FORMULATION AND EVALUATION OF PALM OIL ESTERS BASED NANO-EMULSION FOR TOPICAL DELIVERY OF KETOPROFEN

By

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
C	Carbon atom
ºC	Degree of centigrade
cm	Centimetre
cm^2	Centimetre square
CV	Coefficient variance
conc.	Concentration
d	Diameter
DW	Distilled water
Eq	Equation
Fig.	Figure
g	Gram
HLB	Hydrophile-lipophile balance
HPLC	High performance liquid chromatography
hr.	Hour
Kp	Permeation coefficient
L	Limonene
M	Molar
mg/ml	Milligram per millilitre
mg/kg	Milligram per kilogram
µg/ml	Microgram per millilitre
µm	Micrometre
µl	Microlitre
mins	Minutes

ml	Millilitre
mm	Millimetre
NE	Nano-emulsion
NSAIDs	Non-steroidal anti-inflammatory drugs
nm	Nanometre
ng	Nano-gram
ng/ml	Nano-gram per millilitre
n	Number of replications
o/w	Oil in water
o/w/o	Oil in water in oil
%	Percentage
POE	Palm oil esters
rpm	Rotation per minute
®	Registered trade mark
SAA	Surfactant or it's mixture
S.D.	Standard deviation
t	Time
T	Temperature
TEM	Transmission electron microscopy
v/v	Volume per volume
w/o	Water in oil
w/o/w	Water in oil in water
w/w	Weight per weight

LIST OF PUBLICATIONS

JOURNAL PUBLICATIONS:

1. **M.H.F.Sakeena,** Elrashid S.M., Muthanna F.A., Ghassan Z.A., Kanakal M.M., Lia laila., Munavvar A.S., Azmin M.N. (2010). Effect of limonene on permeation enhancement of ketoprofen in palm oil esters nanoemulsion**.** Journal of Oleo Science. 59 (7); 395-400.

2. **M.H.F.Sakeena,** Muthanna F.A., Ghassan Z.A., Kanakal M.M., Elrashid S.M., Munavvar A.S., Azmin M.N. (2010). Formulation and *in-vitro* evaluation of ketoprofen in palm oil esters nanoemulsion for topical delivery. Journal of Oleo Science. 59 (4); 223-228.

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PRESENTATIONS (ORAL & POSTER):

1. **M.H.F.Sakeena,** M. F. Abdulkarim, G.Z.Abdullah, J.Akram, Munavvar Zubaid A.S., Azmin M.N. Formulation and stability of ketoprofen loaded nano-sized emulsion prepared by using palm oil esters (POE) as oil phase.3rd International conference on Postgraduate education, Pulau Penang, December 2008 (Oral).

2. **M.H.F.Sakeena,** M. F. Abdulkarim, G.Z.Abdullah, Munavvar Zubaid A.S., Azmin M.N. Pre-formulation of ketoprofen-loaded palm oil esters in water emulsion for topical delivery. 22nd Federation of Asian Pharmaceutical Associations Congress (FAPA), Singapore. November 2008 (Poster).

FORMULASI DAN PENILAIAN EMULSI NANO BERASASKAN ESTER MINYAK SAWIT UNTUK PENYAMPAIAN KETOPROFEN SECARA TOPIKAL

ABSTRAK

Dalam kajian ini emulsi dengan nisbah surfaktan atau dengan campuran (Tween 80®, Tween 85®, dan Span 20®) yang berbagai disediakan dengan menggunakan ester minyak sawit (POE) sebagai fasa minyak. Ketelusan cahaya melalui campuran tersebut digunakan sebagai penanda visual menandakan emulsi nano telah terbentuk dan dipastikan dengan mengukur saiz titisan melalui spektroskopi penyerakan laser (Nanophox). Kawasan gel pada diagram fasa segitiga yang dihasilkan diguna sebagai satu criteria bagi menentukan kesesuaian nilai HLB agen emulsi nano untuk POE dan untuk memilih pelbagai formula yang berpotensi. Telah diketahui bahawa kawasan gel yang terbesar terhasil daripada HLB 15, 13.72 dan 12. Daripada diagram 3 fasa tersebut, 3 poin yang sama iaitu A, B dan C telah dipilih dan campuran POE dan surfaktant seperti terkandung dalam mereka diguna untuk menentukan kelarutan ketoprofen. Kelarutan ketoprofen dalam POE dengan surfaktan HLB 15 pada poin C telah ditentukan sebagai yang tertinggi (134.33 mg/ml). Berdasarkan kepada kelarutan dan data fasa segitiga, surfaktan HLB 15 dengan POE telah dinilai sebagai campuran minyak-surfaktan yang paling baik untuk menyediakan emulsi nano mengandungi ketoprofen. Kajian terhadap pembebasan dan pemindahan drug melalui membrane selulosa metil asetat dan kulit tikus dalam 'Franz diffusion cells' telah dilaksanakan. Ketoprofen yang terbebas dan dipindahkan dibandingkan dengan produk yang berada di pasaran. Profil pelepasan dan pemindahan *in vitro* tersebut menunjukkan peratusan ketoprofen yang

