Title

DEVELOPMENT AND FORMULATION OF WAX-BASED TRANSDERMAL DRUG DELIVERY SYSTEMS.

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Summary

Development and Formulation of wax-based transdermal drug delivery systems.
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Topical and transdermal formulations are promising platforms for the delivery of drugs. The advantages include high patient compliance and reduced variability in absorption. Disadvantages include the low numbers of suitable drug candidates due to potency and ability to permeate the skin. Also, for topical administration, variability in applied dose can be problematic. A unit dose topical or transdermal drug delivery system that optimises the solubility of drugs within the vehicle provides a novel dosage form for efficacious delivery that also offers a simple manufacture technique is desirable.

This study used Witepsol® H15 wax as a base for the delivery system. One aspect of this project involved determination of the solubility of ibuprofen, flurbiprofen and naproxen in the wax using microscopy, Higuchi release kinetics, HyperDSC and mathematical modelling techniques. Correlations between the results obtained via these techniques were noted with additional merits such as provision of valuable information on drug release kinetics and possible interactions between the drug and excipients.

A second aspect of this project involved the incorporation of additional excipients: Tween 20 (T), Carbopol® 971(C) and menthol (M) to the wax formulation. On *in vitro* permeation through porcine skin, the preferred formulations were; ibuprofen (5% w/w) within Witepsol® H15 + 1 % w/w T; flurbiprofen (10 % w/w) within Witepsol® H15 + 1 % w/w T; naproxen (5 % w/w) within Witepsol® H15 + 1% w/w T + 1 % C and sodium diclofenac (10 % w/w) within Witepsol® H15 + 1 % w/w T + 1 % w/w C + 5% w/w M.

Unit dose transdermal tablets containing ibuprofen and diclofenac were produced with improved flux compared to marketed products; Voltarol Emugel® demonstrated flux of 1.68×10^{-3} cm/h compared to 123×10^{-3} cm/h for the optimised product as detailed above; Ibugel Forte® demonstrated a permeation coefficient value of 7.65×10^{-3} cm/h compared to 8.69×10^{-3} cm/h for the optimised product as described above.

Keywords: solubility, HyperDSC, transdermal, wax, unit dose, NSAID

Dedication

This piece of work is dedicated to the memory of my dad; Mr. David Oladunjoye Oladiran (deceased) and to my mother; Mrs R.O.Oladiran and finally to my master and Lord; Jesus Christ; for giving me the priceless legacy; to attend school and learn.

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Mandeep Singh Sidhu; a final year Masters of Pharmacy student under the supervision of Dr H.K.Batchelor, carried out some of the mathematical modelling as part of his Masters of Pharmacy degree programme.

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Also, it worthy to mention my folks in the Lord: the Craig family, who allowed God to use them as a vessel to honour, Pastor Alimi, Brother Luke, Pastor Moses and all other brethren of Destiny International, Birmingham UK, who I can not state here, thank you all! May the Lord bless you richly in all measures in Jesus name, Amen.

Penultimately, I value my family; Tundun, David, Tolu, Akin, Damilola and my mum; Mrs Oladiran, for their great sacrifice to make this dream come true. Their effectual prayers and advice kept me going during the myriads of challenges along the way. I pray the Lord bless our home and may His will be fulfilled in our lives in Jesus name.

Ultimately, to Him who gives me His beauty for my ashes and makes me to prosper, I give all glory, honour, adoration and praises forever. May He receive all glory over my life, home, future and endeavours in Jesus name, Amen.

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Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder (ADHD)
APC	Antigen Presenting Cells
BP	British Pharmacopoeia
cm	Centimeter
DSC	Differential scanning calorimetry
FDA	Food and Drug Administration (United States)
g	Grammes
h	Hours
HCl	Hydrochloric acid
Hyper-DSC	Hyper- differential scanning calorimetry
HPLC	High -Performance Liquid Chromatography
J	Flux
K_H or k_H	Release rate constant
m^2 kg/s^2	Joules
min	Minutes
ml	Milliliter
mmol	Millimolar
NSAID	Non-steroidal anti-inflammatory drug
n	Sample size
P	Partition coefficient
pKa	$-\log_{10}$ dissociation constant
RPM	Revolutions per minute

r^2	Correlation coefficient
s.d.	Standard deviation
T	Temperature
UV	Ultraviolet
v/v	Volume in volume (ml/100ml)
w/v	Weight in volume (g/100ml)
%	Percentage
µg	Microgrammes
ug	Microgrammes; this convention is used within figures in place of µg on occasion
°C	Degrees celcius
µl	Microlitres
USP	United States Pharmacopoeia

Chapter One

Introduction

1.1. Introduction

The drug development process culminates in the design of dosage forms (medicines) that are not only readily available for patient use but also enhance the efficiency of the active ingredient while minimising the side effects. Drugs can be administered through several routes including oral, intra-muscular, inhalation, intra-venous, topical or transdermal.

The oral route of drug delivery is most commonly used although it is associated with limitations in terms of extensive first pass metabolism in the gut or liver, unsteady plasma profiles especially for drugs with short half lives and increased systemic side effects such as ulceration of the gut (Ranade and Hollinger, 2003). Injectables are painful and costly due to sterilisation plus the need for expert administration in many cases. Delivery via the lung is growing in interest but is less popular than skin administration. In addition, with drug delivery to the skin, patient compliance has been reported to be very high due to ease of therapy termination in problematic cases (Ranade and Hollinger, 2003). A second reason for increased compliance is associated with reduced dosing schedules for systemically absorbed drugs delivered across the skin; this is particularly relevant for drugs where long term plasma concentrations are required coupled with short elimination half-lives.

Topical administration is most often selected for surface skin disorders such as acne where local action is required whereas transdermal administration is selected for delivery of therapeutic agents into the systemic circulation. The formulations used for topical and transdermal applications can be similar, for example creams, although more often patches are used for transdermal delivery. This thesis reports the development and *in vitro* evaluation of a wax-based dosage forms administered to the skin for either topical or transdermal drug delivery.

1.2. The Skin

Healthy human skin is a metabolically active complex organ that functions mainly as the body's first line of defence against assault via the processes of continual regeneration, immunological and histological responses. Covering a surface area of between 1.5 to 2.0 m^2, it accounts for one-sixth of the total body weight (Williams, 2003). It also confers aesthetic qualities, provides protection against UV rays, helps to regulate body temperature through sweat glands and maintain homeostasis by controlling water loss. Structurally, it can be regarded as a set of bio-membranes arranged in parallel with varying properties based on physiological factors such as body location, age and health (Williams, 2003).

1.2.1. The structure of the skin

Anatomically, human skin is regarded to consist of three main structural layers; epidermis, dermis and subcutaneous layer. Figure 1.1 shows a cartoon representation of human skin.

Figure 1.1: Schematic cross section of the skin (http://skincancer.dermis.net)

1.2.3. Subcutaneous layer

The subcutaneous layer is found below the dermis and known as the subcutaneous fat layer because it is made up of lipocytes. The main cellular components of this layer are the adipocytes (Walters and Roberts, 2002). It serves as the linkage between the overlying dermis and underlying body constituents via collagen and elastin fibres; thus, anchoring the skin to underlying muscles. The lipocytes are readily available high-energy reservoirs, which help to insulate the body and also provide mechanical support against physical injury. The fat layer thickness is variable depending on site and sex; it has been reported to be very thick in the buttocks of women and abdomen of men (Williams, 2003). The fatty layer often serves as a reservoir for highly lipophilic drugs.

1.2.4. Dermis

Structurally, the dermis is a supporting tissue made up of an array of collagen and elastin fibres. It gives the skin its suppleness and elasticity with a myriad of blood vessels and nerve endings within it (Walters and Roberts, 2002). It is about 3-5mm thick (Williams, 2003).

Other functions of the dermis are inflammatory / immune response, temperature regulation via adipose cells and by arteriovenous anastomoses which shunts skin blood flow between arteries and veins (Ryan, 1973), responses to external stimuli by extensive nerve endings (Charkoudian, 2003; Steinhoff et al., 2003) and waste removal by the well connected lymphatics system (Cross and Roberts, 1993, Walters and Roberts, 2002). Katz and Poulsen (1971) stated that the three main appendages found on the human skin; hair follicles, sebaceous gland and sweat glands are rooted in the dermis.

Combs (1966) and Cowan et al., (1979) identified fibroblasts and mast cells as the main cell types of the collagen- rich dermis. The dermis is bathed by a vascularized connective tissue containing mucopolysaccharides. It is primarily proteoglycans that aid the structural network of the skin.

1.2.5 Skin appendages

These are a collection of structures that make up 0.1% of the total skin surface area and are intermittently located from the dermis to appear on the stratum corneum. The main ones are: hair follicles with associated sebaceous glands and sweat glands (apocrine and eccrine) (Monteiro-Riviere et al., 1991), sebum, made by sebeceous glands, lubricates the skin. The eccrine gland is used when the body is cooling and apocrine gland secretes skin lipids (Walters and Roberts, 2002). The type and number of appendages depends on body site and inter-species (Monteiro-Riviere et al., 1991).

1.2.6. Epidermis

The epidermis is located above the dermis and it is composed of a complex layer of keratinized cells that are constantly being eliminated by exfoliation because they are not connected to any blood vessels. It is variable in thickness with the greatest thickness (0.8mm) around the load bearing palms and soles of the feet (Williams, 2003). It is made up of about 80% water (Williams, 2003). Histologically, the epidermis comprises of four basic layers: stratum germinativum, stratum spinosum, stratum granulosum and stratum corneum.

1.2.7. Stratum germinativum (basal layer)

At the borderline between the epidermis and underlying dermis is an array of continually dividing cells mainly; keratinocytes and other cells melanocytes, that produce melanin which helps in protection against UV lights (Jimbow et al., 1993), merkel and langerhans cells (Williams, 2003). The keratinocytes produce millions of new cells daily being fed by nutrients from underlying blood vessels in the dermis. They move upwards progressively towards the skin surface as they are being pushed by cells underneath making them to become more specialized keratinized cells after losing their vital organelles. Langerhans cells (also known as the dendritic antigen presenting cells; APCs) are called to action during skin sensitisation and keratinocyte proliferation (Aiba and Katz, 1990). The other important cells are the Merkel cells that are located in the basal

region and function as sensory receptors for the peripheral nervous system (Tachibana, 1995). Five layers of keratinocytes representing the different stages of differentiation have been identified (Lavker and Sun, 1982; Monteiro-Riviere, 1991; Borradori and Sonnenberg, 1999; Walters and Roberts, 2002). Keratinocytes are made in the basal layer and proliferating cells migrate towards the stratum corneum while undergoing a series of desquamation during which phospolipids are replaced by sphingolipids (long chain saturated fatty acids). Keratinocytes are connected to the basement membrane by hemidesmosomes and to other keratinocytes by desmosomes.

1.2.8. Stratum spinosum

Situated above the germinal layer are irregularly shaped cells formed from the upward migrated keratinocytes. Together with the latter, they constitute the Malpighian layer. Within this layer, cells are differentiated more and become polygonal in shape.

1.2.9. Stratum granulosum

Beneath the stratum corneum, migrated keratinocytes flatten out and begin to die (as they are far from nutrient source); synthesizing keratin and membrane-coated granules that are the main precursors for intercellular lipid lamellae in the process.

1.2.10. Stratum corneum

The stratum corneum; also known as the 'horny layer' is made up of overlapping, flat, scale-like and dead cornified cells which are filled with keratin to make the skin surface a tough and waterproof material. Its unique constituents have been characterized to be 75-80% protein, 5-15% lipid and 5-10% unidentified dry mass (Wilkes et al, 1973). The water content of this layer varies between 10-30%. This is the layer that serves as the main barrier to the body; it thus presents a major challenge in the delivery of drugs through the skin. Elias (1981) represented the structure of the stratum corneum with a 'brick and mortar' model in which the keratinized cells are entrenched in a mortar of lipid

bilayers (Michaels et al., 1975; Menon and Ghadially, 1997); this highly lipophilic barrier prevents excessive water loss to the environment and protects against the transdermal penetration of drug molecules (Yardley and Summerly, 1981; Lampe et al., 1983). Working together in a coordinated fashion, the keratinocytes, lipid lamellae and water maintain the barrier integrity of the skin. The lipid bilayers are made up of primarily cholesterol (27%), ceramides (41%) and free fatty acids (9%) (Williams, 2003).

1.3. Species differences in skin

Different membranes (either artificial or animal) have been utilized as models for human skin *in vitro*. Amongst the most common reported in the literature are hairless mouse or rat skin, reptile, rabbit and human cadaver skin (Ferry et al, 1995; Zakzewski et al, 1998; Ngawhirunpat et al, 2004 and Ostacolo et al, 2004). Significant differences have been reported in the penetration of drugs across the skin of different animals (Monteiro-Riviere et al., 1990). This variability has been attributed to inter-species variation in the structure of the skin. However, the pig- skin has been recognized as an established model for human skin due to the similarity in thickness of the stratum corneum (Monteiro-Riviere et al., 1990); which is thinner in smaller laboratory animals such as rat and mice. Aside from the ease of acquisition, porcine skin also has an added advantage of being histologically the closest to the human skin (Williams, 2003).The stratum corneum of pig skin is similar to that of humans, although they differ in lipid contents, permeability is similar and the pig ear is a choice site for comparison (Williams, 2003). Schmook et al (2001) reported that pig skin was the most suitable model for human skin when four topical dermatological drugs (salicylic acid, hydrocortisone, clotrimazole and terbinafine) with widely varying polarity were compared using Franz cells: the fluxes through the skin and concentrations in the skin were of the same order of magnitude for both tissues.

In a recent study, Ngawhirunpat et al (2004) investigated the transport and metabolism characteristics of ethyl nicotinate (EN) in rabbit, rat, guinea-pig, pig, shed-snake and human skins, they reported that there was no significant difference in skin permeation and metabolism of the permeant between human and pig skin. In another study, Andega

et al (2001) concluded that the permeability of porcine skin to melatonin, in the presence of saturated and unsaturated fatty alcohols, was qualitatively similar to human skin but quantitatively different with some fatty alcohols.

Previous workers (Sintov and Botner, 2006; Rabu, 2003) have placed a question mark on the relevancy of using non-freshly prepared porcine skin that has been stored at 4 or -20°. Rabu (2003) suggested that cryoprotection of skin at −22 °C with 10% glycerol at time of storage could be very helpful for the long-term storage of skin. However; possible permeation enhancing effect of glycerol on the skin is a limitation of this preparation technique.

