FORMULATION AND CHARACTERIZATION OF PALM OIL ESTERS BASED NANO-CREAM FOR TOPICAL DELIVERY OF PIROXICAM: STUDY OF *IN VITRO* RELEASE AND *IN VIVO* ANTI-INFLAMMATORY AND ANALGESIC EFFECTS

by

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FORMULASI DAN PENCIRIAN KRIM-NANO BERASAKAN ESTER MINYAK SAWIT UNTUK PENYAMPAIAN PIROKSIKAM SECARA TOPIKAL: PELEPASAN *IN VITRO* DAN KESAN ANTI-INFLAMOTORI DAN ANALGESIK *IN VIVO*

oleh

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Tesis yang diserahkan untuk
memenuhi keperluan bagi
Ijazah Sarjana Sains

UNIVERSITI SAINS MALAYSIA

JUN 2010

This work is dedicated to my beloved parents Fawzy and Nawal, my wife Asraa, my daughter Dania and son Harith, my brother and sister.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	Percent
±	Plus minus
H	Viscosity
°C	Degree celsuis
1/s	1/second
ANOVA	Analysis of variance
AUC	Area under the curve
B	Beta
Buff	Buffer
C0	Initial Hind Paw Thickness
C_0	Initial drug concentration
Cm	Centimeter
cm/sec	Centimeter/second
cm^2	Square centimeter
cm^3	Cubic centimeter
Conc.	Concentration
COX	Cyclooxygenase
Ct	Hind paw thickness at time
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	Formulation
*E*r	Enhancement ratio
et al.	And others
F	Shear stress
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
hr	Hour
HSD	Harmonic sample distribution
Jss	Steady state flux
kg	Kilogram
kHz	Kilo hertz
Ko/w	Amount of drug in the oil / amount of drug in buffer
Kp	Permeability coefficient
Log p	Partition coefficient
M	Molar
m	Meter
mg	Milligram
mg/kg	Milligram per kilogram

mg/ml	Milligram per milliliter
µg	Microgram
µl	Microlitter
µm	Micrometer
µs	Microsiemens
ml	Milliliter
ml/min	Milliliter per minute
mM	Millimolar
mm	Millimeter
mPa	Millipascal
mv	Millivolt
NaOH	Sodium hydroxide
ng	Nanogram
nm	Nanometer
NSAID	Nonsteroidal anti-inflammatory drug
O/W	Oil in water
OH^-	Hydroxide ion
p	Probability
Pa	Pascal
pKa	Acid dissociation constant
POEs	Palm oil esters
rpm	Revolution per minute
S	Solution of oil and surfactants to measure the drug solubility
SD	Standard deviation
SEM	Standard error of mean
SANS	Small angle neutron scattering
SAXS	Small angle X-ray scattering
sec	second
Surf	Surfactant
$T_{50\%}$	Half time of drug release
TEM	Transmission electron microscopy
TEWL	Transepidermal water loss
UV	Ultraviolet spectrophotometry
UV-VIS	Ultraviolet-visible spectrophotometry
V	Volt
W/O	Water in oil
w/v	Weight by volume
w/w	Weight by weight
X_{10}	10% of reading of particles size measurements by nanophox
X_{50}	50% of reading of particles sizes measurements by nanophox
X_{90}	90% of reading of particles sizes measurements by nanophox

FORMULASI DAN PANCIRIAN KRIM-NANO BERASAKAN ESTER MINYAK SAWIT UNTUK PENYAMPAIAN PIROKSIKAM SECARA TOPIKAL: PELPASAN *IN VITRO* DAN KESAN ANTI-INFLAMOTORI DAN ANALGESIK *IN VIVO*

ABSTRAK

Sejak beberapa tahun kebelakangan ini, terdapat peningkatan dalam penggunaan sistem pembara topikal yang depat membantu penelapan drug melalui kulit. Drug yang terlibat biasanya yang mempunyai masalah bila diambil secara oral, contohnya: mudah terhidrolisis dalam saluran gastrousus, biokereroleban yang rendah atau menyebabkan tindak balas mudarat. Satu daripada drug tersebut ialah piroxicam. Ia merupakan anti-inflamasi, tik dan analgesik yang amat efektif, tetapi menyebabkan pendarahan dan ulser gastrousus. Satu daripada sistem pembira ini yang berpotensi menelapkan drug secara trans-dermal ialah krim-nano, iaitu suatu emulsi separa pepejal ber titisan 20-200 nm. Tujuan utama kajian ini dijalankan adalah untuk memformulasikan suatu krim-nano novel yang mengandungi piroxicam untuk penyampaian topikal dan menggunakan minyak ester sawit (POEs) suatu minyak sawit terderivertis yang baru sebagai fasa minyak.

Dalam kajian awal, rajah-rajah fasa pseudoternari bagi campuran air, POEs dan surfaktan bukan ion pelbagai nilai HLB telah hasilkan dan beberapa formula krim-nano telah dipilih. Keterlarutan dan pekali pembahagian piroxicam dan kesan drug ini terhadap cirik formulasi formula yang dipilih pag dikaji. Kestabilan pada suhu yang

berbeza dan sifat formulasi yang lain termasuk perlakuan reologi, potensi zeta, saiz titisan dan ciri-ciri struktur formulasi juga dikaji.