mencukupi terbebas melalui membrane selulosa tersebut. Pelepasan dan pemindahan ketoprofen dari emulsi nano tanpa peningkat penyerapan lebih rendah dengan signifikan berbanding dari produk di pasaran. Peningkatan kepekatan limonen sebagai ajen peningkat penyerapan didapati meningkatkan penembusan ketoprofen dari emulsi nano yang diformulasikan. Keputusan yang diperolehi menunjukkan bahawa emulsi nano dengan kadar 3% limonen menghasilkan ketelapan ketoprofen yang serupa melalui kulit tikus bila dibandingkan dengan formulasi yang berada di pasaran dan dipilih sebagai formulasi optimum. Ujian anti-inflamatori dan analgesik dari formulasi optimum yang dipilih dilakukan terhadap tikus. Keputusan ujian tersebut menunjukkan bahawa emulsi nano mengandungi ketoprofen dan 3% limonen mempunyai kesan anti-inflamatori dan analgesik lebih tinggi dan signifikan berbanding control. Akan tetapi tiada perbezaan yang signifikan berbanding produk di pasaran. Dari hasil kajian ini dapat dirumuskan bahawa emulsi nano POE mengandungi ketoprofen yang telah diformulasikan mempunyai potensi yang besar sebagai penghantar topikal ketoprofen.

FORMULATION AND EVALUATION OF PALM OIL ESTERS BASED NANO-EMULSION FOR TOPICAL DELIVERY OF KETOPROFEN

ABSTRACT

In this study emulsions with varying ratios of surfactant or its mixtures (Tween 80®, Tween 85® and Span 20®) were prepared using POE as the oil phase. The transparency of the mixtures was used as visual indications that nano-emulsions were formed and were reconfirmed by measuring the droplets size using laser scattering spectroscopy (Nanophox). The gel area of the triangle phase diagrams produced was used as one of criteria to determine the suitable HLB value of the nano-emulsion's emulsifier for POE and for selection of several promising formulae. It was noted that largest gel area produced by HLB 15, 13.72 and 12. From these three phase diagrams three common points namely A, B and C were selected and mixtures of POE and surfactant constituted in them were used to study the solubility of ketoprofen. The determined solubility of ketoprofen in POE with surfactant HLB 15 point C was the highest (134.33 mg/ml). Based on the solubility and triangle phase diagram data, surfactant HLB 15 with POE was judged as the best surfactant- oil mixture for preparing ketoprofen loaded nano-emulsion. The study on drug release and transfer through the cellulose membrane and rat skin in Franz diffusion cells were carried out. The *in vitro* release profile shows sufficient percentage of ketoprofen was released through the membrane. The transfer of ketoprofen through rat skin from nano-emulsion containing no enhancer was found to be significantly lower than from the product available in market. Utilization of limonene as a penetration enhancer increased the permeation of ketoprofen from the formulated nano-emulsion with increasing concentrations of limonene. The results obtained showed that nano-emulsion with 3% limonene produced similar and comparable skin permeation of

ketoprofen with marketed formulation thus selected as the optimum formula. The anti-inflammatory and analgesic effects of the selected optimum formulation were performed on rats. The results indicated that ketoprofen nano-emulsion had significant anti-inflammatory and analgesic effects than control. However, there is no significant different between the effects of POE nano-emulsion formulation and marketed formulation. From these results it can be concluded that the POE nano-emulsion of ketoprofen formulated has great potential for topical delivery of ketoprofen.

CHAPTER 1

INTRODUCTION

1.1 EMULSIONS

Emulsion is a dosage form that has been traditionally used for a considerable period in pharmacy. Emulsions can be design for oral, parental and topical use. Emulsion is defined as heterogeneous system comprising at least two immiscible liquid phases where one liquid dispersed as globules (dispersed phase) in the other liquid (continuous phase) (Otto *et al*., 2009, Sherman, 1983). Colloidal drug delivery systems such as micro-emulsion and nano-emulsion are increasingly focused by formulators in recent years. It is an important formulation type for poorly water soluble drug in pharmaceutical dosage form (Shakeel *et al*., 2008, Shafiq *et al*., 2007) because, optimization of the solubility of poorly water-soluble drugs in pharmaceutical dosage forms presents a challenge, due to restrictions of solvents suitable for drug delivery. Formulation can also plays a key role in influencing the absorption and bioavailability of poorly water soluble drug (Moshfeghi and Peyman, 2005, Service, 2004). Depending on the nature of the dispersed and continuous phase, different types of emulsions can be distinguished as following:

1.1.1 OIL IN WATER (O/W) EMULSIONS

Emulsions with an oleaginous internal phase and aqueous external phase are referred to as oil-in-water (o/w) emulsions. These types of emulsions are important in the preparation of poorly water soluble actives /drugs for cosmetic and medicinal

formulations. O/w emulsions are formulated for parental and topical administration but also for oral and ocular drug deliveries (Tamilvanan and Benita, 2004). These are the type of emulsions most commonly used and popular especially in topical delivery because they have initial cooling effects due to evaporation of water, thus giving a good feeling to the skin (Miller *et al*., 1999). Moreover o/w emulsions do not make the skin look very shiny and are less likely to block the pores. These properties will increase their acceptance by the users (Swarbrick and Boylan, 1996).

1.1.2 WATER IN OIL (W/O) EMULSIONS

Emulsions having an aqueous internal phase and an oleaginous external phase are termed as water-in-oil (w/o) emulsions. These are less popular emulsions when compared to o/w emulsion. This type of emulsion forms a thin film on the skin surface and this will control dehydration (Mitsui *et al*., 1997).