1.4. The basic transdermal permeation process

Williams (2003) described the permeation of substances (chemical drugs in the case of pharmaceutical formulations) across the skin into the systemic circulation; whereby a therapeutic agent can permeate the skin through a series of multiple partition and diffusion steps. Initially, the drug molecules will first partition from the delivery vehicle (which could be a simple aqueous solution or a complex systems such as an emulsion) into the outermost layer of the stratum corneum. Subsequently, a myriad of partition processes occur at the interface between stratum corneum/ epidermal, epidermal / dermal and the dermal / capillary junctions to eventually get the drug molecules into the systemic circulation. In between the partition processes, the permeant molecules also undergo a series of diffusion processes through series of pathways depending on the physicochemical properties of the drug. Lipophilic permeants are more favoured than hydrophilic substances due to the fatty nature of the skin.

The initial step of the permeation process is dynamic as it involves the constant replacement of permeated molecules at the vehicle / stratum corneum interface via random redistribution and diffusion within the vehicle. Whilst this stage could be limited by the permeant-vehicular effect, subsequent steps of the permeation process are controlled mainly by factors such as skin metabolism and nature of permeant.

1.5. In vitro evaluation of the performance of topical /transdermal formulations

The most important decision that is made in designing topical/transdermal drug delivery systems is the choice of vehicles or formulations that are to be used. In addition to the cosmetic considerations the choice of vehicle has a significant impact on the rate and which a drug is delivered to and then into skin. This rate and extent of drug delivery to and into the skin is measured extensively using *in vitro* testing apparatus.

1.6. Franz cell apparatus

For many years, it has been generally accepted that *in vivo* permeation experimentation for the study of transdermal formulations has several difficulties; notable amongst which are, ethical issues, influence of metabolism (either by the skin or its constituent micro-floral), and safety precautions (Williams, 2003); hence, *in vitro* techniques involving the use of static diffusion cells (Franz, 1975 and Franz, 1978) have taken the lead in research (Williams, 2003). The penetration of substances through the skin (a multi-layered membrane) is not easy, but has been simplified and described using mathematical models such as Fick's law of diffusion. A modified form of this law can be applied to small finite dosing situations. In the studies presented within this thesis, the mathematical equations that explain pseudo steady state skin penetration are highlighted, as they are more applicable. Pseudo steady state is a situation when the concentration gradient of the drug across the skin is constant, thus, producing a steady flux, depicted by the linear portion of figure 1.3.

Franz cell apparatus that separates an upper donor compartment from a lower receptor compartment using a bio-relevant membrane is often used to measure drug release. From Fick's first law of thermodynamics, the amount of permeant that penetrates through a unit area of the membrane per unit time is a function of the concentration gradient across the membrane.

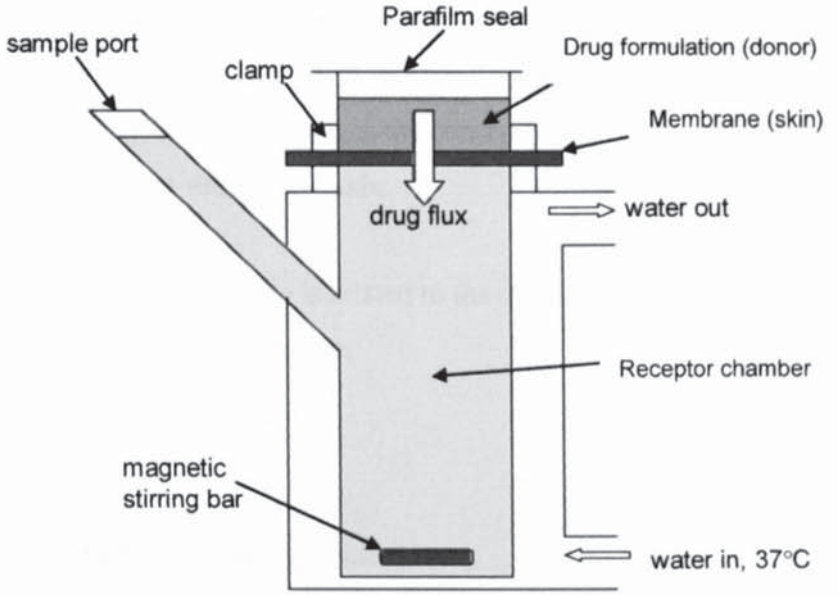


Figure 1.2. Diagrammatic representation of two- chamber diffusion cell used for assessment of drug permeation.

The permeation of drugs can be described mathematically according to equation 1 derived from Fick's law;

$$\frac{dM}{dt} = \frac{DPCv}{h} \qquad \text{(Equation 1)}$$

where dM/dt is the rate of permeation through a membrane, in this case the skin.
D is the diffusion coefficient of the drug; P is the partition coefficient of the drug between the vehicle and the skin and h is the thickness of the skin. Cv is the maximum solubility of the drug in the vehicle, it can be seen that improving the saturation solubility of the drugs in the matrix will enhance the effective concentration present at the skin surface.

For most transdermal studies, a plot of M against corresponding time, t will produce a slope; where the gradient of the slope is taken as the flux, J.

$$\frac{dM}{dt} = J = \frac{DPC_v}{h} \qquad \text{(Equation 2)}$$

Equation 1 above suggests that increasing the concentration of the drug within the vehicle will increase the flux of the drug, as D and h are constants. However it is important to look closely at the relationship between the concentration in the vehicle and the partition coefficient between the vehicle and skin.

The permeability coefficient; Kp is related to the diffusion coefficient and the partition coefficient by the following equation;

$$K_p = \frac{DP}{h} \qquad \text{(Equation 3)}$$

Therefore, equation 1 can also be written as;

$$\frac{dM}{dt} = J = KpCv \qquad \text{(Equation 4)}$$

The permeability coefficient, Kp is often used to compare the rate of permeation of the same drug from a range of vehicles. The permeability coefficient is also useful to note the variability between formulations of difference concentrations, as flux is dependant upon concentration whereas this term is accounted for in the calculation of Kp.

Williams (2003) reported in his review that the above equations can only be true and hence applied provided that the stratum corneum is the major rate limiting barrier, the transappendageal transport of permeant is negligible, the passive diffusion is the main driving force of permeation, there is no vehicular effect on the nature of the stratum corneum, the drug dissolves in the stratum corneum, the diffusion coefficient is independent of concentration, time and distance and that it is assumed the stratum corneum thickness is uniform. This is not always applicable especially in situations when the stratum corneum barrier is broken such as use of iontophoresis.

The essence of manipulating the derived data from experimental studies to calculate Kp and D can only be explained by the use of these parameters. D is a parameter that determines how fast and the extent to which a molecule transverses the skin. It is often

difficult to calculate due to problems associated with estimating the accurate skin thickness. Hence Kp is often applied for comparison of the rate of permeation through the skin between two or more formulations. Kp gives an indication of the actual penetration.

1.7. Finite and Infinite dosing

During *in vitro* permeation process such as in Franz diffusion studies, permeant molecules move from the donor formulation unto the surface of the skin before diffusing through the several layers to reach the systemic circulation. During in vitro experimentation, the initial diffusion step is seen on a classic profile of the plot of amount of drug penetrated across membrane against time as the lag time that indicates little or no drug in the receptor media. However, a steady state flux is obtained as the flux increases steadily over the period of the experiment as long as a concentration gradient of the drug from the donor formulation to the receptor media is maintained. But in situations when there is depletion of the permeant, this often leads to a reduction of the thermodynamic activity (chemical gradient). This will lead to reduction in drug flux. This case is called finite dosing.

It is widely believed that to achieve a constant chemical gradient, saturation of the permeant in the donor vehicle is important, hence, most transdermal formulations contain excess solutes that are believed to replace absorbed molecules and this is referred to as infinite dosing. A typical profile of an infinite dosing situation with a steady state flux is shown in figure 1.3.

In situations where saturation of the permeant was not achieved in the donor formulation, a quick absorption often results in depletion in the vehicle and the flux attains a plateau as shown in figure 1.4.

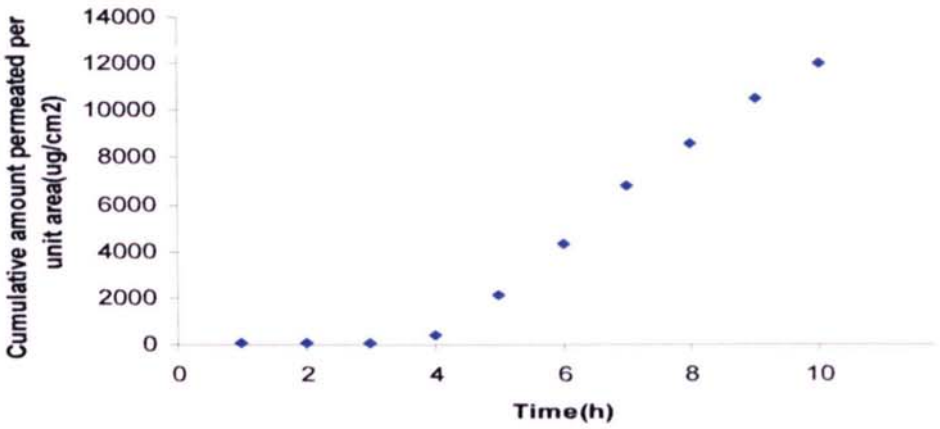


Figure1.3. Permeation profile of infinite dose application to the skin.

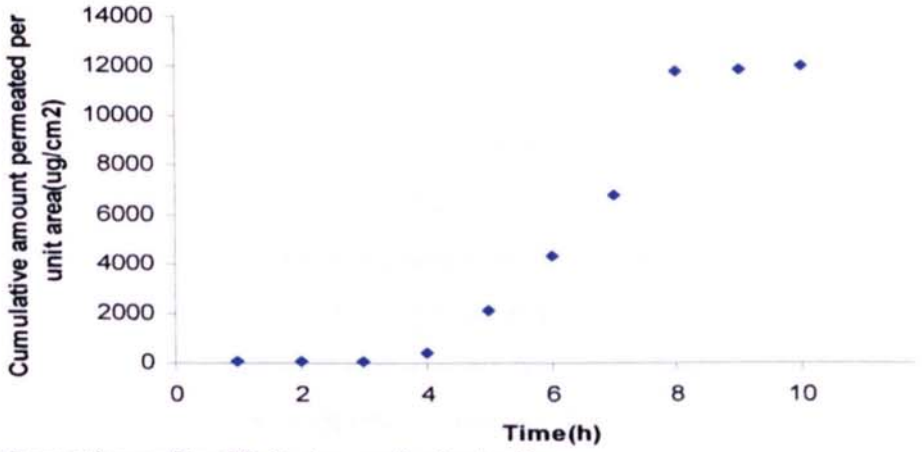


Figure 1.4. Permeation profile of finite dose application to skin.

1.8. Drug delivery into and across the skin

The skin is the largest organ in humans and is a complex barrier that function mainly to keep the 'inside' in and prevent ingress of foreign materials including drugs administered to it. The advantage of easy accessibility of this organ ought to make this route of drug administration more popular however it is often overlooked in the field of drug delivery because the flux of drugs through the skin is primarily influenced by drug related factors. Moreover, even for drugs with the desired physico-chemical properties, the skin rate-

limiting membrane, the stratum corneum, is often a tough barrier to overcome. Hence, in recent years, research has been focused on overcoming this barrier in order to enhance drug administration into the skin for local effect or through the skin into the systemic circulation.

1.8.1. Percutaneous absorption pathways

There are three major pathways recorded in the literature by which chemical agents can permeate through the skin, namely: transappendageal (also known as the shunt route), transcellular and the intercellular routes. Transportation normally occurs through any or a combination of all these routes.

1.8.2. Transappendageal route

This is a pathway that has been recognised as a minor route for the dermal and transdermal delivery of drugs but could act as key route especially during finite dosing (commonly seen in clinical situations), application of iontophoresis (due to lower resistance of the appendages such as hair follicle and sweat ducts) and for targeted delivery of substances via vesicular formulations (Williams, 2003). The main advantage of this route is that it involves transportation through hair follicles and sweat ducts by-passing the tough stratum corneum. Iontophoresis is a non-invasive approach of delivering high concentrations of charged and often high molecular mass molecules across the skin with the use of repulsive electromotive force. The technique normally involves applying a small electrical charge to an iontophoretic chamber; which contains a similarly charged active agent and its vehicle. It is a complex and expensive method that relies on active transportation within an electric field. Hence, it is not commonly used like the simpler chemical means which often involve delivery of small molecules via the transcellular route but it is now attracting more interest as an active means of penetrating the skin barrier.

1.8.3. Transcellular route

This has been recognised as the most challenging but also the most common pathway for molecules to transverse the skin since transportation has to occur via a series of partitioning into and diffusion through keratin-filled cells and subsequent partitioning into the rate limiting inter-cellular lipid bilayers. The usefulness of this pathway to overall flux has being linked mainly to the physicochemical properties of the permeant.

1.8.4. Intercellular pathway

From the studies carried out by Abraham et al (1995) and Roberts et al (1996), this route has now been identified as the most important for the transdermal delivery of small uncharged molecules. Although transportation through it is similar to that of transcellular route in that it involves movement of permeant molecules across the lipid bilayers of the stratum corneum, permeation by this route can be best described by the fluid mosaic model proposed by Forslind (1994). In this model, as seen with the plasma membrane of most cells in the body; each lipid-bilayer is made up of structurally and functionally asymmetric membranes that are embedded with hydrophilic proteins that aid in cross-membrane transport. However, unlike most cells, the lipid bilayers of stratum corneum are phospholipid-free but contain a lot more ceramide molecules (Williams, 2003).

1.9. Transdermal drug delivery

Transdermal drug delivery is an approach that involves delivery of drugs to the systemic circulation via the skin. It offers many advantages above oral and other conventional administration routes in that applications are non-invasive in nature and drugs are not subjected to first pass metabolism in the liver, gut or lungs (Roberts et al, 2002).

In addition, with transdermal drug delivery, patient compliance has been reported to be very high due to ease of therapy termination in problematic cases (Ranade and Hollinger, 2003). A second reason for increased compliance is associated with reduced dosing

schedules; this is particularly relevant for drugs where long-term plasma concentrations are required coupled with short elimination half-lives.