Berdasarkan kajian awal yang dilakukan pada beberapa formulasi yang dipilih, suatu formula dengan komposisi asas 25:37:38 POEs; fasa akues: campuran surfaktan (80% Tween 80: 20% Span 20 HLB 13.72) telah diguna untuk menghasilkan beberapa krim-nano dengan menggunakan penampan fosfat masing-masing pada pH 4, pH 6 dan pH 7.4 sebagai fasa luaran. Kemampuan formulasi tersebut untuk melepaskan piroxicam menembusi membran selulosa asetat dan ketebalan kulit tikus dinilai secara *in vitro* dengan menggunakan sel pembauran Frantz (Frantz diffusion cell). Formulasi E15 dan E16 dikenal pasti sebagai berpotensi dan kesan-kesan farmakodinamik dinilai serta dibandingkan dengan satu sediaan yang terdapat di pasaran. Kesan anti-inflamasi diukur dengan menggunakan kaedah edema tapak kaki belakang yang diindus oleh karagenan dimana isipadu edema diukur berdasarkan ketebalan tapak kaki. Aktiviti analgesis ukurkan sebagai respons ambang kesakitan.

Sebagai kesimpulan, krim-nano yang dirumus melalui penggunaan POEs sebagai fasa minyak dan penampan fosfat pH 6 dan 7.4 sebagai fasa luarnya serta mengandungi 0.5% piroxicam, didapati mempunyai cirri-ciri reologi, saiz titisan serta kestabilan yang sesuai. Krim-nano (pH 7.4) E16 menunjukkan perpindahan drug secara *in vitro* melalui membran selulosa asetat dan ketebalan kulit tikus yang lebih baik jika dibandingkan dengan krim-nano (pH 6) dan sediaan rujukan yang diperolehi dipasaran. Hal ini mungkin disebabkan nilai fluksnya yang lebih tinggi. Krim-nano (pH 7.4) E16 menunjukkan aktiviti anti-inflamasi dan analgesis terbaik di kalangan sediaan-sediaan yang diuji.

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ABSTRACT

During recent years, there has been increased interest in the use of topical vehicle systems that could assist drug permeation through the skin. The drugs of interest usually those causing or having problems when given orally, e.g., easily hydrolyzed in the gastro-intestinal tract, poor bioavailability or to cause adverse reactions. One such drug is piroxicam, a highly effective anti-inflammatory, anti-pyretic and analgesic but causes gastro-intestinal ulcers and bleeding. One of the most promising vehicle systems for trans-dermal permeation of drugs is a nano-cream, a semisolid emulsion with droplet size of 20-200 nm. The main purpose of this study is to formulate a novel nano-cream containing piroxicam for topical delivery and to use palm oil esters (POEs) a new derrivetised palm oil as the oil phase. In the initial study, pseudoternary phase diagrams of water, POEs and non-ionic surfactant mixture of several HLB values were constructed and several promising nano-cream formulae were selected. The stability under different temperatures and other properties of the formulations including the rheological behaviour, zeta potential, droplet size and structural characteristics of the formulation and Solubility and partition coeffecient of piroxicam were studied.

Based on the initial studies conducted on selected several formulae, a formula with 25:37:38 basic composition of palm oil esters: aqueous phase: surfactant mixture (80% Tween 80: 20% Span 20 HLB 13.72) was used to prepare several nano-creams using phosphate buffer pH 4, pH 6 and pH 7.4 respectively as the external phase. The abilities of these formulae to deliver piroxicam through cellulose acetate membrane and full thickness rat skin were assessed *in vitro* using Frantz diffusion cell. E15 and E16 were identified as the promising formulae and their pharmacodynamic effects were evaluated and compared with a preparation available in market. Anti- inflammatory effect was measured by using carragennan-induced hind paw edema method where the edema volume was measured in term of paw thickness. The analgesic activity was measured as the pain threshold response. In conclusion, the 0.5% nano-creams formulated by using the POEs as the oil phase and phosphate buffer pH 6 and 7.4 as external phase were found to have suitable rheological, droplet size and stability properties. Nano-cream (pH 7.4) E16 had shown a better *in vitro* transfer through cellulose acetate membrane and full thickness rat skin as compared to nano-cream (pH 6) and a product available in market. This could be due to the higher flux calculated. The E16 nano-cream (pH 7.4) showed the best anti-inflammatory and analgesic activities among the preparations tested.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Since the last decades, the treatment of illness has been accomplished by administrating drugs to humans via various means which are namely oral, sublingual, rectal, parental, inhalation and topical delivery, etc. Topical delivery can be defined as the application of 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 the drug to the surface of the skin, within the skin or to tissue under the skin.

The topical application of medicines was introduced in the ancient history. Antibiotics and hormones topical delivery were administered in the 1940s. 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 local effect such as steroid for 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

Topical delivery systems include various dosage forms for example: cream, ointment and gel. These systems have numerous benefits over other conventional dosage forms as listed in 1.2.1. As for other dosage forms, these topical systems also have several disadvantages (Walters and Roberts, 2002). Their disadvantages are listed in 1.2.2.