1.1.3 W/O/W OR O/W/O EMULSIONS

Multiple or w/o/w and o/w/o emulsions are defined as emulsions with both types (o/w and w/o) exist simultaneously, where a dispersed phase is contained within another dispersed phase and they have at least two types of surfactants (Florence and Whitehill, 1982). The entrapment of active ingredient in the inner dispersed phase can lead to the delayed release and extended duration of action.

1.1.4 NANO-EMULSIONS

Nano-emulsions are a class of emulsions with very small and uniform droplets size, typically in the range of 20 – 500 nm (Porras *et al*., 2008, Usón *et al*., 2004). Due to their small droplets size, most of nano-emulsions are transparent or translucent, resembling micro-emulsions (Usón *et al*., 2004). In contrast to micro-emulsions, nano-emulsions are not thermodynamically stable (Tadros *et al*., 2004), but they may have high kinetic stability because their small droplets size makes them stable against sedimentation and creaming (Tadros *et al*., 2004). All of these characteristic properties have led to an increase use of nano-emulsions in many different applications related to chemical, pharmaceutical and cosmetic.

In the literature, this type of liquid/liquid dispersions are also referred to as submicron emulsions (Sznitowska *et al*., 2001) mini-emulsions and ultrafine emulsions (Nakajima *et al*., 1990). The term nano-emulsion is increasingly used because it gives a clear idea of the nano-scale range of the droplets size, avoiding misinterpretations with other kinds of dispersions such as micro-emulsions (Solans *et al*., 2005).

The nano-emulsion delivery system is an effective formulation option, especially for poorly water soluble actives or drugs (Shakeel *et al* ., 2007). The major challenge of poorly water soluble substances is their efficacy can be severely limited by instability or poor solubility in the vehicle. Nano-emulsion system offers enhance drug solubility and bioavailability of lipophilic and poorly water soluble drugs (Shafiq *et*

al., 2007). The nano-sized droplets leading to an enormous increase in total interfacial areas associated with nano-emulsion would influence the transport properties of the drug (Shakeel *et al*., 2008, Shafiq-un-Nabi *et al*., 2007, Lawrence and Rees, 2000,).

Nano-emulsions can be prepared by using a high-energy dispersion method (Leong *et al*., 2009, Mao *et al*.,2009) or by spontaneous emulsification method or titration method (Shafiq-un-Nabi *et al*., 2007, Shakeel *et al*., 2007, Shafiq *et al*., 2007, Sadurní *et al*., 2005). Preparation of nano-emulsions by spontaneous emulsification method offers much more advantages than using high-energy method. High energy method using mechanical energy such as high-shear energy or high pressure energy could be too costly and formed high amount of 'foams' during processing, which will led to difficulty in handling (SolÃ¨ *et al*., 2006). Preparation of nano-emulsion by spontaneous process is simple and can be achieved with mild agitation (Shafiq-un-Nabi *et al*., 2007). In this process nano-emulsions can be prepared by adding water to an oil and surfactant mixture and agitate simply by a magnetic stirrer at a constant temperature (Shakeel *et al*., 2007).

The transparency of nano-emulsions help us to assess them for any microbial growth and allow inspection for the presence of undissolved drug. The transparency is also useful in topical preparation, because a clear system is aesthetically more pleasing. In a nutshell nano-emulsions are potential drug carriers for oral, topical and parental administrations. They offer several advantages such as spontaneous formation, ease

of manufacturing and scale up, kinetic stability, transparency, improved drug solubilization and enhanced bioavailability.

Table 1.1 shows a summary of some work carried out on nano-emulsions which were reported in literature during last two decades. It is noted that in recent years i.e. 2009, 2008 and 2007, the published papers has increased enormously on nano-emulsion than in '90s (see Table 1.1). Most of these papers reported they used the non-ionic surfactants such as Tween 80® to formulate nano-emulsion. It is also important to note that, most of nano-emulsions formulated for topical delivery were oil-in-water type formulations.

Table 1.1: Summary of some literatures on nano-emulsion published in the past years.

Oil	Emulsifier	Type	Drug / active	Route	References
Palm oil esters	Span 20®	o/w	lipophilic drugs	Transdermal	Abdul Rahman *et al*., 2009
Capmul MCM	Tween 80®	o/w	Risperidone	Transmucos	Kumar *et al*., 2009b
Oleic acid	Tween 20®	o/w	Amlodipine	Transdermal	Kumar *et al*., 2009a
Virgin coconut oil	Emulium kappa	o/w	Kojic dipalmitate	Topical	Al-Edresi, 2009
Soybean oil	Tween 80®, Span 80®	o/w	Cisplatin	Intravesical	Hwang *et al.,* 2009
Lauroglycol 90	Transcutol	o/w	Caffeine	Transdermal	Shakeel and Ramadan, 2009
FCaproyl	Tween 80®		Nitrindipine	Intranasal	Jain and Patravale, 2009
Oleic acid	Tween 20®		Domperidone	Transdermal	Akhter *et al*., 2008
Benzylc	Solutol	o/w	Ketoprofen	Transdermal	Kim *et al*., 2008
Palm oil esters	Tween 20® , Span 80®	o/w	lipophilic drugs	Transdermal	Basri *et al*., 2008
Soybean oil	Tween 80®		Camphor, Menthol	Transdermal	Mou *et al*., 2008,
Soybean oil	Tween 80®	o/w	Aspirin	Oral	Subramanian *et al*., 2008
Oleic acid	Tween 80®	o/w	Carvedilol	Transdermal	Dixit *et al*., 2008
Sefsol	Tween 80®	o/w	Celecoxib	Transdermal	Baboota *et al*., 2007
Sefsol	Tween 80®	o/w	Ramipril	Oral	Shafiq *et al*., 2007
Castor oil	Tween 80®	o/w	Aceclofenac	Transdermal	Shakeel *et al*., 2007
Camphor EL	Miglyol	o/w	Lidocaine	not mention	Sadurní *et al*., 2005
not mention	not mention	o/w	Stroidal and non-steroidal drugs	Transdermal	Friedman *et al*., 1995
not mention	not mention	o/w	Diazepam	Transdermal	Friedman *et al*., 1994