1.9.1. History of Transdermal Drug Delivery

In the last century, research in skin drug delivery has been focused on mechanisms of penetration, chemical enhancers, *in vitro* and *in vivo* correlations, site-specific variation and use of appropriate models to predict *in vivo* efficacy (Hadgraft and Lane, 2005). A good foundation was laid in the 1950s when Hadgraft and Somers (1956) and Vallette (1953) identified that a good topical penetrant should have optimum physicochemical properties such as log P value between 1 and 3.

In the 1960's, investigations into understanding the barrier properties of skin were intensively carried out and the success of these studies led to detailed explanations of the penetration process using established mathematical principles of thermodynamic activity (Higuchi, 1960, 1961 and 1962). McKenzie (1962 and 1966) and McKenzie and Stoughton (1962) showed the essence of blanching (vasoconstriction) effect for topical delivery of steroids. Furthermore, Clendenning and Stoughton (1962) identified the importance of partitioning as a major force in skin delivery and they reported the link between structural –activity relationship and skin permeation. Also, Vickers (1963 and 1972) highlighted the role of the skin reservoir function on topical application of steroids. Feldmann and Maibach (1967 and 1969) reported variability in skin permeability from one body site to another. A key revelation was the factors that bring about the huge variability often associated with delivery of drugs through the skin. These factors include; differences in skin thickness, density of appendages (hair follicles and glands), vascularity and metabolic enzymes. Thus, different regions of skin in the same individual may display different pharmacokinetics of percutaneous drug penetration. More importantly, it is now widely recognized that extrapolation of percutaneous penetration data within a species may not be practical (Williams, 2003). For example, use of approved products for adult use in infants without consideration of these intra-species variations could be risky (Zernikow et al 2007 and EMEA, 2007).

In the 1970s, Katz and Poulsen (1971 and 1972) shed more light on how the kinetics of drug release from semi-solids followed Fick's second law. The mechanisms of percutaneous absorption and especially the importance of the shunt route, was detailed by Scheuplein (1965, 1967), Scheuplein and Blank (1973) and Scheuplein and Ross (1974). Michaels et al., (1975) and Elias and Friend (1975), after thorough investigation of human skin, revealed that the major resistance to drug penetration is the outermost layer, the stratum corneum (SC). Michaels et al., (1975) also postulated theories that helped in the understanding of drug passage through the stratum corneum into the viable epidermis and dermis, including the "bricks and mortar" theory, representing keratinocytes held together by a lipid bilayer (Menon and Elias, 1997).
Another key development was the use of static cells for *in vitro* experimentation and correlation with *in vivo* studies asreported by Franz (1975 and 1978). Also, Barry and El Eini (1976) showed how suitable model membranes could replace the skin.

The role of chemical enhancers has been and is still being extensively investigated. Stoughton (1964 and 1965) identified DMSO as a good solvent and penetration enhancer for most permeants. Scheuplein and Morgan (1967) identified that hydrated skin is more permeable than the un-hydrated skin.

Within the last few decades there has being a huge progress in transdermal drug delivery due to a better understanding of the permeation mechanisms. This was brought about by advances in technology both directly on the skin barrier, as well as indirectly in modification of skin permeability to drugs using chemical excipients. Furthermore, the use of penetration enhancers that co-diffuse into the skin and alter the properties of the stratum corneum by either altering the lipid packing of the bilayers and hence influence D (D is the diffusion constant; a parameter that measures the extent of movement of drug molecules through the skin) or change the solubility properties of the drug and hence influence P (P is the partition coefficient, a factor that indicates the distribution of drug molecules from the donor formulation to the skin) has been extensively studied (Chiarini et al,1984; Potts and Guy, 1992; Walker et al, 1996, Harrison et al, 1996; Cronin et al , 1999; Beetge et al 2000 and Williams and Barry, 2004).

Drug permeation through the skin has been elucidated to involve a series of partitioning and diffusion processes via the intercellular route, which contains lipid bilayer membranes, composed of lipophilic lipid chains with hydrophilic domains (Williams 2003).

Within the last decade, work with passive transdermal patches has progressed and efforts have been focused on enhancing penetration via the use of specially designed devices that physically break the stratum corneum using a variety approaches, termed active transdermal delivery. These approaches expand the potential for delivering drugs non-invasively by actively transporting the drug across and through the skin such as electroporation, iontophoresis, sonophoresis and magnetophoresis (Barry, 2001). There have also been advances in chemical penetration enhancers for passive drug delivery (Barry, 2001).

Another major milestone is the range of biophysical techniques that are now used to study skin permeation. Examples are DSC (Differential Scanning Calorimetry); (Potts et al, 1991), X-ray scattering (Cornwell et al, 1996), NMR (Nuclear Magnetic Resonance), (Thomas et al, 2003) and FTIR (Fourier Transform Infrared Spectroscopy), (Potts et al, 1991).

With these developments, and with special interest in overcoming problems associated with the inherent side effects exhibited by many drugs when they are administered via conventional routes, research is now turning in favour of transdermal delivery evidenced by more annual growth in the research & development of transdermal products in recent years than other formulations (Thomas and Finnin, 2007). The focus now is the development of transdermal drug delivery systems (TDDS) that are specifically designed to obtain therapeutic level of drugs in the systemic circulation.

The success of transdermal drug delivery is evidenced by the considerable interest and the fact that there is a growth market currently worldwide for transdermal pharmaceutical products for the treatment of a wide range of conditions (Thomas and Finnin, 2004).

1.9.2. Marketed Transdermal formulations

Transdermal treatment is available for a wide variety of conditions including: hypertension, angina, motion sickness, female menopause, male hypogonadism, severe pain, local pain control, nicotine dependence, and recently, contraception and urinary incontinence (Thomas and Finnin, 2004).

Below is a list of current formulations available on the market or undergoing clinical trials selected randomly. The list is not exhaustive and only a few are reported here due to their relevance to the current project as topical or transdermal formulations.

Compound	Delivery System	Stage of Development	Company website with relevant information
Alprostadil	Gel – Alprox-TD	Marketed in China	NexMed; http://www.nexmed.com
Buprenorphine	Patch – Transtec®	Marketed in Europe	Grunenthal; http://www.grunenthal.com/
Dihydrotestosterone	Gel – Andractim®	Marketed in France and Netherlands	Unimed/Solvay; http://www. solvaypharmaceuticals-us.com/
Estradiol	MDTS®	Phase II	Acrux; http://www.acrux.com.au/
Estradiol/progestogen	Gel	Phase II in USA	Antares; http://www. antarespharma.com
Ethinylestradiol & Norelgestromin	Patch – Ortho® Evra	Marketed in USA	J&J; http://www.jnj.com/
Fentanyl	Patch – iontophoresis: E-TRANS®	Pre-registration	Alza/J&J; http://www.alza.com/
	MDTS	Phase I	Acrux; http://www.acrux.com.au/
hGH	Microneedle – Macroflux®	Phase I	Alza/J&J; http://www.alza.com/
Hydromorphone	Patch – thermal	Phase I in USA	Altea; http://www.alteatherapeutics.com
Insulin	Sonophoresis	Preclinical	Imarx; http://www.imarx.com/ corporate.asp
	Patch –Thermal	Phase I in USA	Altea; http://www.alteatherapeutics.com
Lidocaine	Patch – Lidoderm®	Marketed in USA	Endo; http://www.endo.com
Methyltestosterone	Patch	Phase II in USA	Noven; http://www.noven.com
Nicotine	Patch	Marketed in the UK and US	Nicorette (Pharmacia); Nicotinell (Novartis); NiQuitin (GSK)
Oxybutynin	Patch –Oxytrol	Launched in USA	Watson/Theratech; http://www.theratech.com
Parathyriod hormone	Patch – Thermal®	Phase I in USA	Altea; http://www.alteatherapeutics.com
Rotigotine®	Patch	Marketed in the UK and US	Aderis/Schwarz; http://www.aderis.com/
Selegiline	Patch –STS®	Pre-registration	Watson/Mylan; http://www.mylan.com/
Testosterone for males	Gel –Testim®	Marketed in USA	Auxilium/Bayer; http://www.auxilium.com

Testosterone for females	Patch	Phase III in USA	Watson/P&G; http://www.pg.com/main.jhtml
Vaccines	Patch	Preclinical	Alza; http://www.alza.com/
Diclofenac sodium	Gel	Launched in the EU	Shire http://www.shire.com/shire/Products/index.jsp
Eflornithine	Cream	Launched in the EU	Shire http://www.shire.com/shire/Products/index.jsp
Emsam® (Seleginine)	Patch	Approved in US	Somerset pharmaceuticals http://www.fda.gov/bbs/topics/NEWS/2006/NEW01326.html
Nicotine	Patch	Approved for use in the EU and US	GSK and Novartis www.mednet3.who.int/prioritymeds/report/background/tobacco.doc
Dermestril® (oestradiol)	Patch	Marketed in the UK	www.novartis.co.uk/pharma/
Oestrogel	Gel	Marketed in the UK	www.novartis.co.uk/pharma/
Crinone® (Progesterone)	natural progesterone vaginal gel®	Marketed in the UK	www.novartis.co.uk/pharma/
Evorel Sequi® (oestradiol, norethindrone acetate)	Patch	Marketed in the UK	www.novartis.co.uk/pharma/
Nuvelle TS®	Patch	(Phase I - oestradiol and phase II- oestradiol & levonorgestrel) in the UK	www.novartis.co.uk/pharma/

Table 1.1: List of some current transdermal products on the market. Abbreviations: MDTS, metered-dose transdermal system; TD, transdermal delivery.

The FDA approved a total of 35 transdermal products over the past 20 years, the majority of which use passive transport that allows drug to be continually absorbed into the skin via natural diffusion processes.

The Food and Drug Administration approved scopolamine patch for treatment of motion sickness in 1979. Nitro-glycerine patches were approved in 1981 and smoking cessation nicotine patches were introduced in 1991. Emsam® (selegiline®), the first skin (transdermal) patch for use in treating major depression was approved in 2006. This once a day patch works by delivering selegiline, a monoamine oxidase inhibitor systemically via the skin. At its lowest strength, emsam® can be used without the dietary restrictions that are needed for all oral monoamine oxidase inhibitors that are approved for treating major depression. Also in 2006 the FDA approved daytrana® (methylphenidate transdermal system), the first and only non-oral medication for the treatment of attention deficit hyperactivity disorder (ADHD). In 2007 the U.S. Food and Drug Administration announced the approval of Neupro® (rotigotine transdermal system), a skin patch designed to treat symptoms of early Parkinson's disease. Neupro® is the first transdermal patch approved for the treatment of symptoms of Parkinson's disease

1.10. Typical topical and transdermal formulations

Topical and transdermal formulations have been and are still being developed to overcome the barrier integrity of the skin. In recent years, the heightened focus of research has moved from simple aqueous solutions to complex micro-emulsions or liposomal formulations. These formulations are developed and/or manipulated using a scientific approach for effective delivery of drugs either through or into the skin taking into consideration physicochemical properties of the permeant and the vehicle to ensure optimum bioavailability of the former. However, the stability and compatibility of any excipient with the active ingredient as well as the aesthetic value of the formulation are important.

Generally, topical products are used in the treatment of localised disorders such as skin infections. Topical formulations are available in a variety of forms, including gels, creams, ointments, solutions, lotions, suspensions, pastes, emulsions, patches and foams. Water miscible creams have generally been employed for moist or weeping lesions whereas ointments have been used for dry, lichenified or scaly lesions or where a more occlusive effect has been required. Lotions on the other hand have been applied where minimal application to a large or hair bearing area has been required or for treatment of exudative lesions. Creams are generally more acceptable to patients since they are easy to apply. Pastes are generally more applicable for treatment of localised lesions because of their stiff consistency. Patches are used when long term controlled systemic delivery of drugs is desirable but not often for local delivery.

Topical and transdermal formulations can either be a single or a multiple phase system. Single-phase systems are those in which the active ingredient is formulated in an aqueous, oily or miscible co-solvent vehicle. Generally, such formulations are liquids with relatively poor residence time on the skin and hence delivery is often for a short time. Often, the vehicle evaporates leaving behind a thin film of the formulation with high concentration (hence increased thermodynamic coefficient) of the active to drive permeation through the skin. Examples of drugs formulated into single-phase topical solutions are malathion to treat mice and chlorhexidine to disinfect the skin.
Multiple phase systems are formulations with more than one phase available for the active to distribute into or between; typically they are semi-solid preparations. There are many types of such formulations but the easiest a two-phase system to describe is an oil-in-water emulsion, for example as found in an aqueous cream. The permeation process of a hydrophilic drug from such system will be enhanced when the water on the skin surface evaporates via increased thermodynamic activity. On the contrary, a reduced permeation of the same drug will be seen when formulated in a; water –in-oil two-phase system because the oil will form an additional barrier for the drug to diffuse through prior to contact with the skin.

According to Mills and Cross (2006), the key factors to consider in terms of drug delivery to the skin are the intended sites of action for the active such as: (i) local effects (e.g, corticosteroids for dermatitis); (ii) transport through the skin for systemic effects (e.g, fentanyl, nicotine, methylphenidate, oestradiol and testosterone patches); (iii) surface action (e.g., sunscreens and anti-infective), (iv) targeting deeper tissues (e.g., non-steroidal anti-inflammatory agents [NSAIDs] for muscle inflammation), and (v) the case of accidental exposure (e.g., solvents in the work place, agricultural chemical, or allergens). The main properties of common topical and transdermal formulations are described with reference to specific examples from the literature.

1.10.1. Creams

A cream is a bi-phasic semi-solid emulsion commonly used for topical administration of drugs and comprises of one phase dispersed in the other. The system is usually stabilised by an emulsifier to prevent cracking. The continuous and non- continuous phases could be water or oil giving this dosage form an advantage of delivering both lipophilic and hydrophilic drugs. If a water soluble permeant is delivered from an oil/water cream, as the water evaporates, the saturation of the permeant is increased in the continuous phase, thus enhancing its penetration while the reverse is the case if the same permeant is to be delivered from a water in oil cream, the continuous phase serves as another barrier to its permeation. In addition, as the emulsion becomes unstable, emulsifiers that are formed can trap the permeant in the continuous phase. In most cases creams are oils dispersed within an aqueous phase. Sintov and Shapiro (2004) studied the bioavailability of lidocaine after administration of topical EMLA (eutectic mixture of local anaesthetic) cream. They observed that in vitro transdermal flux of lidocaine through rat skin was significantly improved by microemulsion composed of the glyceryl oleate–PEG-40 stearate combination rather than glyceryl oleate–PEG-40 hydroxylated castor oil. The reason adduced for this result was the co-surfactant/ surfactant ratio. Several other studies have been carried out using creams as a dosage form for topical administration of drugs. For example, Kreuter et al (2004) discovered that pimecrolimus 1% cream was an efficacious and safe treatment option for cutaneous lupus erythematosus; Tian (2004)

noticed that EMLA cream relieved venous puncture in patients on clinical observations; Gupta and Myrda (2004) observed that topical application of Perillyl alcohol cream was an effective chemoprevention therapy against skin cancer. As a principle, the mechanism by which this dosage form drives drug permeation is via increase in the solubility of the drugs in the vehicle and the skin with a corresponding increase in the concentration gradient, the vehicle drives transfer both into and across the skin. Within the UK the majority of topical products are available as creams. The disadvantages of this dosage form are limited shelf life due to un-stable emulsion composition, quantification of exact amount for treatment and the messiness on application to the skin.