1.2.1 Advantages of Topical Drug Delivery Systems

These dosage forms:

- Bypass the first pass metabolism which is a potential hindrance with the use of oral administration of drugs.
- Promote a patient's compliance as they are convenient and easy to apply.
- Are able to avoid the risks and inconveniences of intravenous injections.
- Can forgo varied conditions of absorption in the gastrointestinal tract GIT, like pH changes, the presence of enzymes, gastric emptying time etc.
- Can avoid the fluctuation in drug plasma levels, inter- and intra subject variations.
- Enable easy termination of the medications, when needed.
- Can be applied to a relatively large area for application of drugs in comparison with buccal or nasal cavity.
- Are suitable for self-medication.

1.2.2 Disadvantages of Topical Drug Delivery Systems

These dosage forms may:

- Cause skin irritations leading to contact dermatitis due to the use of the drug and/or excipients.
- Cause poor permeation of some drugs through the skin because of the complexity of the skin tissue.
- Be less effective for systemic delivery.
- Be used for systemic effect only when very small plasma concentration of drug is required for action.
- Be denatured by the skin enzymes present mainly in the epidermis.
- May not be absorbed easily through the skin if the size of drug particles is large.

1.3 Factors Affecting the Absorption of Drugs through Skin

The physicochemical properties of the drug and topical dosage form formulated play major roles in affecting the rate and extent of drug absorption by the skin. Moreover, the physiological conditions of the skin most probably affect the drug transportation to the systemic circulation (Wiechers, 1989). Some of these factors are also mentioned in Section 1.3.3.

1.3.1 Physicochemical Properties of Drug Substances

The physicochemical properties of the topical administered drugs are:

- Partition coefficient: Ideally, the partition coefficient into octanol-water system should be between 1 to 4 for a successful topical drug delivery (Naik *et al*., 2000).
- Drug solubility: The higher the solubility of the drug is in the vehicle, the greater is the permeation of the drug through the skin.
- Concentration: When the drug concentration in the vehicle is above the saturated level, then there would be an increase in the permeability of the drug. The mechanism of enhancement via drug supersaturation would therefore be based simply on the increased thermodynamic activity of the drug in the vehicle, i.e. an increased driving force for its transition out of the formulation into and across the stratum corneum. However, the major limitation of this technique is that once the drug concentration is increased, then the supersaturated formulations are typically subject to recrystallization of the drug substance (Williams, 2003).
- Particle size: The smaller the particle size is, the greater is the surface area and the permeation of the drug through the skin. In this regard, the nano and micro-emulsion systems are of prime interest.
- Polymorphism: The most soluble polymorph of the drug should be chosen for the preparation of the formulation as it would affect the permeability through the skin.
- Molecular weight: A drug with a molecular weight less than 400 dalton would easily permeate through the skin tissues (Naik *et al*., 2000).

1.3.2 Release Properties of Topical Drug Delivery

If drug molecules present in the internal phase of emulsion system, then the drug needs to be partitioned from the internal phase to the external phase first before being absorbed by the skin. It is important for the release from the dosage form to be rapid if a fast absorption and effect are needed. The release of drug from a dosage form may be affected by the composition of drug delivery system. Some of the excipients in the formulation may enhance the absorption and some may retard the absorption of the drugs. For **e**xample, propylene glycol of low molecular weight may reduce permeation and absorption of drug while polyethylene glycols may cause the opposite effect.

1.3.3 Physiological and Pathological Conditions of Skin

The physiological and pathological properties of the skin which could affect the drug permeation are:

- Effect of horny Reservoir layer: The horny layer of the skin may bind with drug molecules and act as a depot for the drug in therapy.
- Lipid film: The lipid film formed naturally on the skin surface would act as a protective layer to prevent moisture loss from the skin.
- Skin hydration: Hydration of the stratum corneum by an occlusive cover applied over it would prevent moisture loss from the skin and would enhance the absorption of drugs.
- Skin temperature: When there is an increase in body temperature, it results in an increase in the rate of skin permeation. This may be due to an increased thermal

energy which may enhance drug diffusion, and may increase the solubility of the drug in skin tissues at higher temperature as well as the vasodilation of the blood vessels.

- Regional variation: The difference in the nature and thickness of the skin in different parts of the body would lead to the difference in permeability to drugs. The rate of permeation increases in an anatomic order: Plantar anterior fore arm, scalp, ventral thigh, scrotum and posterior auricular area.
- Pathologic injuries of the skin: When there is an injury to the skin, in particular to the stratum corneum, there would be an increase in permeability.
- Cutaneous drug metabolism: The metabolising enzymes present in the viable epidermis may metabolise the drug to an inactive metabolite before reaching the circulation. This would lead to a reduced topical bioavailability and decreased pharmacodynamic activity of the drug. For example, Testosterone is 95% metabolised in the viable epidermis layer (Hadgraft, 2001).

1.4 Penetration Enhancement

Barrier function of the skin would normally not allow most of the foreign molecules including drugs to penetrate into the skin. Therefore, different approaches have been proposed by various researchers to enhance the penetration of the drug molecules through the skin (Thomas and Finnin, 2004). The enhancement of drugs permeation through the skin can be achieved either by chemical enhancers or by physical methods.

