron size (Kentish *et al.*, 2008, Yuan *et al.*, 2008). On the other hand, the low energy method comprises phase inversion and temperature inversion techniques. Phase inversion is achieved by the gradual addition of the external phase to inverse the system into a nano-sized dispersion (Maestro *et al.*, 2008). While, in temperature inversion technique, the temperature is firstly raised and then reduced to change the hydrophilic nature of the surfactant that results in a nano-scaled dispersed system (Solans *et al.*, 2005).

O/w nano-scaled emulsions have been extensively studied and widely used for parenteral and topical applications (Benita and Levy, 1993b). Topically, they are appropriate in delivering lipophilic drugs as the inner oil phase of nano-scaled emulsions can solubilize and accommodate these lipophilic drugs (Sonneville-Aubrun *et al.*, 2004). In contrast, w/o nano-scaled emulsion has only been mentioned in publication recently (Gutiérrez *et al.*, 2008).

Micro-emulsions and nano-emulsions offer many advantages to be used in delivering drugs topically. They have a high drug-solubilization power and their various constituents provide synergistic effect to enhance drug delivery. Oil, water and surfactants mixture or surfactant-co-surfactant mixture can combine synergistically to enhance the drug flux (Kreilgaard, 2001). Additionally, the small droplets size is the driving force which had contributed to the high interest and active investigation of these topical drug delivery systems.

1.5.3.1. Oils Used as Enhancers of Nano-scaled Emulsion Topical Delivery Systems

Some oil substances, such as oleic acid (fatty acid) and isopropyl myristate (isopropyl ester) frequently used to produce micro- and nano-scaled emulsions have been studied extensively as they have been shown to have drug-permeation enhancing properties. Chemically, oleic group is an unsaturated alkyl chain comprising 18 carbon atoms and demonstrating a cis-configuration. Oleic acid was found to increase the flux of 5-fluorouracil through human skin by 56 times (Goodman and Barry, 1989). Additionally, this oil improved significantly the topical absorption of amino acid and naloxane (Aungst *et al.*, 1986). Likewise, isopropyl myristate has been mentioned as a considerable permeation enhancer for topical delivery of steroids (Peltola *et al.*, 2003, Djordjevic *et al.*, 2004).

Ester wax, a naturally occurring wax found in jojoba oil, is widely used as an oil phase in the preparation of various creams and ointments. Physically, this oil is highly stable and has the capability to produce emulsion of reduced droplets size (Chung *et al.*, 2001). Its ability to enhance the permeability of drugs through the skin is derived from its protective and occlusive effects on the skin, which consecutively improves skin hydration that leads to a higher penetration rate (Lautenschläger, 2003).

1.5.3.2. Aqueous Phase in Nano-scaled Emulsion Topical Delivery Systems

Several researchers have reported the usage of water and phosphate buffer pH 7.4 as the aqueous phase in producing micro-emulsions for topical applications (Alvarez-Figueroa and Blanco-Méndez, 2001, Trotta *et al.*, 2003). In all aqueous solutions, water is involved as the major component, which can loosen the tightly bounded lipid lamellae of stratum corneum as its absorption leads to the hydration of this layer (Walters and Roberts, 1993a).

1.5.3.3. Surfactants Used in Nano-scaled Emulsion Topical Delivery Systems

Basically, the lipid bilayer of stratum corneum renders it a strong barrier to various exterior substances; therefore, materials that can disturb the lipid bilayer are capable of making stratum corneum a less effective barrier. Ionic and non-ionic surfactants were found to show such effects. Irreversible disturbance of these lipid bilayers is extremely discouraged as it could lead to serious skin complications. Ionic surfactants were found to promote irreversible changes; therefore, they are not preferred to be used topically. On the contrary, non-ionic surfactants are safe. They enhance drugs flux effectively and widely used in topical drug delivery systems. However, dissimilar skin species and drug models respond differently to non-ionic surfactant enhancing effect (Black, 1993, Endo *et al.*, 1996, Williams, 2003). Table 1.1 illustrates some of the researches that were carried out on micro-emulsions using different drugs, oil phases, surfactant systems, aqueous phases and membrane models.

Table 1.1:

Overview of micro-emulsion formulations reported by various researchers

Drug	Micro-emulsion			Skin Source
	Oil phase	Surfactant/ co-surfactant	Water	
[3H] H2O	Octanol	dioctyl sodium sulphosuccinate	Water	Human
5-Fluorouracil	Isopropyl myristate	Octadecyltrimethylammoni-um bromide	Water	Mouse
8-Methoxsalen	Isopropyl myristate	Tween 80®, Span 80®, 1,2-octanediol	Water	Pig
Apomorphine hydrochloride	Isopropyl myristate, decanol	Epikuron 200®, 1, 2-propanediol benzyl alcohol	Water, Aerosil 200®	Mouse
Ascorbic acid	Isopropyl palmitate, cetearyl octanoate	Dodecylglucoside cocoamide propylbetaine, phosphatidylcholine/2-ethyl-1,3-hexanediol	Water	-
Ascorbyl palmitate	Mygliol 812®	Labrasol®/Plurol Oleique®	Water	-
Diclofenac	Isopropyl palmitate	Lecithin	Water	Human
Diphen-hydramine hydrochloride	Isopropyl myristate	Tween 80®, Span 20®	Water	Human
Felodipine	Isopropyl myristate, benzyl alcohol	Tween 20®, Taurodeoxycholate	Water, Transcut-ol, carbopol	Mouse

Table 1.1. Continue

Glucose	Octanol	Dioctyl sodium, sulphosuccinate	Water	Human
Hemato-porphyrin	Isopropyl myristate, decanol, hexadecanol, oleic acid, monoolein	Lecithin, monohexylphosphate, benzyl alcohol	Water	Mouse
Indomethacin	Isopropyl palmitate	Lecithin	Water	Human
Ketoprofen	Oliec acid, Triacetin, MYvacet	Labrasol, Cremophor, RH	Water	Rat
Lidocaine	Isostearylic isostearate	Labrasol, Plurol, Isostearique	Water	Rat
Methotrexate	Decano	Lecithin, benzyl alcohol, Labrasol / Plurol	Water, PG	Mouse
Methotrexate	Ethyl oleate	Isostearique	Aq. 145 mM NaCl (pH 7.4)	Pig
Methotrexate	Isopropyl myristate	Tween 80, Span 80, 1,2-octanediol	Water	Pig
Nifedipine	Benzyl alcohol	Tween 20, taurodeoxycholate	Water, Transcutol, PG	Mouse
Prilocaine hydrochloride	Isostearate	Labrasol, Plurol, Isostearique	Water	Rat
Propanolol	Isopropyl myristate	Polysorbate 80	Water	Artificial
Prostaglandin E	Oleic acid	Labrasol, Plurol, Oleique	Water	Mouse
Prostaglandin E	Gelucire	Labrafac, Lauroglycol	Transcutol, Water	Mouse
Sodium salicylate	Isopropyl myristate	Tweens 21®, 81 and 85/ bis- 2-(ethylhexyl) sulphosuccinate	Water, gelatin	Pig
Sucrose	Ethyl oleate	Labrasol, Plurol, Isostearique	Aq. 154 mM NaCl	Mouse

*This table is adapted from (Kreilgaard, 2002).