1.10.2. Pastes

These are pharmaceutical preparations containing a high percentage of powder suspended in a fatty base that could either be a liquid or a semi-solid. The main mechanism that drives the permeant through the skin is the hydration of the stratum corneum brought about by the occlusive nature of pastes. While there are few workers that have investigated the application of this dosage form for topical delivery of drugs, there are even fewer paste formulations available commercially. For example, Mujumdar and Misar (2004) looked at the anti-inflammatory activity of Jatropha curcas roots in mice and rats applied as a topical paste. Recently, Ortega et al (2006) in a randomised controlled clinical trial investigated the effect of a topical antimicrobial paste (applied to oral mucosal membranes rather than skin) on healing after extraction of molars in HIV positive patients. Ljungberg et al (1998) compared the effectiveness of both dextranomer paste and bead formulations (they are made up of larger granules) applied topically for the delivery of dextranomer for treatment of venous leg ulcers. It was found that clinical efficacy of the dextranomer paste was comparable to that of the beads and this was due to improvement in extent of granulation in the former.

1.10.3. Gels

These semi-solid formulations are prepared from a continuous liquid phase made viscous by thickening agents which are mostly polymers such as carbopol® or hydroxylpropyl-methylcellulose (HMPC). The thickening agent also acts as a control release modifier for the active ingredient. Many transdermal products are available commercially in the form of gels for example oxa gel and voltarol emulgel notably for the administration of non-steroidal anti-inflammatory drugs (NSAID) such as diclofenac. Recently, commercial gels for delivery of estradiol and testosterone have also been developed (Thomas and Finnin, 2004). Ammar et al (2006) developed a new hydrocarbon based gel delivery system for the topical administration of aspirin as an antithrombotic drug. Amongst the most successful application of gel was the development of lidocaine gel for topical anaesthesia (Kirber, 2000; Soliman et al, 2004; and Kafali, 2003).

Brown et al (2001) compared the effect of hyaluron with other glycosaminoglycans on the dermal partitioning and percutaneous penetration of diclofenac and ibuprofen. The study revealed that hyaluronic acid significantly enhanced the partitioning of both diclofenac and ibuprofen into human skin when compared to an aqueous control, pectin and carboxymethylcellulose based formulations.

1.10.4. Patches

There are three main types of transdermal patch available on the market. Namely, drug in adhesive, drug in a matrix and drug as a reservoir type of patches, modifications of these gives rise to various marketed products.

(i) Drug in adhesive

The adhesive layer of this system contains the drug (homogenously distributed) and therefore not only adhere the whole system to the skin but also controls the release of the formulation. Modification can give rise to a single or a multiple layered patch. These patches help in maintaining a near constant concentration gradient as delivery progresses

with time if high initial drug loadings are used. An example of a drug-in-glue patch is Deponit used for delivery of glyceryl trinitrate (Williams, 2003).

Aveva gel matrix transdermal delivery system is designed as a drug in adhesive product. Although, it is currently marketed as once a day patch for management of angina and post Myocardia Infarction, it offers advantages of being reliable and gentle above conventional products of its class in that it can be removed and re- used up to six times (Dubin, 2007).

(ii) Drug in matrix

In a matrix system, the formulation is a semisolid matrix containing a drug solution or suspension with the adhesive layer partially surrounding the formulation. The drug is present at high concentrations to maximise the concentration gradient and therefore the flux.

(iii) Drug as a reservoir

The main difference between this and the drug in matrix patch system is that the formulation has a separate layer. The formulation in the drug layer is a liquid compartment containing a drug solution or suspension separated by the adhesive layer. The backing layer also backs this patch. In clinical applications, reservoir type patches cannot be cut to sizes for usage while drug in matrix type patches can be, due to the delivery system arrangement.

Patches have been extensively investigated for transdermal drug delivery; for example, Bian et al (2003) evaluated patch formulations for the topical administration of gentisic acid; which is an aspirin metabolite used as an anti-oxidant. Gammaitoni et al (2004) reported on the effectiveness of lidocaine administered in patch form for the treatment of lower back pain and osteoarthritis in open clinical trials. Alessandri et al (2006) also investigated topical diclofenac patch for postoperative wound pain in laparoscopic gynaecologic surgery. Even topical delivery of vaccines in the form of patches has been considered (Partidos et al, 2003). The advantages of skin delivery of vaccines especially

in the form of patches was documented by Partidos et al (2003) as; increased compliance, painless immunization, avoidance of cross-infection since administration is bloodless, as well as the simplicity and benefit of eliciting both systemic and mucosal immunity.

The latest advancement in patch technology often incorporates other approaches of overcoming the barrier integrity of the stratum corneum such as iontophoresis (Dubin, 2007).

1.10.5. Transdermal tablets

Normally tablets are associated with the oral route of administration. However, Mills et al (2001) proposed a novel approach for the delivery of drugs via a metered-dose tablet that can be applied directly to the skin; which also solves the problem of inaccurate dosing. A notable example of this type of tablet technology is pandermal®. Pandermal® is described as melt-on, unit-dose, solid state delivery systems for topical or transdermal use that offers the advantage of being mess-free and removes patch-related adhesion, irritation and residual dose problems with enhanced drug pay load capacity (www.Pharmakodex.com, 2007). This technology is currently being developed for delivery of anti-inflammatory analgesia, smoking cessation and musculo-skeletal drugs to or through the skin.

1.11. Recent advances in transdermal drug delivery

The latest advances in topical/transdermal drug delivery can be categorised into several strategies including the use of active technologies including microneedles to physically permeate the stratum corneum, electro-assistance for drug delivery and sonophoresis, using acoustic waves to improve permeation of the skin. Advances in chemical manipulation of the stratum corneum has also lead to advances in passive transdermal devices where high concentration gradients are used in combination with penetration enhancers to maximise skin penetration.

Highlighted below are some of the latest technologies on the market and those under development; this list is not exhaustive but attempt is made to mention a few in this thesis.

1.11.1. Iontophoresis

Viscusi and Witkowski (2005) define iontophoresis as a process by which electrical field is applied to drive charged particles across skin. Barry (2001) and Curdy et al (2001) stated that this approach to transdermal drug delivery works via two main mechanisms; namely, electrical repulsion of ionised charged drugs from the anode followed by drug flow into the stratum corneum via electro-osmosis. Barry (2001) identified that penetration of small size, positively charged lipophilic drugs was markedly enhanced by this approach.

Minkowitz (2007) recently reported the development of a novel patient controlled iontophoretic transdermal system; called ionsys ™ for the delivery of fentanyl hydrochloride to manage acute post-operative pain. Alza pharmaceuticals received FDA approval for this device in May 2006. Each unit of the device consists of a plastic upper case which houses the 3-V Lithium battery and electronics, while the lower plastic casing contains two hydrogel reservoirs covered with a polyisobutylene adhesive that serves as an anchor to the skin. The dosing button controls the release of fentanyl HCl from one of the hydrogels; while the other contains pharmacologically in-active ingredients. The advantages of this system above previously known iontophoretic devices (such as intra-venous or intra-muscular pre-programmed patient controlled analgesia, are better patient tailored dosage; thus, improves patient safety, on –demand delivery in synchronisation with circadian rhythms and user satisfaction in addition to expected reduced cost due to avoidance of associative operational cost. Another active device that operates using the iontophoretic principles is LidoSite, which was developed by Vyteris for dermal anaesthesia (Dubin, 2007).

1.11.2. Micro-needles

These are small needles specially designed (150 μm in length) employing microelectro-mechanical systems to pierce the rate limiting stratum corneum in order to assist drug penetration through the skin. The needles are often made with silicone, silicone dioxide, glass, titanium or metal into either in – plane or out of plane geometry. Henry et al (1998) demonstrated that their use enhanced the permeability of human skin by a factor of 4 with almost no painful effect. The success of this approach had led to a wave of interest with commercial prospects.
The main challenge of this technique is being able to produce ultra-sharp needles and pierce the visco-elastic stratum corneum (Mikszta et al, 2002). High power injectors are now being integrated with micro-needles and used for transdermal delivery of drugs (Teo et al, 2006).

One notable technology in this area is the 3M's Micro-structured Transdermal System (MTS®); a microneedle system developed for delivery of molecules that would necessarily not pass through the skin, including vaccines (Dubin, 2007). The key advantage is targeted delivery of vaccines to antigen presenting cells thereby improving efficiency and reducing dosing needs (Dubin, 2007).

1.11.3. Sonophoresis

The application of low level ultrasound energy or sonophoresis, for a short time period (< 90 seconds) increases permeability of the stratum corneum by creating reversible channels through the skin, permitting the delivery of drug molecules through the transdermal route. SonoPrep® is an ultrasonic skin-permeating device developed by Sontra Medical Corporation that incorporates this technology. It has been developed for delivering SonoPrep topical anaesthetic that significantly reduces time required to achieve anaesthesia. Further applications of ultrasound transdermal delivery are likely to be expanded to include transdermal insulin, heparin, vaccines and continuous glucose

monitoring. TransPharma Medical currently has some products in the pipeline using this technology (Dubin, 2007).

1.11.4. Direct scraping of stratum corneum

This technology is sometimes called dermabrasion. It can be done via chemical peel (aluminium oxide), adhesive tape stripping or laser technology (Friedland and Buchel, 2000). Akhomea et al (2007) recently employed direct scraping of the stratum corneum as a means of enhancing the permeability of drugs across the skin. They observed that the barrier of the skin to permeation of the selected drugs was significantly reduced. The question remains as to the feasibility of this technique in clinical situations without compromising the protective function of the skin against un-wanted organisms and substances.

1.11.5. Needleless injection

This involves the use of gas-powered guns or spring-actuated mechanisms to deliver small and sometimes large particles into the skin. Although, there are many devices under development, there are many questions that need to be answered especially as regards safety prior regulatory approval (Williams, 2003). The leading products in development include lignocaine and levobupivacaine for local anaesthesia, proteins (follicle stimulating hormone and β-interferon) and hepatitis B DNA and other vaccines (Barry, 2001).

1.11.6. Patchless patch system

Aside from the problem of effective permeation, a second limitation associated with transdermal drug delivery is safety of formulations due to difficulty experienced with accurate dosing. Inaccurate dosing of some transdermally applied actives such as topical anaesthetics has been linked with grave reactions like seizures, irregular heartbeats and

death in some cases (FDA, 2006). A notable recent approach to overcome this challenge is the use of metered dose technologies, MDTS® (Acrux, 2007); which are user-friendly devices developed to deliver specific amount of a medication to a certain area of the body. Formulating drugs for transdermal applications by employing MDTS® technology such as that of Acrux (http://www.acrux.com.au) is interesting. This technology is a passive patchless patch system; which consists of a penetrating chemical enhancer, volatile solvent and the drug. This technology consists of a liquid spray or aerosol formulation for forming a non-occlusive reservoir of drug and enhancers within the skin (as opposed to on top of the skin with a transdermal patch). Patchless patch® drug and enhancer form a reservoir within the skin which provides sustained enhancement of the drug into the bloodstream

The reported advantages include low risk of skin irritation, low cost of production, dosage flexibility and cosmetic acceptability. This is suitable for unit dose delivery of systemic agents but not always appropriate for localised delivery.

1.11.7. Magnetophoresis

Magnetophoresis is a novel approach for enhancing drug delivery across biological barriers; this method employs magnetic field to deliver diamagnetic materials through the skin. A study by Murthy (1999) demonstrated enhanced flux of benzoic acid (a diamagnetic substance across rat skin where the flux increased with magnetic field strength.

1.11.8. Radio frequency

Radio frequency- microchannel™ is a technology being developed by TransPharma. The system is made up of a battery-operated handheld electronic unit with attached disposable low cost microelectrodes that creates micro-pores through the stratum corneum when activated. This prepares the skin for application of specially designed patches (dry protein). The initial application of this technology is with viaDerm, where a phase II

clinical trial of viaDerm hPTH (1-34) has recently started (http://www.transpharma-medical.com/proprietary.html).

1.11.9. Thermal Energy

Altea Therapeutic's PassPort® system works by forming microchannels through the stratum corneum. This technology uses short bursts of focused thermal energy to create hundreds of tiny micropores on the surface of the skin, which then facilitate the flow of the chosen drug into the body. The principle of pore formation involves ablation of the stratum corneum. The advantage of this technology above existing patches is the continuous delivery of substances normally administered via needle injection, rapid onset of therapeutic effects and rapid elimination of drug via removal of patch (Dubin, 2007) in case of adverse reaction or overdose. In addition, Dubin (2007) reported that the applicator is equally designed to ensure dose control and monitoring.
This technology sets a high standard for transdermal delivery systems and already on-going clinical trials are being carried out in the USA for: insulin patch to treat diabetes, fentanyl citrate patch for management of pain, apomorphine hydrochloride patch for management of Parkinson's disease.

1.11.10. Wax-based formulations

Wax-based formulations are able to withstand high loadings of drugs, as they are not required to adhere to the skin; rather melt on the surface providing a high local concentration of drug. The also offer the advantage of simple manufacture and can be formulated as unit dose tablets for transdermal use.

Skyepharma, (2007) recently developed a semi-solid dosage formulation known as dermastick™. It is a wax stick within which the active ingredient is homogenously distributed and it works to facilitate controlled application to the affected skin. This means that there is no risk of irritation by application to unaffected skin regions and is

therefore suitable for administration to infected skin areas or to others including children or pets.

Currently there are no drugs incorporated into pandermal® tablets and there is no existing data on the efficacy of this formulation compared to alternative drug delivery strategies. There are ongoing trials evaluating a range of drug products within this technology.