1.4.1 Chemical Penetration Enhancers

A chemical skin penetration enhancer increases skin permeation by reversibly altering or damaging the nature of the stratum corneum. The alteration includes increased hydration of stratum corneum and/or a change in the lipid and lipoprotein structure. The various chemical penetration enhancers are:

Solvents: These solvents enhance the permeation process possibly by swelling the polar pathway through the stratum corneum and/or by fluidizing the lipids of the membrane. Examples of these solvents include water, alcohol, methanol ethanol, propylene glycol, glycerol isopropyl palmitate.

- **Surfactants:** These compounds are proposed to enhance the permeation by reversibly affecting or damaging the strateum corneum. The commonly used surfactants are:
    i. Anionic surfactants**:** They are strong permeation enhancer but they are irritants to the skin. They may even 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. So, they are very rarely used for permeation enhancement.
    iii. Nonionic surfactants: These are the surfactants with least potential of causing skin irritation. Examples are Tweens and Spans etc. which are widely used in topical delivery prepartions (Black, 1993).

1.4.2 Physical Methods for Enhancing Topical Drug Delivery

The physical techniques that can enhance drug permeation are as follow:

- **Iontophoresis**: Iontophoresis is a technique that is used to transport ionic or charged molecules into a skin tissue through a passage of an electrolyte solution containing ionic molecules that will be delivered by using an electric current of appropriate polarity (Prausnitz *et al.*, 1996).
- **Electroporation**: This method involves an application of transient high voltage electrical pulse (250 v) to cause rapid dissociation of the stratum corneum. The rapid dissociation involves structural and conductance changes in the cell membranes thereby increasing the pore size for enhanced absorption (Prausnitz *et al.*, 1996).
- **Sonophoresis**: This method involves the usage of ultrasound waves of low frequency (25 kHz).
- **Phonophoresis**: In this technique, an ultrasound-coupling agent is placed over the area on the skin to be treated. The area of skin is then massaged with an ultrasound source. The movement of drugs through skin under the ultrasound perturbation is known as phonophoresis.
- **Vesicular Concept**: The various vesicles used for this purpose are liposomes, niosomes and transferosome. These vesicles can enhance the permeability of the drug by reversibly altering the cell membranes.
- **Microfabricated Microneedles Technology**: In this technique, drug loaded in silicon microneedles are used. After the introduction into the skin, these

microneedles will create conduits for the transfer of the drug through the stratum corneum. Once the drug passes the stratum corneum, it rapidly diffuses further and enters the systemic circulation (Prausnitz, 2004, Teo *et al.*, 2006).

1.5 Semisolid Topical Drug Delivery Systems

The two main semi solid dosage forms of topical medication are cream and ointment. The ointment is a topical delivery system where the drug is dissolved in a lipophilic vehicle, such as liquid paraffin. The ointment is a water-free system. Inversely, cream is a water-containing system with the presence of oil and surfactant or emulsifier. It can be classified as water in oil (W/O) cream when the oil constitutes the external phase and oil in water (O/W) when the water is the external phase. Therefore, creams can be defined as O/W or W/O emulsion that have a colloidal gel structure (junginger, 1994). Emulsion, micro-emulsion and nano-emulsion systems are considered as an efficient carrier for many drugs to be delivered topically (Williams, 2007).

1.5.1 Emulsion

Emulsion is a mixture of two immiscible liquids. One will act as the external or continuous phase, while the other is dispersed in droplet forms. The mixture is stabilized by the addition of emulsifying agent which maintains the dispersion of the immiscible liquids. The dispersed phase droplets' sizes range between 0.1 to 10 mm. Despite its instability problems, emulsion has 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 system formed spontaneously without an extensive mechanical input. The size of the dispersed phase droplets ranges between 15 - 200 nm (Kwon and Bourne, 1997, Esposito *et al.*, 2003, Cai *et al.*, 2007). It is thermodynamically stable since the spontaneous formation process happens without a high energy input during the mixing process. The free energy of the micro-emulsion system is equal to (γ A) where A is the total of new interfacial area formed after the formation of the emulsion and γ is the interfacial tension of the system. A is high but γ is near to zero since the surfactant should reduce efficiently the interfacial tension, thereby making the free energy of the system almost to zero. Usually, single surfactant cannot reduce the interfacial tension to the extent that the system can be formed spontaneously (Moulik and Paul, 1998). Thus, there is a need for the addition of second surfactant or co-surfactant.

Double alkyl chain surfactant and some non-ionic surfactants were found to be able to produce this emulsion system (Biruss and Valenta, 2008). The addition of another surfactant or co-surfactant can reduce the interfacial tension so as to be of negative value. The surfactant mixture or surfactant with co-surfactant can work synergistically in reducing the interfacial tension through the formation of a mixed layer at the interface.