1.2 TRANSDERMAL DELIVERY

Transdermal drug delivery has many advantages over most of other conventional routes of administration which include; avoidance of the hepatic first-pass metabolism, enhanced therapeutic efficacy, better patient medication compliance and reduced systemic side effects (Park *et al*., 2000). Therefore, there has been an increased interest in recent years in pharmaceuticals and cosmeceuticals fields to use topical formulations to deliver drugs or actives through the skin.

1.2.1 THE HUMAN SKIN

The skin is the largest organ of the body, accounting for more than 10% of body mass and the one that enables the body to interact most intimately with its environment. It is one of the most extensive, readily accessible organs and is the heaviest single organ of the body which combines with the mucosal linings of the respiratory, digestive and uro-genital tracts to form a capsule which separates the internal body structures from the external environment (Barry, 1983).

A diagrammatical cross-section through human skin is presented in Figure 1.1. The skin consists of three layers namely the epidermis, dermis and subcutaneous tissue. There are also several associated appendiges such as hair follicles, sweat ducts, apocrine glands and nails (Buck, 2004; Wiiliams, 2003;Barry, 1983).

The epidermis is divided into four anatomical layers namely stratum basale, stratum spinosum, stratum granulosum and stratum corneum (Wiiliams, 2003, Barry, 1983)

as shown in Figure 1.1. The stratum corneum is the heterogeneous outermost layer of the epidermis and is approximately 10-20μm thick. The cells of the stratum corneum, keratinocytes originate in the viable epidermis and undergo many morphological changes. The keratinocytes are metabolically active and capable of mitotic division (Buck, 2004, Wiiliams, 2003).

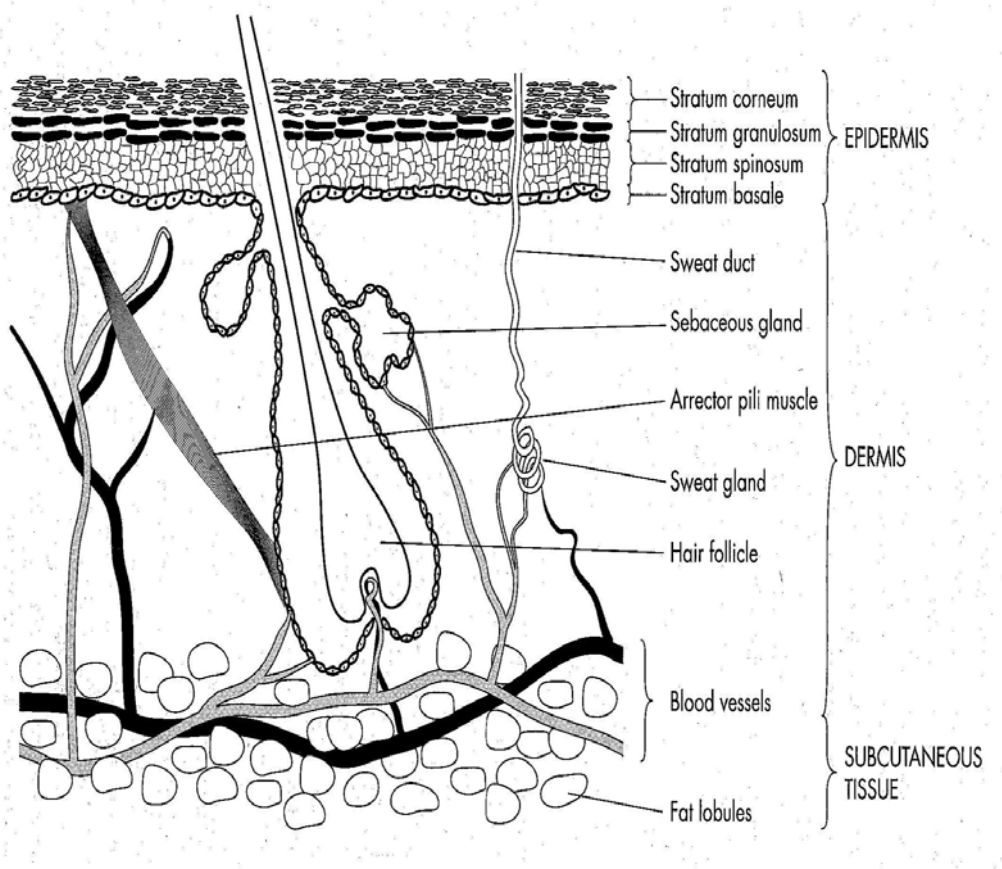

Figure 1.1: A diagrammatical cross-section through human skin (Skin cross section diagram (2010),World Wide Web: Google™)

The dermis (or corneum) at 3 to 5 mm thick, is much thicker than the overlying epidermis which it supports and thus makes up the of the skin (Barry, 1983). The dermis, which provides the elasticity of the skin, contains immune cells and has the

vascular network that supplies the epidermis with nutrients that can carry absorbed substances in to the body (Buck, 2004). Blood vessels, nerves and lymphatic vessels cross this matrix and skin appendages (endocrine sweat glands, apocrine glands and pilosebaceous unit).