Recently, Kimura et al (2007) developed a similar stick-type transdermal delivery system of ketotifen fumarate for treatment of allergic conjunctivitis, asthma and rhinitis. This system consisted of yellow beeswax, isopropyl myristate and a range of enhancers to include lauric acid, l-menthol, limonene, oleic acid, sodium lauryl sulphate, polyoxyethylene oleyl ether and polyoxyethylene lauryl ether as penetration enhancers all blended together. The mixture was then poured into a rip tube container and allowed to set at room temperature. This formulation demonstrated rapid flux through hairless mouse skin and polyoxyethylene oleyl ether, from the enhancers used, most enhanced the skin permeation of the drug. The permeation enhancement was mainly due to the increase in the drug solubility in the stratum corneum and the resulting increase in the partition coefficient of the drug.

1.12. Influence of permeant physicochemical properties on transdermal drug delivery

There are many factors that have been reported in the literature that affect the permeability of drugs through the skin, amongst the most important are; solubility, melting point, partition coefficient and molecular size.

1.12.1. Solubility

The majority of topical and transdermal dosage forms are formulated as semi-solids. It is important to characterize the solubility of drugs within such delivery systems in order to understand and predict the *in vivo* performance of the product. Generally it is assumed that if an increase in solubility is achieved within a topical/transdermal dosage form then

the rate of delivery or flux of the drug will increase thereby resulting in an improved clinical response. The permeation of drugs can be described mathematically according to equation 1,

$$\frac{dM}{dt} = \frac{DPCv}{h} \qquad \text{(Equation 5)}$$

where dM/dt is the rate of permeation through a membrane, in this case the skin.
D is the diffusion coefficient of the drug; P is the partition coefficient of the drug between the vehicle and the skin and h is the thickness of the skin.
Cv is the maximum solubility of the drug in the vehicle, it can be seen that improving the saturation solubility of the drugs in the matrix will enhance the effective concentration present at the skin surface. Thus, improving drug solubility within the vehicle has been used to promote transdermal delivery (Henmi et al, 1994; Megrab et al, 1995; Valenta et al, 2000 and Raghavan et al, 2000). Higuchi (1960) postulated that the highest thermodynamic potential, which usually corresponds to a saturated system should be utilised to achieve the maximum efficacy. The thermodynamic activity of the drug can be maximised by increasing the solubility of the drug in the vehicle, as this is the driving force for the permeation of drug through the skin. However, with supersaturated formulations, thermodynamic stability and drug recrystallisation are issues often considered during product development.
Determination of drug solubility in semi-solids has become recognised as a significant problem in drug development and excess drugs are commonly added to formulations to produce a saturated system which is often wasteful, and also leads to increased cost of medicine production. Hence, determining the solubility of drugs in formulations is vital; a selection of the available techniques is discussed in detail in the sections below.

1.12.1.1. Visible microscopy to measure solubility in semi-solids

Microscopy provides a simple method where the concentration at which drug crystals are first observed indicates the upper limit of solubility of the drug in the vehicle. The down

side of this technique is that data derived are always qualitative. Many samples need to be prepared around the solubility concentration to get a reliable approximation of solubility. In addition observations are subject to interpretation where errors may occur. Also, this method relies on transparent samples and ability to prepare slides from this. As well as crystals, other solids can be observed for amorphous materials.

1.12.1.2. X-ray powder diffraction to measure solubility in semi-solids

X-ray powder diffraction (XRPD) is a standard method frequently used in the pharmaceutical industry by which crystalline materials are detected and differentiated from amorphous compounds. It is a non–destructive method that has been used to study solid phases of active pharmaceutical ingredients (Suryanarayanan et al, 1992). Biradar et al (2006) recently employed XRPD as an analytical method for studying the effect of different processing and formulation variables on the saturation solubility of roxithromycin in aqueous systems. Ahmed et al (2004) employed XRPD to study penciclovir solubility in eudragit films. Latsch et al (2004) have also reported using this approach to determine solubility of norethindrone acetate in transdermal patches. One major disadvantage of this method is its complexity as well as the fact that active must be in crystalline form. Also the need for expensive apparatus, longer time and laborious preparation of samples (as they must be in the form of films) and the need of trained personnel are further known demerits of this technique.

1.12.1.3. Conventional differential scanning calorimetry (DSC) to measure solubility in semi-solids

There are many thermal techniques that have been applied in pharmaceutical analysis; the most common is differential scanning calorimetry (DSC) with its many variant forms. The most widely used is conventional DSC in which heating rates are based on a linear scale (Victoria and David, 2003). The use of differential scanning calorimetry (DSC) for the quantitative measurement of the solubility of drugs dispersed in semi-solid (polymeric) matrices was first described by Theeuwes et al. (1974); the DSC method was

based on the simple principle that the fraction of drug solubilised within the matrix does not contribute to the melting endotherm (heat of fusion, ΔH_f) associated with the dispersed drug fraction. Hence, by analysing a range of formulations having different concentrations of the drug and integrating the resultant peak seen in the thermogram within the region of the melting point of the drug, it is possible to calculate the heat of fusion (ΔH_f) associated with the thermal process. Plotting the measured ΔH_f values against the drug concentrations for the range of formulations and, then extrapolating the resultant curve to the concentration axis, the solubility of the drug in the polymer was estimated. Victoria and David (2003) analysed ethosuximide suppository formulations using DSC, they observed that at higher drug concentrations (>30%w/w) a separate endothermic melting peak was observed for the drug indicating that it was no longer soluble within the wax matrix, however they did not advance on this to determine the actual solubility of the drug within the wax.

The reported limitations of this technique are prolonged time of analysis, complexity and insensitivity of the technique; in addition the solubility estimate is that at the melting temperature of the drug; which is often not of practical relevance. Also if the vehicle melts at a much lower temperature than the drug then this also leads to insensitivity. However advantages include small sample size required, qualitative data are derived, and a linear regression can be plotted from a series to provide a single estimate of solubility.

1.12.1.4. Temperature modulated differential scanning calorimetry (TMDSC) to measure solubility in semi-solids

This technique involves the small sinusoidal modulation of temperature in conjunction with the usual linear ramp seen in conventional DSC (Hurtta and Pitkänen, 2004). There has been no reported study that details the application of this technique in the measurement of drug solubility. However the use of TMDSC to ascertain miscibility of pharmaceutical drugs in polymers or polymer –polymer interactions at molecular level has been reported in the literature (Feldstein et al, 2003; Hamon et al, 2001).

1.12.1.5. High-speed differential scanning calorimetry (Hyper-DSC) to measure solubility in semi-solids

Hyper-DSC differs to other DSC techniques as Hyper-DSC involves controlled fast heating and cooling rates of >200°C min^{-1} and up to 500 °C min^{-1} (Hurtta and Pitkänen, 2004). This fast scan rate means that kinetic events are minimised, as there is insufficient time for kinetic events (including polymorphic changes and further solubilisation) to occur during the temperature scanning process. This technique significantly increases the sensitivity of thermal analysis due to the increased scan rate with concomitant higher heat flow to reach the desired end point in reduced time. The sensitivity of this technique was demonstrated in a recent study where low levels of amorphous content of pharmaceutical substances in predominantly crystalline samples were quantified via hyper-DSC (Hurtta and Pitkänen, 2004; Saunders et al, 2004). McGregor et al (2004) successfully utilized this novel as a fast analytical tool for studying the polymorphic behaviour of metastable polymorphs. The method was based on the measurement of the specific heat change in the melting point region by hyper-DSC.

HyperDSC was used to determine solubility of drugs in semi-solids; for example, Gramaglia et al (2005) applied this technique to quantify the solubility of metronidazole in a polymeric controlled delivery system where silicone elastomer was the matrix. The prediction of solubilisation seen when this approach is used is of particular importance when looking at drug solubility in wax-based systems that are designed to melt at body temperature, where the melting temperature of the drug is much higher than that of the body. In addition, this technique has been reported (Gramaglia et al (2005) to have the benefit of inhibiting any thermal events that make contribute to further solubilisation as seen when conventional DSC methods were applied. Furthermore, as a high throughput method (runs taking under 1 minute compared to over 20 minutes for conventional DSC), it has been used for analysis of small sample mass with greater sensitivity (Gabbott, 2003). Moreover, it has recently been demonstrated that hyper-DSC is a better tool than conventional DSC in determination of drug solubility within semi-solids (Saunders, 2004).

1.12.1.6. Higuchi release kinetics to measure solubility in semi-solids

Higuchi (1960, 1961) first pioneered the theory of diffusion release of a drug from a polymeric matrix or an ointment. Higuchi (1961) reported that there was a linear relationship between the amount of drug released and the square root of time depending on the state (either dissolved or dispersed) of the drug within the matrix provided that the drug was homogenously distributed and that its loading concentration is much greater than its solubility within the release media and as long as sink conditions applied (Higuchi, 1961). Sink conditions is a state at which the volume of dissolution media is far greater (possibly by a factor of five to ten) than the volume at the saturation point of the drug contained in the drug delivery system being tested. The same concept has been used for many years to calculate the solubility of a drug in a vehicle. In brief, the rate of drug release differs according to the drug form in the vehicle; soluble drugs exhibit different release to the suspended. Therefore, a plot of release rate versus loadings shows an inflection at solubility.

$Q = k_H t^{½}$ (Equation 6)

Where Q is the amount of drug released after time, t and k_H is the release rate constant, the slope of a plot of Q versus $t^{½}$; although this value differs according to whether the drug is in suspension or solution.

For a solution $k_H = \frac{2AD}{\pi}$ (Equation 7)

For a suspension $k_H = D(2A - C_s)C_s$ (Equation 8)

Where Cs is the concentration at which the drug is soluble in the matrix, A is the area of diffusion and D is the diffusion factor.

A plot of k_H versus concentration will have an inflection at the point where the drug is no longer soluble but suspended within the matrix and thus offers a means of measuring drug solubility. Thus, the point at which the two curves (one for k_H of dissolved formulation and the other for k_H of suspended drug formulation) plotted on the same graph met can be extrapolated to the concentration axis and taken as the solubility of the drug.

1.12.1.7. Thermodynamic activity and skin permeation as a tool to predict solubility in semi-solids

Thermodynamic activity is a concentration -like parameter, which measures the capacity of molecules to move from one medium to another across a particular membrane. When the thermodynamic activity is greatest the highest flux will result, where flux is defined as the rate of drug that permeated through a defined area of skin within a specified time. Thermodynamic activity is far less than unity for a substance which is not in its saturated state; the flux of a substance through skin should be greatest when the thermodynamic activity (which drives diffusion) is close to unity, for example in the saturated state. The flux from a saturated solution is at a maximum and therefore should be the same for a drug at its saturated solubility within a matrix provided that the vehicle does not alter permeation and there is no interaction between the drug and its matrix.
Experimentally, it is possible to determine the flux of a drug from a series of concentrations of the drug in the matrix below its solubility to above its solubility and construct a plot of the relationship between the flux and the concentration. If this plot is compared to the flux from a saturated solution of the drug the flux that relates to this value can be deemed to be the flux from the vehicle where the solubility is at a maximum and therefore the thermodynamic activity is unity. Jasti et al (2004) applied this method using lidocaine as a model drug within a polyacrylate pressure-sensitive adhesive.

1.12.1.8. Hildebrand solubility parameters as a tool to predict solubility in semi-solids

Hildebrand theoretical solubility parameters involve the principles of intermolecular attractions; which are attractions between one molecule and a neighbouring molecule. Burke (1984) reported that a solution could only be formed when solvent molecules overcome the intermolecular attractions in a solute. Hence, the solvent molecules have to be aligned within the solute molecules while in addition; the solvent molecules must separate the solute molecules from one another.

Sloan et al (1986) considered the role of solubility parameters (δ) in predicting drug flux (J) and skin permeation.

The Hildebrand solubility parameter is a solubility value, which is reflected by the total van der Waals forces. It provides a numerical value that indicates relative solvency behaviour of a specific solvent and is derived from the cohesive energy density (CED) of a solvent, which in turn is derived from the energy of vaporisation (ΔEv). The energy of vaporisation (ΔEv) measures the amount of energy needed to be added to separate molecule from a liquid into a gas. It was discovered that low intermolecular forces result in a low energy of vaporisation.

Mathematically the solubility parameter is defined as the square root of the cohesive energy densities, which corresponds to the energy of vaporisation per unit volume.

$$\delta = \left(\frac{\Delta Ev}{Vm}\right)^{1/2}$$

(Equation 9)

where Vm represents the molar volume and Ev is the energy of vaporisation.

The solubility of a solid in a vehicle can be expressed by the following equation (Martin, 1993):

$$-\ln X_2 = \frac{\Delta Hf}{RT}\left(\frac{T_o - T}{T_o}\right) + \frac{V_2\phi_1^2}{RT}(\delta_1 - \delta_2)^2$$

(Equation 10)

where X_2: molar fraction solubility; Φ_1: volume fraction of solvent; V_2: molar volume of solute; R: gas law constant; T: temperature in degrees Kelvin; T_0: melting point of the solid; ΔHf: molar heat of fusion; δ_1: the solubility parameter of the solvent; δ_2: the solubility parameter of drug.

For a particular temperature, the first term of the equation is constant. Therefore, the solubility would be expected to increase with a decrease in the difference $(\delta_1 - \delta_2)^2$ as the greatest dissolution is achieved when the forces of attraction between the molecules of two components are similar.

Burke (1984) stated that the cohesive energy density (CED) is a numerical value that indicates the energy of vaporisation in calories per cubic centimetre (or similar units) and is a direct reflection of the degree of van der Waals forces 'holding' the molecules of a liquid together. Furthermore, Greenhalgh et al (1999) defined it as the quantity of energy needed to separate the atoms/molecules of a solid or liquid to a distance where the atoms/molecules process no potential energy.

Equation 11 shows the formula for CED:

$$CED = \frac{\Delta Ev}{V_m}$$

Equation 11: CED (Joules/g/cm^3)

Where:

ΔEv is the energy of vaporisation

Vm is the molar volume

Hildebrand proposed that the square root of CED (equation 12) is a numerical value that can be used to indicate solvency behaviour of a specific solvent, when compared to another:

$$\delta = \left(\frac{\Delta Ev}{Vm}\right)^{1/2} = (CED)^{1/2}$$

(Equation 12)

Using solubility parameters to predict solubility is attractive due to the simplistic nature and can be applied to low molecular weight materials and polymers. The energy of vaporisation cannot be measured for many polymers since a polymer would decompose before its energy of vaporisation could be measured. However calculations using the Fedors group contribution method (Fedor, 1974) can be used to calculate Hildebrand solubility parameters since data for this does compare well with other methods that are used (Greenhalgh et al, 1999).