In general, there are three main structures that can be accounted for the micro-emulsion system. These are the O/W (where oil micro-droplets are dispersed in the water external phase), the W/O (where water micro-droplets are dispersed in the oil external phase) and the bi-continuous micro-emulsion (where the water and oil phase are presented as a continuous phases separated by a surfactants rich interfacial layer)

(Moulik and Paul, 1998). Bi-continuous micro-emulsion is usually related with the system containing an equal amount of oil and water. Surfactant is the essential factor for determining the type of micro-emulsion. Viscous micro-emulsion can be produced through the usage of particular surfactants. The use of nonionic surfactant mixture results in the formation of viscous micro-emulsion with a gel structure. A combination of many structures was found to have been formed in micro-emulsion system. These systems include combination of hexagonal and cubic crystalline liquid structures which are formed in the micro-emulsion produced (Carlfors *et al.*, 1991, Bolzinger *et al.*, 1998). Nonionic O/W micro-emulsion can form a highly viscous gel structure through the formation of a network between the H-bonds of the polyoxyethylene chain of the surfactants surrounding the oil droplets (Podlogara *et al.*, 2004). Lecithin can form a highly viscous W/O micro-emulsion system through the formation of a worm like micelles, where the oriented chains from each micelle will attach the chain of the other micelle to form a network (Paolino *et al.*, 2002).

1.5.3 Nano-Emulsion

Nano-emulsion is another emulsion system comprising of two immiscible liquids in which one liquid is dispersed as fine droplets in another liquid . It differs significantly from the conventional emulsion in its droplets size which range between 20-200 nm in diameter. For its production, energy input is necessary espacially when the concetration of surfactant is not very high. Although the formed dispersion is thermodynamically unstable, it is kinetically stable due to the very small particle size that reduces the

sedimentation or creaming of the dispersed droplets (Solans *et al.*, 2005). O/W nano-emulsions have been extensively studied and widely used for the parenteral and topical application (Benita and Levy, 1993). It is suitable for the topical delivery of lipophilic drugs where the lipophilic drugs are dissolved in the inner oil phase (Sonneville-Aubrun *et al.*, 2004). W/O nano-emulsion on the other hand has only been mentioned in publication recently (Gutiérrez *et al.*, 2008).

The high energy method and the low energy method are the main techniques used in the preparation of nano-emulsion. The high energy method includes the input of high energy for the emulsification of the system in order to produce nano sized dispersion droplets . Either sonication or high pressure homogenization is used to provide a high energy that is sufficient enough to transform and break up the internal phase of the system into droplets of submicron in size (Kentish *et al.*, 2008, Yuan *et al.*, 2008). The phase inversion method is a low energy technique where the external phase is added gradually to inverse the system to nano sized dispersion (Maestro *et al.*, 2008). In another low energy technique, i.e. the temperature inversion method,the temperature is initially raised and then reduced to change the hydrophilic nature of the surfactant that results in a nano dispersion system (Solans *et al.*, 2005).

Although the nano-emulsion system is kinetically stable, this system can be destabilized by subjecting it to the flocculation and Ostwald ripening phenomenon. The energy conserved within the system is the main cause for the degradation of the product (Akabori *et al.*, 1978).

Micro-emulsion and nano-emulsion have many advantages to be used as topical drug delivery system. The high solubilization of different compounds into this dosage form, the synergistic effect of various components to enhance drug delivery, as well as the small droplet size are the driving forces which had contributed to the high interest and active investigation of these topical drug delivery systems. The main ingredients; oil, water and surfactant mixture or surfactant, co-surfactant mixture can combine synergistically to enhance the drug flux (Kreilgaard, 2002).

1.5.3.a Oils Used as Enhancers of Nano-Emulsion Topical Delivery Systems

Fatty acids (such as oleic acid and isopropyl esters like isopropyl myristate) present in the micro and nano-emulsion are topical enhancer oils that have been studied extensively. Oleic is an unsaturated C18 alkyl chain which has cis-configuration. It was found that oleic acid has the ability to raise 5 fluorouracil flux through the human skin by 56 folds (Goodman and Barry, 1989). Oleic acid was also detected to improve the topical absorption of amino acid and naloxane efficiently (Aungst *et al.*, 1986). Similarly, 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 is an oil phase used in the preparation of creams and ointments. Ester wax is found naturally in the jojoba oil. This type of oil has unique properties of high physical stability and in the reduction of emulsion droplet size (Chung *et al.*, 2001). It has a protective and occlusive effect on the skin which in turn can hydrate the skin and increase permeability through the skin (Lautenschläger, 2003). The use of jojoba oil in

the formulation of lipogel was discovered to improve fluconazole flux by 1.5 folds as compared to the use of cutina oil (Kreilgaard, 2002).

1.5.3.b Aqueous Phase in Nano-Emulsion Topical Delivery Systems

Water as well as phosphate buffer pH 7.4 were mainly incorporated as the aqueous phase of topical delivery micro-emulsion system by various researchers (Alvarez-Figueroa and Blanco-Méndez, 2001, Trotta *et al.*, 2003). The absorption of water leads to the hydration of the stratum corneum that enhances drug permeation through the loosening of the tightly lipid lamellae (Walters and Roberts, 1993).