1.2.2 ABSORPTION THROUGH THE SKIN

For transdermal delivery to be effective, drugs have to enter into the viable skin in sufficient quantities to produce a therapeutic effect. The possible pathways for transdermal drug delivery are intercellular routes and shunt routes as have been reported by Williams (2003). Drugs are transported through appendages such as hair follicles, transcellular transport through the corneocytes and intercellular transport via the extracellular matrix (Barry, 1983). Although the routes of drugs penetration through the stratum corneum include follicular regions, sweat ducts and intact stratum corneum, they are influenced by skin condition, skin age, blood supply, species variation, hydration and concentration of the drug being administered. Furthermore, the characteristics of the applied agents also influence the penetration (Buck, 2004).

1.2.3 NANO-EMULSIONS FOR TOPICAL DELIVERY

Nano-emulsions as a topical carrier, offer many significant advantages such as good permeation ability and high drug-loading capacity (Tadros *et al*., 2004). Several NSAID's were successfully incorporated into nano-emulsion system (Table 1.1.).

Nano-emulsions are reported to be suitable for efficient delivery of active ingredients through the skin because the large surface area of the emulsion system allows rapid penetration of actives (Tadros *et al*., 2004). Due to their small droplets size, nano-emulsions can penetrate through the 'rough' skin surface and this enhances the penetration of actives or drugs. The transparent nature of the nano-emulsion system, their fluidity (at reasonable oil concentration) as well as the absence of any thickeners may give them a pleasant aesthetic character and skin feel preferred by patients and consumers (Tadros *et al*., 2004).

Several drugs have been incorporated into nano-emulsion system for transdermal delivery (Akhter *et al*., 2008, Shakeel *et al*., 2008, Shakeel *et al*., 2007, Schwarz *et al*., 1995, Friedman *et al*., 1995, Friedman *et al*., 1994, Subramanian *et al*., 2008). These researchers have concluded that nano-emulsion formulation may enhance the permeation through skin over the conventional formulation.

Even though nano-emulsions show several advantages for topical delivery; some attempts are reported in literature to enhance the topical uptake of the drug from nano-emulsion, for example, positive submicron emulsions with permeation enhancement effect were studied (Youenang Piemi *et al*., 1999). There is a finding (Mou *et al*., 2008) that nano-emulsion system with terpenes as enhancers (5% camphor, 5% menthol) shows good stability and powerful permeation ability for the topical delivery of a lipophilic drug.

The success of a transdermal drug delivery system depends on the ability of the drug to penetrate the skin in sufficient quantities to maintain the therapeutic level. Absorption through the skin is limited by a poor penetration of drugs across the stratum corneum (Park *et al*., 2000) therefore it is very important to overcome the skin barrier properties or stratum corneum. Many strategies have been studied in order to overcome the skin barrier or stratum corneum and to enhance the permeability of drugs through the skin. A popular approach is the use of penetration enhancers, which enhance the permeability of the stratum corneum (Barry, 2001, Southwell and Barry, 1984). Penetration enhancers interact with the lipids of the outermost layer of the skin which lead to better penetration of co administered drugs (Bialik *et al*., 1993) and increased the rate of drug penetration (Guy and Hadgraft, 1987).

1.2.4 USE OF TERPENES IN TOPICAL DELIVERY

Terpenes like menthol, cineole and limonene were used extensively in transdermal delivery system for many years to enhance the penetration of lipophilic drugs (Jain *et al*., 2002, Takayama *et al*., 1991). Terpenes, constituents of volatile oils exhibit excellent permeation-enhancing effects to facilitate transdermal drug delivery (Vaddi *et al*., 2002) and have been shown to enhance the permeation of both lipophilic drugs, such as testosterone and hydrophilic drugs, such as propranolol (Vaddi *et al*., 2002).

Terpenes are a very safe and effective class of penetration enhancers, obtained from natural sources. The FDA classifies them as generally regarded as safe (GRAS) (Vaddi *et al*., 2002). They cause no skin toxicity or if any, only mild irritation (Krishnaiah *et al*., 2003). Even though terpenes are considered to be skin irritants; they did not cause lasting erythema (Okabe *et al*., 1990).

In an earlier report (Rhee *et al.,* 2001) a transdermal preparation was developed to deliver ketoprofen with permeation enhancers namely; limonene, cineole, menthol and camphor. Out of this four terpenes used, only limonene resulted in a powerful enhancing activity. It increased the permeation ability of ketoprofen by 3 fold over the control (Rhee *et al*., 2001) and found to be a good penetration enhancer for ketoprofen by many researchers (Okabe *et al*., 1992, Okabe *et al*., 1990). Based on these reports, limonene is chosen as a penetration enhancer to be used in this study to enhance the permeation of a model drug (ketoprofen) through rat skin.

1.3 CHOICE OF KETOPROFEN AS A MODEL DRUG

Several model drugs have been used to study nano-emulsions as illustrated in Table 1.1. In this study, the nano-emulsion to be formulated will be used to entrap and deliver poorly water soluble drug topically and to improve its penetration through the skin to exert the expected effects. Ketoprofen is poorly water soluble and is suitable candidate to be incorporated into nano-emulsion formulation, thus ketoprofen is chosen as model drug.