Calculation of the molar vaporisation energy of a material using the group contribution method involves the summation of molar vaporisation enthalpies of structural fragments in the material. The molecular volume of the material is derived from its density and molecular weight (molecular weight/density) or similar to the calculation of vaporisation energy, it can be calculated using the volume of the molecular fragment present in the material in an additive fashion. Once the solubility parameter values have been obtained, predictions about their miscibility can be made.

The limitations of the Hildebrand solubility parameter comes from the method used to calculate the energy of vapourisation using the Fedors group method; this method relies on segmenting molecules into functional groups that are appropriate and there can be discrepancies in calculations according to the operator involved in the procedure; therefore it is important to recognise that these values are not absolute.

1.12.1.9. Infrared attenuated total reflectance (IR-ATR) spectroscopy as a technique to predict solubility in semi-solids

IR-ATR has been used to measure water/solvent diffusion and can also be used to measure the concentration of drugs within formulations. IR-ATR can continuously monitor the rate at which a drug or excipient diffuses over time; therefore a liquid containing high levels of the drug of interest can be put in contact with the semi-solid vehicle and the drug uptake by the vehicle can be monitored. The equilibrium concentration of drug into the semi-solid vehicle can be taken as a measure of the

solubility of the drug within the vehicle. This method was used by Cantor et al (1999) to measure the solubility of testosterone in acrylic pressure sensitive adhesives.

1.12.1.10. Comparison of techniques used to measure solubility in semi-solids

Several approaches have been used in attempts to measure solubility in semi-solids and these include microscopic examination of drug crystals within the matrix (Gopferich and Lee, 1992); conventional differential scanning calorimetry (DSC) (Theeuwes et al, 1974; Jenquin and McGinity, 1994); infra-red attenuated total reflectance (IR-ATR) spectroscopy (Cantor, 1999); Higuchi release data (Higuchi, 1960; 1961), (Chowhan and Pritchard, 1975); X-ray powder diffraction (XRPD) (Suryanarayanan et al, 1992); Hildebrand solubility parameters (Liron and Cohen 1984; Sloan et al 1986); the relationship between thermodynamic activity and flux (Jasti et al 2004) and hyper-differential scanning calorimetry (HyperDSC) (Gramaglia et al, 2005). Table 1.2 compares the techniques described above and lists the main advantages and disadvantages of each.

In a recent study to measure penciclovir solubility in films, Ahmed et al (2004) found that visible microscopy was the simplest method but provided only qualitative data; whilst DSC, XRPD and Higuchi release provided additional quantitative information such as release kinetics and drug characterisation, although they were time consuming and/or complex.

Technique	Advantages	Disadvantages
Visible Microscopy	Quick, simple and non destructive Highly sensitive Can observe crystal shape, distribution and homogeneity Gives good estimate of drug solubility Active does not have to be crystalline	Data is qualitative
DSC	Observed changes in drug melting point Impurity or polymorphs melting transition Sample size required is less than 10mg Qualitative data gives idea of solubility range Linear calibration plot can be constructed	Time consuming Complex and destructive Active has to be crystalline Moderate sensitivity Estimate of drug solubility at melting point
XRPD	Non-destructive Quantitative data gives idea of drug solubility Linear calibration can be plotted Amorphous compounds do not interfere Useful in identifying compounds and polymorphs	Time consuming and complex Moderate sensitivity Quantitative data gives poor idea of solubility value Data analysis is complex Many sources of error Active must be crystalline Large sample size required
Higuchi release	Active may be crystalline or amorphous Gives good estimate of solubility Data is quantitative Gives good release kinetics of formulation	Time consuming Complex and destructive Sample size of 0.2g required
Hyper-DSC	Very sensitive Gives qualitative data Linear calibration can be constructed Useful in identifying interaction between drug and matrix or excipients Small sample size required	Requires expert personnel Instrumentation is very expensive Validation of assay is challenging Sample is destroyed Active must be crystalline
Relationship between thermodynamic Activity and flux	Simple Gives good prediction of flux of formulation Linear calibration can be plotted	Time consuming Based on ambiguous assumptions Not reliable for vehicles that affects permeation of drugs Prone to many errors
Hildebrand solubility parameters	Simple Give good indication of possible interaction between composition of a formulation Does not require sample preparation	Can not be used for metal salts May not be reliable if structure of formulation components is unknown

Table 1.2. Advantages and disadvantages of the techniques used for the solubility studies.

1.12.2. Melting point

A drug with a low melting point generally has a high aqueous solubility and its solubility will be reduced in the skin since the skin contains less amounts of water (Williams, 2003). Reduced solubility in the vehicle will maximise thermodynamic activity, which is the driving force for permeation of drugs through the skin (as described in section 1.6). Several workers (Stott et al, 2001; Stott et al, 1998 and Hamad et al, 2006) have utilised eutectic systems, an approach that involves formulations with lower melting points than the constituting compositions to enhance the transportation of therapeutic agents across the skin. A eutectic system can be defined as mixtures of two components that do not interact to form a new chemical compound but which, at certain ratios, inhibit the crystallisation process of one another resulting in a system with a lower melting point than either component.

Stott et al (1998) reported that terpene formed hydrogen bonds with ibuprofen to produce a eutectic system (at a ratio 6:4) and that the flux of ibuprofen was increased significantly compared to a saturated solution of the drug. Stott et al (2001) further showed that at 1:1 molar ratio, an interaction between the carbonyl group of the fatty acids (lauric and capric acids) and the amino group of the β-blocker (propanolol) lead to the formation of a fatty-acid propanolol salt with a lower melting point than was predicted from each of the components thereby indicative of a eutectic mixture. This was used to explain the improved permeation of the drug in the presence of fatty acids. Kang et al (2000) have shown that binary mixture of lidocaine and menthol significantly enhanced the permeation of the drug through snakeskin. Lodzki et al (2003) demonstrated that cannabidiol and phosphatidylcholine formed a eutectic system, which enhanced the penetration and accumulation of the drug through and in nude mice skin. A well-known marketed eutectic system technology product is the EMLA cream (eutectic mixture of local anaesthetics), which contains lidocaine and prilocaine.

1.12.3. Partition coefficient

The skin permeation process involves a series of partition and diffusion processes into and out of several layers of cells constituting the skin. One such factor that has been used to predict how well a molecule can undergo these processes is the n-octanol/water partition coefficient, or log P (o/w), which is a measure of how well a substance partitions between a lipid (octanol) and water. The log P value of a drug molecule has been used to predict its distribution within a biological system (Potts and Guy, 1992).

Barry (1987) revealed that the partition coefficient is a determinant factor, which dictated the penetrating route of permeant. Lipophilic molecules are favoured to transverse the skin via intercellular rather than the intra-cellular routes favoured by hydrophilic molecules. However, an ideal transdermal molecule is expected to have an optimum log P value of 1 to 3 (Williams, 2003). At this value, permeants are expected to partition easily into and out of the stratum corneum and into the viable epidermis.

The key factor of log P values in transdermal delivery is better explained by the fact that lipophilic molecules tend to permeate the skin faster than hydrophilic molecules (Jack, 1991). Goosen et al (1998) carried out a study to find the correlation between physicochemical characteristics and the transdermal absorption of NSAIDs; they found that there was an increase in absorption of drugs as the lipophilic character (log P) increased. Lee et al (2001) found that removal of stratum corneum via laser technology enhanced the penetration of hydrophilic drugs more than lipophilic molecules, suggesting that the fatty stratum corneum is the most significant barrier to hydrophilic drugs.

It is therefore, possible to associate permeability coefficient of homologous series of molecules with their solubility within intercellular lipids. However, Beall (1994) in a study to deliver the members of a series of 1-alkyloxycarbonyl-5-fluorouracil (5-FU) derivatives into and through skin discovered that the most effective member of the series at delivering aqueous solutions of 5-FU through skin (flux) was the ethyl derivative, which was twenty-five times as effective as 5-FU, which was also the most water soluble

member of the series. The enhanced flux was due to increased concentration gradient. They concluded that there was a good correlation between flux and water solubility for the entire series but none between flux and lipid solubility. This study reiterates the need for some level of aqueous solubility of permeant in order to transverse the skin.

Gerber et al, (2006) recently demonstrated that an optimum aqueous solubility of not less than 1mg/mL in water was required for effective permeation because there is a need for the permeant to transverse hydrophilic polar head groups of the lipid bi-layers of the skin and moreover, most transdermal formulations are aqueous based formulations. In their studies, Gerber et al showed that the aqueous solubility of acetyl salicylic acid was much greater than its synthesised derivatives and that the flux of the former was much higher than the latter due to optimum aqueous solubility of the former and its high log D (diffusion constant which is related to partition coefficient).

1.12.4. Molecular size

Several workers such as Kasting (1987), Lieb and Stein (1971) and Cronin (1999) have suggested that an inverse relationship exists between membrane diffusivity and penetrant size. In his review, Williams (2003) reported that therapeutic agents selected for transdermal delivery lie within the molecular range of 100 – 500 Dalton.

1.13. Drugs for transdermal delivery

It has been emphasised that an ideal molecule for transdermal delivery should have a molecular size below 500 Dalton with a partition coefficient value between 1-3.5 and an aqueous solubility value of greater than 1mg/ml. Also, the drug should be highly potent with a wide therapeutic window due to the inter-patient variability in skin permeability and a daily dosage of less than 10 mg/day (Williams, 2003). Transportation across the skin is mainly influenced by drug related factors such as the concentration gradient; which is a function of the drug partitioning and diffusivity into and out of the skin respectively. Table 1.3 shows some of the drugs that have being delivered across the skin

as reported in table 1.1 and it is interesting to see that very few, if any, satisfied all the properties defined above.

Drug	MW	Solubility in water	Log P
Estradiol	272.4	0.0039 g/L	3.51
Clonidine	266.6	Soluble	2.27
Nicotine	162.2	17 g/L	1.30
Testosterone	288.4	0.02 g/L	3.59
Buprenorphine	467.6	Slightly	5.09
GTN	228	1.25 g/L	0.12
Fentanyl	336.5	Insoluble	4.14

Table 1.3: Physicochemical properties of selected marketed drugs. MW, solubility and Log P values were taken from http://redpoll.pharmacy.ualberta.ca/drugbank/

1.14. Physicochemical properties of selected non-steroidal drugs

This study investigates the development of vehicles for the delivery of non-steroidal anti-inflammatory drugs, therefore it is important to compare the physicochemical properties of these drugs as it has been reported that these factors have a major effect on the permeation of drugs through the skin (Majumdar et al, 2006). These drugs were selected, as there is a large market for transdermal delivery of non-steroidal anti-inflammatory agents for the treatment of pain. Furthermore, most NSAIDs are relatively potent drugs with reasonable therapeutic window that is a vital factor due to the variability in skin permeability (Williams, 2003). In addition, these drugs satisfy the requirements of an ideal transdermal candidate specified in section 1.11.

Detailed below are the major characteristics that can influence the solubility of ibuprofen, sodium diclofenac, flurbiprofen and naproxen in a matrix as well their pharmacological action. The selected drugs were used due to the wide range of their aqueous solubility value, as it is desirable to know what effect this would have when the selected drugs are formulated into intended dosage forms. Furthermore, the availability of the drug at the time of the study was another factor the influenced the choice of the selected drugs. An example of other NSAIDs that may be selected is ketoprofen.

In Table 1.4, the key physicochemical properties of the selected model drugs are highlighted.

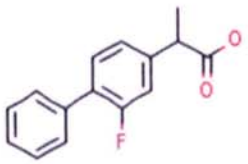
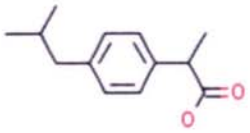
Drug	Flurbiprofen	Ibuprofen
Log P	4.078	3.481
pKa	4.13	4.91
M.Wt (g/mol)	244.261	206.281
M.Point (°C)	110-111	75-77
Chemical Structure		
Water sol. (mg/mL)	8	0.049

Table 1.4a. Physicochemical properties of the selected model drugs; ibuprofen and flurbiprofen. (Information from www.sigmaaldrich.com and from http://redpoll.pharmacy.ualberta.ca/drugbank/cgi-bin/getCard.cgi?CARD=APRD01135.txt)).

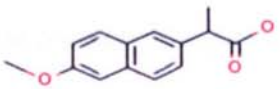
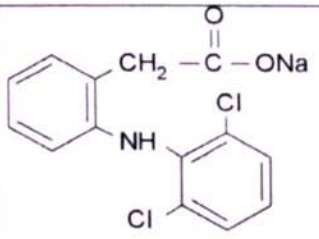
Drug	Naproxen	Sodium Diclofenac
Log P	3.313	3.98
pKa	4.15	4.15
M.Wt (g/mol)	230.259	318.13
M.Point (°C)	153	283-285
Chemical Structure		
Water sol. (mg/mL)	15.9	0.00082

Table 1.4b. Physicochemical properties of the selected model drugs; naproxen and sodium diclofenac. (Information from www.sigmaaldrich.com and from http://redpoll.pharmacy.ualberta.ca/drugbank/).

1.15. Witepsol® H15 as a vehicle for transdermal formulations

Witepsol® H15 belongs to a class of white odourless hard fats composed of triglycerides derived from hydrogenated vegetable oil. It is a commonly used suppository base due to its melting temperature of between 33.5-35.5 °C, a shelf life of about three years and because of its low toxicity profile (Sasol Product Information, 2006). It is a pharmaceutically acceptable excipient (Yahagi et al, 1999, 2000; Yahagi et al, 1999; Berkó et al, 2002; Hanaee et al, 2004; Takatori et al, 2004). Witepsol® bases contain emulsifiers that will absorb limited quantities of water.

The recent research into unit-dose tablet type formulations that also provide high solubility values of drugs that are incorporated into such a base meant that Witepsol® with its pharmaceutical acceptability was an obvious chose as a base for formulations reported within this thesis.