1.5.3.c Surfactants Used in Topical Delivery Systems

Ionic surfactant and non-ionic surfactant can disturb the lipid bilayer of the stratum corneum thereby making it a less effective barrier to the permeation of the drug. Ionic surfactant however, was found to induce irreversible changes in the structure of the stratum corneum lipid bilayer, hence it is not preferable for use in the topical delivery. In contrast, non-ionic surfactants are widely and safely used for the topical delivery system. Different skin species and different drug models respond differently to the enhancing effect of non-ionic surfactant (Black, 1993, Williams, 2003, Masayuki *et al*., 1996). Listed in Table 1.1 are some of the researchers carried out on micro-emulsion using different combination of drug, oil phase, surfactant system, aqueous phase and membrane model.

1.5.4 Techniques Used in the Structural Characterization of the Nano- and Micro-Emulsion Systems

The identification of micro- and nano-emulsion system structures can be carried out by using various instruments like Scanning Electron Microscopy, TEM (Transmission Electron Microscopy) (Danino *et al.*, 2002), SAXS (Small Angle X-ray Scattering) (Glatter *et al.*, 2001) and SANS (Small Angle Neutron Scattering) (Silas and Kaler, 2001). Scanning electron microscopy and TEM are electron microscopy instruments which make use of the beam of highly condensed and monochromic electrons to identify the size, shape, surface feature and structure of the samples. Viscosity and conductivity measurement can also indicate the structure of these systems (Djordjevic *et al.*, 2004).

Table 1.1: Overview of micro-emulsion formulations reported by various researchers.

Drug	Micro-emulsion			Membrane/ skin
	Oil phase	Surfactant/ co-surfactant	Water	
[3H] H_2O	Octanol	dioctyl sodium sulphosuccinate	Water	Human
5-Fluorouracil	Isopropyl myristate	Octadecyltrimethylammonium 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

Continued of Table 1.1				
Felodipine	Isopropyl myristate, benzyl alcohol	Tween 20, Taurodeoxycholate	Water, Transcut-ol, carbopol	Mouse
Glucose	Octanol	Dioctyl sodium, sulphosuccinate	Water	Human
Hematoporphyr-in	Isopropyl myristate, decanol, hexadecanol, oleic acid, monoolein	Lecithine, 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, Transcuto l, 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	Transcuto l, Water	Mouse
Sodium salicylate	Isopropyl myristate	Tween 21/81/ 85, bis- 2-(ethylhexyl) sulphosuccinate	Water, gelatin	Pig
Sucrose	Ethyl oleate	Labrasol, Plurol, Isostearique	Aq. 154 mM NaCl	Mouse

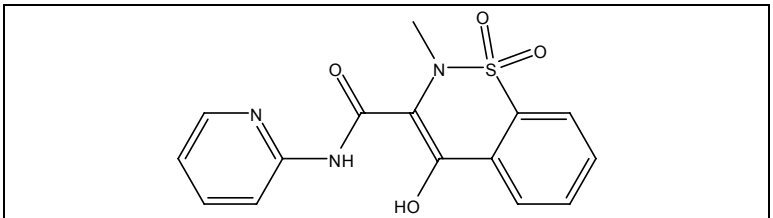
*This table is adapted from (Kreilgaard, 2002)

1.5.5 Properties of Semisolid Topical Delivery System

The topical preparation containing drugs must have special rheological properties. The preparation must stick to the skin when it is applied to ensure drug delivery at the site of application. This means that the topical preparation must be as a solid form under rest condition as well as in the liquid form under stress condition when the mechanical force is applied to remove the dose from the container. Hence, the name semi-solid preparation was introduced to describe these unique dosage forms. Semi-solid preparation is defined as a gel structure preparation containing a drug for topical delivery. A gel structure is formed by the formation of a solid network in which a liquid is incorporated to form a sponge-like structure. The gel form does not flow when the force applied is lower than the elastic strength of the network but will flow at certain viscosity when the applied force exceeded the elastic strength. This unique rheological flow is known as viscoelastic or plastic flow (Barry and Meyer, 1973).

1.6 The Main Ingredients Selected in This Study:

1.6.1 Piroxicam



(4-hydroxy-2-methyl-N-(2-pyridyl)- 2H- 1,2-benzothiazine- 3- carboxamide- 1,1 -dioxide)

Fig. 1.1: Chemical Structure of piroxicam.

1.6.1.a Physicochemical Properties

Piroxicam is a crystalline powder, off-white to light yellow in colour with a bitter taste (USP 27, 2003). Piroxicam can be crystallized from an ethanolic solution through a fast cooling technique to form a needle shaped crystal. On the other hand, the crystallization of the ethanolic solution through the slow cooling technique will result in the formation of cubic crystals. Piroxicam is neither soluble in water nor in cyclohexane. It is sparingly soluble in diisopropyl ether and slightly soluble in ethanol and isopropanol solvent. It is soluble in some polar organic solvents like dimethyl formamide, dimethyl sulfoxide and chloroform and to a less extent in dioxane, acetonitrile and acetone. This drug is a weak acid compound with a pKa value of 5.3 and its partition coefficient (Log P octanol/phosphate buffer pH 7.4) is 1.8 (Mihalic, 1986).

1.6.1.b Pharmacodynamics

Piroxicam is a non-steroidal anti-inflammatory drug (NSAID) which shows its anti-inflammatory and analgesic activity through a non selective inhibition of cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2) receptors.