1.3.1 PHYSICO-CHEMICAL PROPERTIES

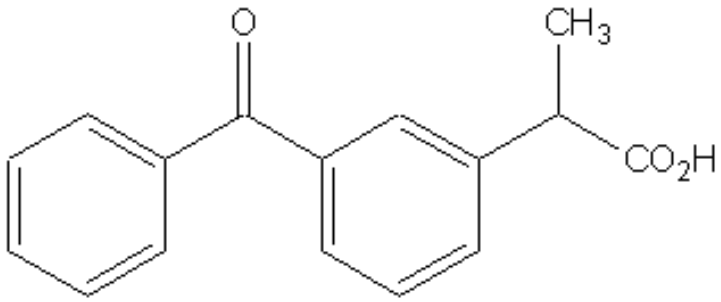

Figure 1.2: Structural formula of ketoprofen

Ketoprofen is, 3-Benzoyl-a-methylbenzeneacetic acid (Goosen *et al*., 1998). Its chemical formula is $C_{16}H_{14}O_3$ and belongs to the aryl propionic acid class. Ketoprofen is a white or almost white, crystalline and odorless powder with a sharp bitter taste (Martindale, 2002). The physico chemical properties of ketoprofen are listed in Table 1.2.

Table 1.2: The physico-chemical properties of ketoprofen (Goosen *et al*., 1998)

Physico-chemical properties	**Ketoprofen**
Chemical formula	$C_{16}H_{14}O_3$
Molecular mass	254.29
Log *p*	0.97
pKa	4.45
Solubility constraint	175.38
Melting point	94.5^0C

1.3.2 PHARMACOLOGY, PHARMACODYNAMICS AND USES

Ketoprofen is a potent non-steroidal anti-inflammatory drug (NSAID). It is used widely for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and acute gouty arthritis (Patrono and Rocca, 2009, Brooks, 1998, Kantor, 1986). It has anti-inflammatory, analgesic and antipyretic pharmacological properties. It has been frequently used to treat mild to moderate pain (Kantor, 1986). Like most NSAIDs, ketoprofen is advantageous because it lacks addictive potential and does not result in sedation or respiratory depression.

Ketoprofen inhibits the cyclooxygenase responsible for the biosynthesis of prostaglandins (Patrono and Rocca, 2009, Vane and Botting, 1996). Ketoprofen is rapidly absorbed after oral administration and maximal concentration in plasma is achieved within 1 to 2 hrs. Food reduces the rate but not the extent of absorption. The drug is extensively bound to plasma proteins (99%), and it has a half life in plasma about 2 hrs. Ketoprofen is conjugated with glucuronic acid in the liver, and the conjugate is excreted in the urine (Hardman *et al*., 2001). Adverse and side effect of ketoprofen includes; gastro-intestinal erosions such as gastric, duodenal and intestinal (Patrono and Rocca, 2009, Hardman *et al*., 2001).

1.3.2.1 ANTI-INFLAMMATORY EFFECTS

In several animal models (rats, mice, rabbits, guinea pigs and pigeons) ketoprofen displayed potent activity against acute inflammation (increased vascular

permeability, edema and erythema), sub acute inflammation and chronic inflammation (Hardman *et al*., 2001, Kantor, 1986). Its anti-inflammatory activity is 20 times more potent than ibuprofen and 160 times potent than aspirin (Hardman *et al*., 2001, Kantor, 1986).

1.3.2.2 ANALGESIC AND ANTIPYRETIC EFECTS

Ketoprofen was shown to be a potent, peripherally acting analgesic. It was also shown to be equivalent to indomethacin, slightly more potent than naproxen and 30 times more potent than aspirin in pain management (Hardman *et al*., 2001, Brooks, 1998 , Kantor, 1986). Ketoprofen is currently marketed throughout the world in a variety of forms: capsules, tablets, injectables solutions, suppositories and gels. Available formulations are tabulated in Table 1.3.

Table 1.3: Ketoprofen formulations available in the market.

Rout of administration	**Dosage form**	**Strength**
Oral	Tablets Enteric coated Capsules Extended(controlled) release	50 mg 100 mg 50 mg 75 mg 100 mg 100 mg 200mg
Parental Rectal Topical	Intramuscular Suppository Gel	100 mg / 2ml 100 mg 2.5 g / 100 g

1.3.3 KETOPROFEN AS TOPICAL DOSAGE FORM

There are several reports in literature about ketoprofen (Cordero *et al*., 2001, Cordero *et al*., 1997), which accomplishes that ketoprofen is suitable for topical dosage form, because it exhibits many physio-chemical properties for percutaneous permeation. Ketoprofen is an excellent candidate for transdermal delivery among various NSAIDs (Cordero *et al*., 1997). Its plasma levels were maintained relatively consistent for about 24 hrs after transdermal application (Chi and Jun, 1989). Ketoprofen has a relatively short half life and has the potential to be delivered topically (Jamali and Brocks, 1990). It's low molecular weight, low melting point and high lipophilicity may contribute to good skin permeability (Cordero *et al*., 1997).

Various transdermal dosage forms containing ketoprofen have been reported, including patches (Yim *et al*., 1996), gels (Chi and Jun, 1991) and ointments (GuÌˆrol *et al*., 1996), but none of these dosage form used palm oil esters (a new enzymatically synthesized palm oil) as one of their main ingredient.