Witepsol® is well known and has being used as a vehicle for delivery of paracetamol in Alvedon suppositories (AstraZeneca, UK). Witepsol® H-15 is a saturated triglyceride that has been reported to promote the absorption and bioavailability of antimicrobials when used as a vehicle for rectal formulations (Bergogne-Bérézin and Bryskier 1999). Witepsol® H15 was chosen for this study as the desirable base due to its availability at the time of the investigations. Other applicable waxes that could be used are bases with glyceride mixtures such as Suppocire® and Gelucire® products. Other Witepsol® grade products (such as Witpeol®; H5, H32, S51, S55, S58 and W25 with melting point below 35° C melting point could also be employed.

1.16. Penetration enhancers to promote skin permeation

Manipulation of a transdermal drug delivery vehicle by increasing the thermodynamic activity of the drug, improving the vehicle / skin partition coefficient and reducing the barrier properties of the stratum corneum are the major means of enhancing penetration of substances through the skin.

In a review, Hadgraft (1999) detailed a conceptual framework (shown in figure 1.5) of how the skin and chemical enhancement approach can be viewed. According to the diagram (figure1.5), it was shown that there are four major approaches to chemical modulation for delivery through the skin, namely; chemical alteration of a drug to a pro-drug, solvent effects, biochemical modulators and vaso-active drugs; each of which works by their effect on the formulation, stratum corneum, viable tissue and the blood respectively.

For better understanding of these approaches, it is vital to briefly describe each of the approaches mentioned above.

The chemical potential of the drug can be improved within a formulation by maximising the thermodynamic potential; this may be manipulated by varying the solubility of the drug within the vehicle.

The incorporation of solvent-like permeation enhancers that demonstrate similarities to the chemical composition of the skin can influence the barrier nature of the skin by disruption of its membrane, thus; resulting in increased permeability to drugs. The most commonly used chemical strategy is the solvent effect. This approach involves the use of chemical substances termed 'penetration enhancers' that act to reduce the barrier properties of the skin either by improving the removal or fluidisation of the skin lipids or increasing the solubility and partition of the permeant out of the formulation into the skin

(Hadgraft, 1999 and Williams, 2003). While many of these chemicals are applied as vehicular systems in the form of micro-emulsions (Lehmann et al, 2001), liposomal based delivery systems (Cerv et al, 1997 and Touitou et al, 2000) and supersaturated solutions (Moser et al, 2001); most are simply blended with other excipients during formulation preparation (Femenía-Font, 2005 and Nokhodchi et al, 2006). However, appropriate penetration enhancing substances should possess acceptable characteristics as reported by Hadgraft (1999), namely; pharmacologically inert, non-toxic, immediate in action, reversible in action, work uni-directionally to allow ingress of permeants and prevent egress of other substances, chemically and physically compatible with the active and other excipients and cosmetically acceptable. However, few chemicals meet all of these requirements.

Walker and Smith (1996) and Wertz (1996) have elucidated that application of vehicles with similar biochemical composition as the skin lipids will not only improve the solubility of the co-administered permeant within the stratum corneum but also increase the lipid fluidity of the intracellular bilayers to allow for easier penetration. In another study, Tsai et al (1996) demonstrated that the use of fatty acid and cholesterol inhibitors; helped to reduce the synthesis of the lipids that maintain the barrier function of the stratum corneum and thus enhanced drug permeation. However, the type of tissue limits this approach because there are variations within and between skin tissues (Williams, 2003).

Vaso-active drugs are chemical agents that are capable of eliciting vaso-constrictive or vasodilative action on the vasculature. Theoretically, this mechanism is thought to be feasible but there are few reports in the literature about the success of this technique (Hadgraft, 1999). However, menthol is a commonly found vasodilator that is included in many transdermal formulations.

To achieve any or a combination (which is attracting more interest) of these strategies, different approaches have being used such as: the long-standing use of chemical

enhancers (Williams and Barry, 2004; Hadgraft and Lane, 2005; Bugaj, 2006 and Liu, 2006).

1.16.1. Surfactants as permeation enhancers

The major mechanisms by which surfactants generally enhance drug permeation are influence on thermodynamic activity of drug, disruptions and existence in separate domains of the stratum corneum lipids (Williams, 2003).
In addition, polysorbate surfactants commonly known as 'Tween' are another group of cosmetically acceptable and pharmaceutically safe substances that have also been widely exploited as penetration enhancers. For example, Cappel and Kreuter (2005) and Alsarra (2005) reported that Tween 20 could act to solubilise the stratum corneum lipids to enhance dermal delivery. Furthermore, in the literature, Williams (2003) explained that structurally, these molecules consist of lipophilic tail and a hydrophilic head group. Furthermore, the non-ionic surfactants are the only known type within this group that is reported to pose no potential risk to human skin (Williams, 2003).

1.17. Strategies to optimise release and permeation from Witepsol® transdermal formulations

In order to enhance the saturation solubility and increase the dissolution rate of selected lipophilic drugs in Witepsol® base, one strategy that could be used is to improve the hydrophilicity of the drugs within the matrix with the use of acceptable excipients. Hence, Tween 20, menthol and Carbopol® 971 were incorporated into the investigated formulations.

1.17.1. Incorporation of Carbopol®

Previous studies have identified that Carbopol® 971 and 934 can increase solubility of the drugs in a base by acting as a co-solvent (Wagner et al 1994; Li et al, 2001; Williams and Barry, 2004 and Yong et al, 2004). Gurol et al (1996) reported that Carbopol® enhanced the release and bioavailability of ketoprofen from different ointment bases. Meanwhile,

Carbopol® is often added in powder form to pharmaceutical formulations for preparation of tablets and gels made of this chemical are commonly used as vehicles for transdermal delivery of drugs (Mura et al, 2000; Shin, 2005 and Yu et al, 2006).

Yahagi et al (2000) in a study on the muco-adhesive nature of suppositories of ramosetron hydrochloride (a 5-HT3: serotonin, receptor antagonist-type anti-emetic) containing Carbopol® reported that incorporation of 2% Carbopol® produced 2.5 times larger area under curve $AUC_{0-24\,h}$ than normal Witepsol H-15 suppositories. In addition, they showed that the Carbopol® improved the water absorbability of the base and facilitated the release of the drug. Barreiro-Iglesias et al (2003) in a study on the controlled release of oestradiol have also shown that Carbopol® / surfactant aggregates acted as efficient carriers of the hydrophobic drug.

1.17.2. Incorporation of Tween

Li and Chen (2002) reported that increasing the concentration of Tween 20 not only increased the equilibrium solubility of drugs in pharmaceutical formulations but also the solubilisation rates of hydrophobic agents. Kawakami et al (2006) found that the solubility of phenytoin in aqueous surfactant solutions increased linearly with increase of the surfactant concentration. Takahashi et al (2002) reported that Tween 20 enhanced the bioavailability of poorly soluble drugs. Saha and Kou (2000) also reported that Tween 20 when formulated with other solubilising excipients improved the soluble donor concentration and bioavailability of a new chemical entity.

Kogan and Garti (2006) recently described the use of Tween 20 in the preparation of microemulsions as effective vehicles for transdermal delivery of certain drugs. In their studies of various microemulsions where model drugs such as retinoic acid, 5-fluorouracil, triptolide, ascorbic acid, diclofenac, lidocaine, and prilocaine hydrochloride were used, Kogan and Garti (2006) discovered that microemulsions were found as an effective vehicle of the solubilisation of certain drugs and as protecting medium for the entrapped of drugs from degradation, hydrolysis, and oxidation. Also, there was

prolonged release of the drugs and prevention of irritation despite the toxicity of the some of those drugs. They attributed the effectiveness of these vehicles to the presence of Tween 20 in that it acted as a typical surfactant.

In another study, Cappel and Kreuter (1991) found that a solution of Tween 20 with other surfactants acting as transdermal delivery vehicles influenced the skin permeability of hydrophilic molecules, with a slight increase observed, however, the permeability of lipophilic octanol decreased as a function of polysorbate concentration due to a decrease in thermodynamic activity as a result of micelle formation. Alsarra et al (2005) have used Tween 20 for transdermal delivery of ketorolac.

1.17.3. Incorporation of Menthol

In a recent study, Yong et al (2004) observed that the influence of menthol on the enhanced transdermal delivery of ibuprofen from a liquid suppository formulation could be linked to the increase in aqueous solubility of ibuprofen in the vehicle. In another study that involved the effect of menthol on release rate of drugs from poloxamer matrix, Yong et al (2004) noticed that menthol improved the dissolution rates of ibuprofen from poloxamer gels. Prior to this, Ho et al (1997) published their findings on the improvement of indomethacin solubility in cream formulations with the addition of menthol and in the presence of other co-solvents. A recent study by Fang et al (2007) also concluded that both menthol and ethanol acted synergistically to enhance the transdermal delivery of anaesthetic tetracaine (from gels made of Carbopol® 940) across rat and human skin (in both experimental and clinical studies).

Furthermore, in another study on the effect of terpenes and their mechanism of action on transdermal delivery of zidovudine, Thomas et al (2004) concluded that even though menthol, like other terpenes, increased the flux of the drug, it did not affect the thermodynamic activity or saturation solubility of this drug in the solvent system vehicles. Nevertheless, Sudo et al (1998) from a study on *in vivo* and *in vitro* transdermal absorption of L-dopa from hydrogel in rats concluded that menthol increased the plasma

amount and flux of the drug to reach a therapeutic level suitable enough to treat Parkinson's disease. Contrarily, Gao and Singh (1998) demonstrated that enhancement in the permeability coefficient of tamoxifen by menthol was due, at least in part, to improvement in the partitioning of the drug in the stratum corneum. In addition, Narishetty and Panchagnula (2005) concluded that menthol enhanced the transdermal permeation of zidovudine and other drugs by transforming SC (stratum corneum) lipids from a highly ordered orthorhombic perpendicular sub-cellular packing to a less ordered hexagonal subcell packing. In another study, using synchrotron x-ray diffraction, Obata et al (2006) found out that the action of menthol in enhancing the flux of drugs through hairless rat stratum corneum could be linked to its effect on decreasing the ration of lipid hydrocarbon chain packing index.

Importantly, it is vital to state that although the present studies reported in this thesis involve only *in vitro* experimentation, there is much literature reporting the vasodilatory action of menthol in *in vivo* transdermal studies involving both humans and animals. There is no reported vasodilatory action of menthol in *in vitro* transdermal experimentation. For example, Brian et al (2006) reported that there was no difference in the plasma level of ibuprofen when compared to *in vitro* results on comparison of two marketed products Ibuleve™ gel (5% w/w Ibuprofen, Dermal Laboratories, UK) and Deep Relief™ gel (3% w/w Ibuprofen, Mentholatum, UK).

Several other studies have looked at the addition of release promoters to lipophilic drug-in-oil formulations. For example, Hanaee et al, (2004) have shown that non-ionic surfactants such as Tween 80 and ionic surfactants like sodium lauryl sulphate increased the release of salbutamol from oily suppositories. Miyake et al (2004) also discovered that incorporation of sodium laurate, taurine or L-glutamine remarkably improved the dissolution of rebamipide from fatty base suppository prepared using Witepsol® H-15.

1.18. Aims and objectives

1.18.1 Aims

The aims of the study were:

- To accurately measure the solubility of drugs in semi-solids for transdermal drug delivery
- To develop a transdermal formulation that:
    - allows delivery of drugs that fall outside the optimum range for existing transdermal drug delivery technology (ie those with a log P much greater than 2)
    - can be formulated as a unit dose for accurate dosing
    - can contain high concentrations of drug that are soluble to maximise the concentration gradient and therefore the flux
    - demonstrates greater *in vitro* efficacy than existing formulations

1.18.2. Objectives

There are few analytical techniques that can determine accurately the solubility of a drug within a wax-based semi-solid; one of the objectives of this study was to compare the use of hyper-DSC as a novel approach to determine drug solubility in fat based semi –solid formulations with other techniques (microscopy, Higuchi kinetics, theoretical models). Each of these approaches is expected to provide estimates of the solubility of ibuprofen, naproxen, and flurbiprofen in Witepsol® based formulations.

Wax-based tablets for transdermal drug delivery offer the potential for a unit dose delivery system that can be applied directly to the skin. Using a wax that melts close to body temperature enables the drug-wax blend to melt when applied to the skin thus delivering drug at the site.

The formulations investigated are to be optimised in terms of drug solubility and release via the inclusion of excipients such as menthol, Carbopol® 971 and Tween 20. The effect of these pharmaceutically acceptable excipients on the release and permeation (through full dorsal porcine skin) of the selected model drugs from the lipophilic-based

formulations would be investigated. The effect on these excipients on the release of the selected drugs directly and further manipulation of the data obtained to calculate the solubility of each drug within the formulations is to be carried out. Mills et al (2001) reported that menthol enhanced the release of diclofenac from wax-based matrix. Cappel and Kreuter (2005) and Alsarra (2005) had also reported in other studies that Tween 20 enhanced the release of drugs and further solubilised the stratum corneum lipids to promote transdermal delivery of such drugs. These reasons justified the choice of these excipients.

Ultimately, the main objective of this project is that this novel formulation should be a more reliable, accurate and cost effective means of dosing drugs both into and across the skin. This product should outperform currently marketed products according to *in vitro* models.

Chapter Two

Materials, Methods and Validation of Methodologies

2.1. Materials

Table 2.1 below shows the details of all the materials that were used.

Material	Supplier	Batch Number	CAS Number	% Purity
Acetonitrile	Fisher Scientific, UK	0606916	200835-5/75-05-8	99
Methanol	Sigma Aldrich, UK	0614272	200-659-6 / 67-56-1	99
Water (double distilled)	Generated in-house	-	-	
Orthophosphoric acid	Sigma Aldrich, UK	80K3723/P6560	231-633-2	99
Acetic acid	BDH, UK	6749620J1/5222		99
Durotak 87-900A	National Starch, USA	90676954		
Durotak 87-201A	National Starch, USA	90302956		
Durotak 87-2677	National Starch, USA	90543390T		
Witepsol® H15	Sasol, South Africa	BW575		
Menthol	Sigma Aldrich, UK	0614272	200-659-6/67-56-1	99
Tween 20	Fisher Scientific, UK	001336	/9005-64-5	70% in water
Carbopol® 971	Noveon, USA	CC15NAJ119		
Ibuprofen	Sigma Aldrich, UK	R/N 60473	15687-27-1	99
Flurbiprofen	Erregierre (Italy)	0302006	5104-49-4	98
Diclofenac Sodium	Fischer Scientific, UK	D6899-25G	239-346-4/15307-79-6	99
Naproxen	Sigma Aldrich, UK	N8280-5G/075K	244-838-7/22204-53-1	98
Disodium Fluorescein	Sigma Aldrich, UK			
Sodium Hydroxide	Sigma Alrich, UK			98
Hydrochloric acid	Sigma Aldrich, UK			37
KH_2PO_4	Acros UK	9896494/498	2319134	99.95
Na_2HPO_4	Sigma Aldrich, UK	0583639	231-448-7/10028-24-7	99
Sodium Chloride	Sigma Aldrich, UK	U05602	7647-14-5	99
Glycine	Fisons, UK		G/0800/5	99
Citric acid	Sigma Aldrich .UK			99
Micropore syringe filter	Kinesis, UK	00058679/F2613		

Table 2.1: Materials used in all the experiments reported in this thesis.