1.6.1.c Pharmacokinetic Properties

The group of enolic acid NSAIDs has a long half life. As for piroxicam, it is 50 hr. Hence, the steady state blood concentration is achieved after 7-12 days after

oral administration. The plasma protein binding is as high as 99%. Piroxicam metabolism is mainly accomplished in the liver through hydroxylation and conjugation with glucouronic acid. It is excreted through the urine with 5% of the excreted drug remaining unchanged. It has been reported that there is a presence of piroxicam in the breast milk of mother who take piroxicam orally. Piroxicam is well absorbed from the GIT and the plasma peak level is achieved after a duration of between 3-5 hr (Martindale, 2002). On the other hand, Betadex a modified tablet form containing piroxicam and β cyclodextrine has the ability to reach the peak plasma level for piroxicam within 30-60 minutes. Topically, Piroxicam is absorbed from gel through the skin slowly but incompletely. The peak plasma level is reached only after 4 days of the application of piroxicam gel twice a day (Marks and Dykes, 1994)

1.6.1.d Dosage and Administration

Piroxicam is available in the market in the form of 10 mg and 20 mg capsules to be taken as a single dose daily. Piroxicam Betadex is offered as a 191 mg of a mixture of piroxicam with β cyclodextrine containing 20 mg of piroxicam that is to be given once daily (BNF 47, 2004). Sublingual tablet of 20 mg is affordable by everyone in the market. Piroxicam is also available as 0.5% gel for topical application. Suppositories containing 20 mg or 10 mg of piroxicam are available in the market too. Besides, a 20 mg/ml intramuscular injection is also available in the market.

1.6.2 Oil

Palm oil esters (POEs) is constituent of a modified form of Palm olein oil known simply as palm oil. A main and important agriculture commodity of which received great attention from researchers especially Malaysian researchers. Structurally, palm oil is a mixture of triglyceride compound. Palm oil is an edibile oil and is used through out the world as main ingredient in many food products. Palm oil, as a raw material, had also attracted many researchers to study its use in topical delivery products and in the effort to synthesize new derivatives with beneficial properties for chemical and pharmaceutical applications. Universiti Putra Malaysia (UPM) had led the way in the production of new derivatives of palm oil (Keng *et al*., 2005). Palm oil had been discovered to react with oleyl alcohol and the reaction was catalysed by the use of lipase enzyme to produce POEs contained high molecular weight esters of alcohol with an even number of carbon ranging from 12-32 (Keng *et al*., 2008). The composition of POEs and its physical properties are represented below in Table1.2 and Table 1.3 respectively.

It was found that POEs has skin hydration activity of 40.7% after 90 minutes of its application (Keng. *et al.*, 2009). The emollient effect of POEs had been proven thereby making this oil highly recommendable for its incorporation into the topical preparation as oil phase.

Table 1.2: Chemical Composition of Palm oil Eesters (Keng *et al*., 2008).

Esters	Composition (%)
Oleyl laurate	0.71- 0.97
Oleyl myristate	3.2- 4.4
Oleyl palmitate	31.1- 39.9
Oleyl stearate	4.2- 4.7
Oleyl oleate	31.4- 35.3
Oleyl linoleate	6.4- 7.1

Table 1.3: Physicochemical Properties of POEs (Keng *et al*., 2008).

Properties	Result
Physical state	Liquid
Appearance	Clear yellow
Solubility in water	Insoluble
Iodine value	76.59
Cloud Point(°C)	19.07
Viscosity (cP)	19.73
Density (g/cm³)	0.85

1.6.3 Surfactants

Non-ionic surfactants are widely used in topical preparation. These surfactants are compatible with acidic and basic media and less liable to hydrolysis and degradation by micro-organism (Idson, 1988). These surfactants are non-toxic surfactants and can be used safely for the topical delivery. It is reported that the recommended dose of Tween and Span are 25 mg/kg for human body per day (Raymond *et al.*, 2003).

Tween 80 is a non-ionic surfactant that is widely used as an emulsifier in the preparation of emulsion for cosmetics and topical applications. Structurally, it consists of four polyoxyethylene chains containing 20 carbons attached to sorbitan monooleate. The polyoxyethylene chains are responsible for the hydrophilicity of this surfactant thereby making it a water soluble surfactant with HLB value of 15. It is a yellowish viscous liquid. According to researchers, 80 is the number related to the oleate group which represents the hydrophobic part of the surfactant.

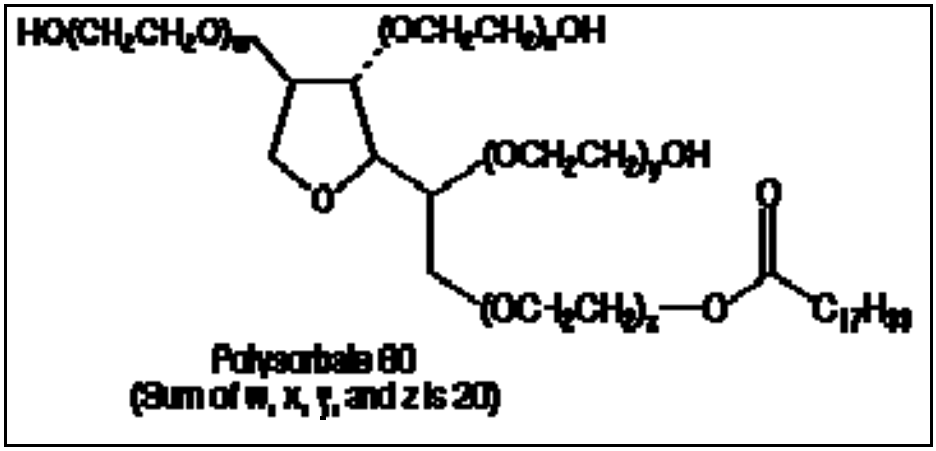


Fig. 1.2: Chemical Structure of Tween 80.