1.3.4 ASSAY OF KETOPROFEN

It is important to quantify the drug in order to analyze how much ketoprofen is released or permeated through the skin (artificial membrane or biological membrane). Several methods have been reported for the analysis of ketoprofen and many numerous chromatographic methods to quantify ketoprofen in different medias

have been published; serum (Alvi *et al*., 1998), plasma (Chi and Jun, 1989) and also in *in vitro* samples for pharmaceuticals (Dejalan *et al*., 2000).

1.4 CHOICE OF OTHER MATERIALS TO PRODUCE NANO-EMULSIONS

1) Palm Oil Esters (POE)

2) Non-ionic surfactants

3) Limonene

1.4.1 PALM OIL ESTERS (POE)

Traditionally palm oil is used, mainly as cooking oil, shortening, butter substitutes, creamer, margarine, and in soap and detergent industries. With the advent of the oleo chemical industries based on palm and palm kernel oil feed stocks during the past decade; diversification of palm oil usage into nontraditional areas is gaining importance. It is beginning to find other uses in the nonfood areas, notably in cosmetics and personal care products. Research bodies in Malaysia such as Malaysia Palm Oil Board (MPOB) and Universiti Putra Malaysia (UPM) are actively modifying palm oil into better constituents having improved properties and new applications.

Palm oil consists of triglycerides, a combination of glycerol and different fatty acids. The fruit of the palm tree *Elaesis guineenis* is the source of two distinctively different oil types. The outer pulp contains palm oil and the nut in the fruit contains

kernels that are the source of palm kernel oil. Palm oil and palm kernel oil differ considerably in their characteristics and properties even though they are derived from the same plant (Keng *et al*., 2009). Palm oil is rich in C_{16} and C_{18} fatty acids, while palm kernel oil is rich in C_{12} fatty acids. These oils / fats can be further fractionated into solid and liquid fractions to yield stearins and olein, respectively. Alcoholysis of triglycerides from palm oil and palm kernel oil to produce wax esters (the new derivatives of palm oil and palm kernel) through enzymatic reaction by lipase is a relatively simple process and moreover, the starting materials are cheap (Keng *et al*., 2008, Basri *et al*., 2007, Gunawan *et al*., 2005).

The synthesis of palm oil esters (POE) was first reported by Gunawan *et al*., in 2004. In their work, they synthesized the esters using palm oil and long chain alcohols catalyzed by lipase enzyme. The work on the modification of palm oil in to palm oil esters (POE) has grown due to the possibility of obtaining a wide variety of high quality natural products under mild reaction conditions utilizing selective enzyme as environment friendly biocatalysts (Keng *et al*., 2009, Keng *et al*., 2008, Basri *et al*., 2007).

$$\text{Palm Oil} + \text{Alcohol} \xrightarrow[\text{Lipase}]{} \text{Palm Oil Esters}$$

(Gunawan *et al*., 2004)

The POE has gained interest among researchers because it can produce nano-emulsion (Abdul Rahman *et al*., 2009, Basri *et al*., 2008) and can be use as a substitute for jojoba oil which is rich in oil esters but very costly. Basri *et al*., (2008) in their study have shown that POE and nonionic surfactants can be used to produce

nano-emulsions and have suggested that POE are suitable for use as a new ingredient for pharmaceuticals and cosmeceuticals especially for topical delivery (Keng *et al*., 2009, Basri *et al*., 2008) because of their small droplets size, high droplets volume and exceptional stability**.**

A molecular dynamic study on nano-emulsions produced by POE and non-ionic surfactants was studied by Abdurrahman *et al*., (2009). From their simulation study on the ability of palm based nano-emulsion to self-assemble and the size of the micelle resulted, they suggested that palm-based nano-emulsion has great potential to be used in transdermal delivery due to its low energy micelle formation pathway. Thus, POE are a new ingredient for pharmaceutical industry (Abdul Rahman *et al*., 2009, Keng *et al*., 2009, Basri *et al*., 2008, , Keng *et al*., 2008,). The oil has no irritation on human skin, it increases the skin hydration due to its moisturizing properties and it shows high thermal stability (Keng *et al*., 2009), it could be used in cosmetic or medicinal formulations to deliver poorly water-soluble lipophilic actives or drugs (Abdul Rahman *et al*., 2009, Basri *et al*., 2008) and is very cheap when compared to jojoba oil.

1.4.2 SURFACTANTS

Amphiphilic molecules or surfactants or emulsifiers are characterized by the presence of both polar and nonpolar regions of the same molecule. This dual nature is responsible for the surface activity of these substances leading to accumulation at hydrophobic interfaces and formation of aggregates (Lawrence and Rees, 2000). Surfactants constitute the most important components of the emulsions. Generally,

these are water soluble surface-active agents comprised of a hydrophobic portion, usually a long alkyl chain, attached to hydrophilic functional groups. Surfactant molecules will arrange themselves at the oil-water interface, reducing the interfacial free energy and tension hence increasing the thermodynamic stability of the emulsion formed (Khair, 2005). Emulsifying agents can be divided into three groups; synthetic agents, naturally occurring materials and finely divided solids. The synthetics groups are divided into anionic, cationic, non-ionic and amphoteric (having both anionic and cationic groups on the hydrophilic portion of the same molecule) agents.