Similarly, Tween 85 consists of four polyoxyethylene chains containing 20 carbons. In fact, 85 is the number related to the tri-oleate chains that represent the hydrophobic part of the surfactant. Tween 85 is water soluble and is an amber coloured liquid surfactant with HLB value of 11.

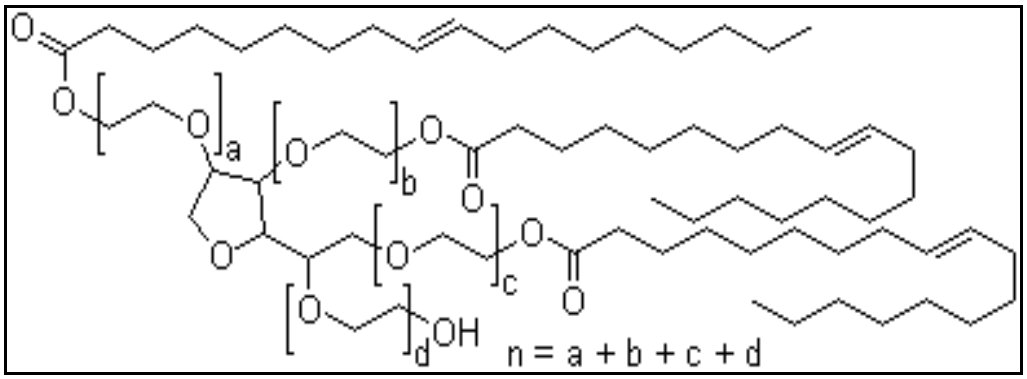


Fig. 1.3: Chemical Structure of Tween 85.

Span 20 and Span 85 are lipophilic non-ionic surfactants wich have emulsifying and solubilizing effects. They are esterified forms of sorbitol and are termed as sorbitan esters. Span 20 is a monolaurate ester of sorbitan. It is a viscous liquid which is yellow in colour with the viscosity value of 3900-4900 mPa at 25°C. The HLB value of sorbitan Monolaurate is 8.6. It is widely used in the formulations used for topical delivery. Span 85 is a trioleate ester form of sorbitan. It is an amber viscous liquid with a viscosity value of 200-250 mpa at 25 °C. It posses HLB value of 1.6.

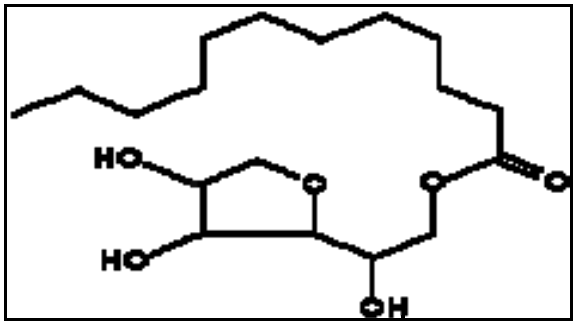


Fig. 1.4: Chemical Structure of Span 20.

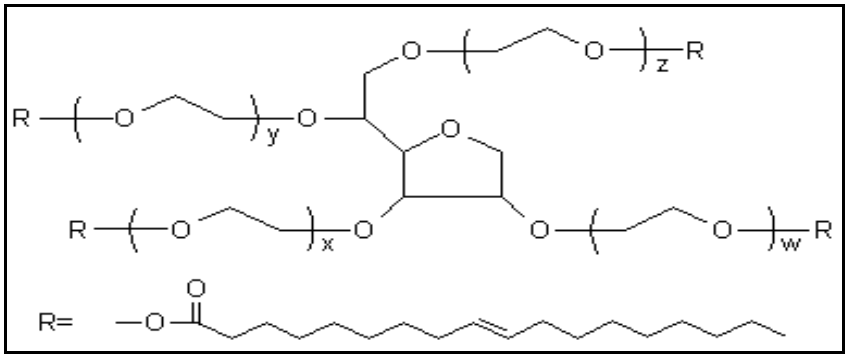


Fig. 1.5: Chemical Structure of Span 85.

1.7 Literature Review of Piroxicam Topical Drug Delivery

Piroxicam is a water insoluble drug with an acidic pKa value of 5.3. Structurally, the pyridine ring that is attached to the amide group also provides a pKa value of 1.86. Therefore, it is possible that piroxicam can act as a zwitter ionic drug at certain pH value. As a weak acid, piroxicam ionizes at pH 7.4 and at physiological pH (Jinno *et al.*,