According to the reports from Abdulrahman *et al*., (2009) and Basri *et al*., (2008), the nano-emulsions could be prepared by mixing POE, water and nonionic surfactants without need of co-surfactants. Abdulrahman *et al*., (2009) used only Span 20® while Basri *et al*., (2008) used Span 20® and Tween 60® in their formulations. Both of these studies attracted us to choose non-ionic surfactants in our study, to formulate POE nano-emulsions without the aid of co-surfactant.

There are several reports that conclude, non-ionic surfactants could be able to form nano-emulsion without the need for a co-surfactant (Lawrence and Rees, 2000). This is helpful because it reduce the complexicity of phase behavior and eliminates the requirements for inclusion of medium chain alcohols / co-surfactants (Lawrence and Rees, 2000). Non-ionic surfactants such as Tween 80®, Tween 85® and Span 20® are selected as the emulsifier in this study due to their lack of their toxicity (Florence and

Rogers, 1971). Moreover these non-ionic surfactants are less susceptible to acidic or alkaline hydrolysis as well as to decomposition by micro-organisms.

Non-ionic surfactants have the ability to produce surfactant mixtures having wide range of Hydrophile-lipophile balance (HLB) values. HLB concept was first introduced by Griffin in 1949 (Pasquali *et al*., 2009) and developed in the 1950s. This concept has a mean of characterizing the surfactant (Pasquali et al., 2009). HLB system provides the formulators with relevant information regarding emulsion formulation (Ishii and Nii, 2005). By HLB method, each agent is assigned to HLB value or number indicating the substance's polarity. The usual range is between 1 and 20. Materials that are highly polar or hydrophilic have been assigned to higher numbers than materials that are less polar or lipophilic.

W/o emulsions can be obtained by using a surfactant with a low HLB (3-6), while o/w emulsions stabilization requires a higher HLB (8-18). Algebraic manipulation is necessary when a blend of surfactants, with known HLBs, is used for a particular emulsification (Pasquali *et al.,* 2009). Emulsifiers such as blend of Tween 20® and Span 20® having high HLB value will form an o/w emulsion. On the other hand Span 60® alone having a HLB of 4.7, tends to form a w/o emulsion. The type of emulsion produced is a function of the relative solubility of surfactant (Martin, 1993). Thus surfactants with high HLB are preferentially soluble in water and resulted in the formation of o/w emulsions while surfactants of low HLB are less soluble in water and tend to produce w/o emulsions

1.4.3 LIMONENE

Limonene is a hydrocarbon lipophilic terpene, obtained from the lemon peel of *Citrus limon*. It is a chiral molecule and as is common with such molecules, biological sources produce one specific enentiomer : D-limonene, ((+) – limonene). Racemic limonene is known as dipentene. (http://www.en.wikipedia.org/wiki/Limonene). Limonene was shown to enhance the percutaneous penetration of ketoprofen in several reports (Rhee *et al*., 2001, Okabe *et al*., 1992, Okabe *et al.,* 1990) and chosen as a suitable candidate to be used in this study as a penetration enhancer of ketoprofen through biological membrane.

1.5 SUMMARY AND PROBLEM STATEMENT

Nano-emulsions offer several advantages as drug delivery systems and according to the studies by many researchers in this field; nano-emulsions have been formulated as parental, transdermal and oral delivery systems as well as topical preparations. Nano-emulsions offer the advantages of having a large surface area, ability to solubilize lipophilic drugs and relatively good stability and may improve the bioavailability of drug.

Ketoprofen is a non-steroidal anti-inflammatory, analgesic and antipyretic drug used for the treatment of rheumatoid osteoarthritis, ankylosing spondylitis and gout. It is more potent than the other non-steroidal anti-inflammatory drugs (NSAIDs) with respect to some effects such as anti-inflammatory and analgesic activities.

Although ketoprofen administered orally is rapidly absorbed, metabolized and excreted, it causes some gastrointestinal complaints such as nausea, dyspepsia and some renal side effects like other NSAIDs. Therefore, there is a great interest in developing topical dosage form of these NSAIDs to avoid the oral side effects and provide relatively consistent drug concentrations at the application site for prolonged period. POE is new oil, which is cheap but rich in esters and is a suitable candidate for use as a substitute of jojoba oil, which is very expensive.

1.6 SCOPE AND AIMS OF THE STUDY

The main aim of this study is:

To formulate o/w nano-emulsions containing non-ionic surfactants as emulsifier and palm oil esters (POE) as internal phase and water as the continuous phase, and ketoprofen as the model drug for transdermal delivery. To achieve this, the study was planned with following objectives.

- To construct ternary phase diagrams with POE and various non-ionic surfactants (different HLB) and water, this will be used to select several promising formulae.

- To determine the solubility of ketoprofen in POE alone and POE combination with non-ionic surfactants.

- To determine the droplets size and transmission electron microscopic properties of nano-emulsion prepared.

- To develop a simple high performance chromatographic (HPLC) method for quantification of ketoprofen in samples collected from Franz diffusion cells after being transferred through methyl acetate cellulose membrane and through rat skin.

- To study the transfer of ketoprofen through methyl cellulose membrane and rat skin (*in-vitro-test*) and to compare with marketed product.

- To study the effect of penetration enhancer (limonene) on optimized formulation and to compare the release with the marketed formulation.

- To conduct the pharmaco-dynamic studies; anti-inflammatory studies by carrageenan induced paw edema test and analgesic studies by hyperalgesia pain threshold test.

- To study and evaluate visually for any irritation caused by the formulation.