It should be noted that where Carbopol® 971 was used in this thesis, it is referred to as Carbopol® 971. Also, to ascertain purity and quality control, the same batch of Witepsol H15® was employed throughout this project.

2.2. Preparation of wax based formulations

2.2.1. Via melting

In preparing the drug-in wax formulation, the wax was melted using a gentle heat and the required amount of drug was added to this melted wax and blended until homogenous as described for the production of suppositories. Once a homogenous mixture was formed this was poured into fixed volume moulds and allowed to fully cool.

2.2.2. Via Granulation

A granulation method was introduced that not only enhanced the reproducibility of the drug content of each formulated batch but also promoted even distribution of drug molecules in the matrix when compared to the melting method. In this method, weighed drug (ibuprofen, naproxen, flurbiprofen and diclofenac) was dispersed gently into the melted wax (Witepsol® H15) at between 30- 37 °C and this was continuously stirred until visibly homogenous before being allowed to set via cooling at room temperature (over a period of hours). Excipients were added (if appropriate) before cooling. Fast cooling was avoided as this tended to harden the mixture too much which then made it difficult to process. The cooled mixture was then crushed into large granules using a mortar and pestle; these granules were stored at 4°C overnight and further milled using a mortar and pestle to form a coarse powder (particles of approximately 200-1000μm diameter). Tablets were prepared by subjecting 100 mg of the powder to one tonne of force for 20 seconds on a conventional KBr table press (Sepac series 15.011) to make cylindrical tablets of 5 mm diameter. Tablets made were stored at 4°C until needed.

2.2.3. Analysis of drug content

A wide range of drug loadings was made as appropriate for each study (see table 2.4 below). To check homogeneity, at least three repeats of each formulated batch were prepared and at least three tablets from each batch were assayed for drug content as described in section 2.7. Section 2.13 describes the method used for determination of the drug stability in the wax formulation.

2.3. Uniformity in weight of tablets

The tablets used for this study were prepared using the method described in section 2.2. European Pharmacopoeia method 2.9.5 (European Pharmacopeia, 2006) was used to determine the uniformity of weight of the formulated tablets, no more than two tablets should deviate by 7.5% of the mean weight and no tablet should deviate by more than 15% of the mean weight. For each batch, 20 tablets were randomly selected and weighed individually using a four decimal place Kern 770 balance (Kern, Germany). This was performed to ensure reproducible manufacture.

2.4. HPLC analysis

Analytical and pharmaceutical grade drugs and high purity HPLC graded solvents were used throughout these studies. Sigma Aldrich (Poole, UK) supplied acetonitrile and orthophosphoric acid. Double distilled water was produced in-house in a Fison distiller. A Hewlett Packard Agilent 1100 HPLC system fitted with G1312A Binary pump, G1313A ALS auto-injector, 0831341A thermo Hypersil ODS column (150 x 4.6) mm and a G1314A VWD (variable wavelength detector) was used for all analysis. Chromatograms were integrated with the Rev.A.09.03 Chemstation (2002) software. This system was employed for the analysis of samples that contained ibuprofen, flurbiprofen and naproxen.

A second HPLC system used in the course of these studies was a Dionex (USA) chromatography system which consists of a high - pressure gradient pump (model GP50), an autosampler (model AS50) equipped with a pre-packed phenomenex 5μ ODS 2column (150mm × 4.6mm, 5μm, Phenomenex, USA), and a variable wavelength UV detector (UVD 170U). The data analysis was performed using Dionex Chromeleon Chromatography management system (Dionex, USA). This system was used for the analysis of samples that contained diclofenac.

Calibration samples were prepared by dissolving each drug in PBS at pH 7.4 (Sorensen's phosphate buffer solution; see appendix 1 for composition). These were used as standards

and were diluted using PBS. This is most appropriate as it mimics the physiological pH of the human body, hence; the release study and permeation experiments were carried out in this media. The standard curve was constructed by plotting the area under the chromatogram against the corresponding drug concentration and the equation of the line was applied for all samples taken from each study. All calibrations covered the concentration region of interest with at least five repeats prepared. The injection volume (this varied depending on the drug; see appropriate sections below) as well as the intra and the inter batch results were highly reproducible. Calibrations curves are shown in figures 2.1; 2.2; 2.3 and 2.4 while the data are shown in tables 2.9, 2.10, 2.11 and 2.12. The limit of detection and limit of quantification for each drug is detailed in section 2.15.1.

2.5. Hyper -DSC methodology

HyperDSC; a variant form of conventional DSC described in chapter one was applied as a relatively new technique to investigate the nature and the solubility of the selected common non-steroidal anti-inflammatory drugs (NSAIDs) in a wax based matrix. The samples analyzed were prepared as described in section 2.2 above. Calibrations were performed in accordance with manufacturer's advice.

Thermal analysis was carried out in a pre-calibrated (for temperature and enthalpy at 500°C/minute using an indium standard; Perkin-Elmer) Diamond DSC (Perkin-Elmer, Pyris series 5.0) according to the manufacturer's specification.

Less than 2mg of the formulated granulated samples of each drug containing varying loading (% w/w) of the active (the exact amount of each drug is detailed in relevant sections below) were placed air tight in hermetically sealed aluminium pans suitable for volatile materials (Perkin Elmer) and analysis was carried out under stated conditions mentioned for each drug as stated below. An empty pan was used as a reference for each run; the resulting thermograms were analysed using Pyris series 5 data software (Perkin Elmer).

A thermogram is the graphical representation of the heat profile that results from a thermal analysis of a material. Endotherm peaks are usually seen on this graph indicating the melting point of the substance being analysed.

Appropriate calibration of HyperDSC is important, recently Vanden and Mathot (2006) published some recommendations for temperature calibration in the heating and cooling mode in order to minimize thermal lag. These recommendations were followed to avoid this problem.

Reproducible results have previously been achieved under these conditions (Gramaglia et al, 2005; Saunders et al, 2004).

Purge gas	Helium
Gas supply rate (ml /minute)	20
Scan rate (°C / minute)	500
Analyser	Pyris Diamond DSC (Perkin Elmer, UK)
Scan range (°C)	-30 to 200 or 250
Software	Pyris software (version 5.0, Perkin Elmer, UK)

Table 2.2. General hyper-DSC parameters.

Low mass of samples (1 - 2.20 mg) and pans specially designed by Perkin Elmer (UK) were used throughout this study due to volatile nature of the base matrix. Preliminary investigations showed that samples were spilled from the pans during the analytical process when high sample mass and conventional DSC pans were used. Helium was used as a purge gas due to its lightweight that has an added advantage of rapid response equilibration time.

Diclofenac formulations were not analysed via this approach due to the high melting point of the drug.

Section 2.15.2 shows the detail validation of this assay.

2.6. Visible microscopy

Formulations were prepared as described in section 2.2 above.
In this study, blends of each drug (as appropriate see section 2.2 above) at different loadings in the wax matrix, were melted and stirred until completely homogenous and then examined at room temperature under a visible microscope (Axioskop 40) with a 4x objective lens for the presence of drug crystals and photographs were taken with an Axio high resolution camera. Images were captured using Axiovision version 3.1 software.

2.7. Drug content

Drug content and matrix interference was determined by dissolving a prepared tablet containing known amount of each drug, in 10 ml of PBS: methanol (50:50) solution and stirred in a water bath maintained at 37°C for 24 hours. At least four repeats for three batches of tablets prepared for each drug was carried out. 1ml of this solution was diluted to 10 ml with PBS medium and analysed for drug loading using appropriate HPLC assayed described above. Solubility of the drugs varied in the media used, however, the recovery was more than 90 % loaded drug amount in each case. Table 2.3 shows the percentage recovery data for each drug.

Drug	Initial drug loaded (mg)	Measured drug amount(mg)	% Recovery
Flurbiprofen	10	9.08 ± 0.91	90.8
Ibuprofen	10	9.24 ± 0.46	92.4
Naproxen	10	9.51 ± 0.57	95.1
Diclofenac	10	9.65 ± 0.12	96.5

Table 2.3. Percentage recovery of each drug from the wax matrix. Data shows mean ± s.d. n = 4.

2.8. Excipient manipulation

Table 2.4 shows the amount of excipients added to the investigated formulations for samples employed during the drug release study reported in chapter eight of this thesis.

These formulations were prepared using the granulation method described in section 2.2.2.

Batch	Tween 20 %(w/w)	Carbopol® 971 %(w/w)	Menthol%(w/w)
1	0	0	0
2	1	0	0
3	2	0	0
4	1	1	0
5	1	2	0
6	2	0	5
7	1	1	5
8	0	0	5
9	0	1	0
10	0	2	0

Table 2.4. Formulations used for release and permeation studies.

2.9. In vitro drug release method

This section describes the materials, method and formulation parameters employed for the drug release study to investigate both the effect of excipients on release and the determination of the drug solubility in the matrix following Higuchi kinetics.
The apparatus used was a dissolution test station and USP II baskets (Hanson Research, USA). Samples were filtered with 0.45μm micropore filters (Kinesis, UK). Analysis was carried out using UV- HPLC.

The release studies were carried out using the USP II basket type method adapted for small volume. A 250 ml beaker was placed into the larger manufacturer's dissolution beaker and held in place with specially designed polystyrene rings. 200ml phosphate buffer solution (pH 7.4) was used as the dissolution medium (kept at 33 °C) was poured into the smaller beaker and one tablet was placed in each of the three baskets. The baskets were rotated at 100rpm and at least three repeats for each drug carried out. 1ml samples were collected at 5-minute intervals up to 30 minutes, then at 10-minute intervals up to 70 minutes and finally the last at the 100^{th} minute. Fresh medium was used to replace collected samples at each time point. Using these conditions, preliminary studies carried

out showed that the dissolution media maintained sink condition within the specified time of the experiment. Concentration data were collected as the area under the curve of the chromatogram obtained after analysis of samples that were converted to concentrations by applying the corresponding calibration curve within the linear range 0.001 to 0.1mg/ml or 1mg/ml as applicable (section 2.4 above). Mean cumulative mass released was calculated as were standard deviations.

Each of the tablets used for these studies had maximum of 45mg of each drug in the wax; at most for the Higuchi approach method for solubility determination studies while 10mg of the drugs made up the tablets used for the lead formulations. Each Franz cell used for the latter studies contained average of 34 ± 1 ml of PBS and 200 ml of the same media was used for dissolution studies in the former investigations.

2.10. Preparation of porcine skin

Full dorsal thickness porcine ear-skin was used as an established model (Simon and Maibach, 2000) to simulate penetration of substances across human skin. The freshly cut porcine ears were collected from a local abattoir prior to steam cleaning and washed under running water. Dorsal portion of the skin was removed with a scalpel and cut into approximately 2cm diameter sections before being stored on aluminium foils at -20°C until required (< 3months). Frozen-thawed skin has been reported to maintain its integrity within this period under the stated conditions (Heard et al, 2006). All skin tissue was used at most with in one month of storage.

Preliminary *in vitro* diffusion studies in our laboratory shows that there was no significant difference between the flux of selected drugs used in this study when 1ml of prepared solutions containing known amount of the selected drugs were applied to fresh porcine skin and those stored at -80° C for up to three months (see table 2.5 below for data). The permeation was carried out using the method described in section 2.11 with the exception of donor solution in this case.

Drug	Flux (μg/cm^2/h) at day one through fresh skin	Flux (μg/cm^2/h) at three months
Ibuprofen	7.31 ± 1.06	7. 68 ± 0.78
Naproxen	3.46 ± 0.52	4.09 ± 0.46
Flurbiprofen	9.11 ± 1.05	9.03 ± 2.11
Diclofenac	10.22 ± 1.47	11.38 ± 0.89

Table 2.5. Effect of storage (at -80° C for up to three months) on skin permeability. Data shows mean ± s.d. N = 3.

2.11. Skin Permeation study methodology

Kika (Germany) supplied the static Franz diffusion cells (model RO 10). Kinesis, UK. Supplied 0.45μm micropore filters. Analysis was carried out using UV-HPLC system described in section 2.3.1.2 above.

Permeation studies were carried out using all glass static Franz type diffusion cells (Friend, 1992). 100 mg of the formulated granules equivalent to 1 tablet was rubbed into the dorsal side of full porcine skin which was then placed over the receiver compartment of individually pre-calibrated diffusion cells with a diffusion area of 3.1428 cm^2 pre-filled with phosphate buffer pH 7.4 (de-gassed to prevent formation of air bubbles). Each cell held 34 ± 1ml of the receptor media. The donor part was clamped into place and 1ml samples were withdrawn at set time points (1, 2, 6, 12 and 24 hours) prior to analysis via HPLC-UV. Fresh receiver medium was used to replace volume after samples were taken in order to ensure sink conditions and maintain contact between the skin membrane and the bathing receiver medium. The fresh medium added was taken into consideration during mathematical calculation of the flux (see equation 13 below). The receiver solution was continuously stirred using a magnetic stirrer while the whole diffusion cells were positioned on the Kika (R0 10P; Germany) magnetic stirrer plate stirred at mode 4 to ensure homogeneity in the receiver solution. The whole experimental set up was connected to a closed circuit circulating water bath (Churchill, England) set at a temperature at 37 °C, providing a skin surface temperature of 32 °C by heat dissipations.

$$M_t = C_t V_T + C_{t-1} V_S \qquad \text{equation 13}$$

Where

M_t = Mass of drug released at time t

C_t = Calculated concentration at time t

V_T = Total volume of dissolution medium (34 ± 1) ml

C_{t-1} = Sum of the calculated concentration of samples collected at time t –1

V_s = Volume of samples taken and equally replaced with fresh medium at time t

The maximum drug loading in a tablet for each drug investigated was kept at 10mg. Naproxen, sodium diclofenac, flurbiprofen and ibuprofen have aqueous solubility of 15.9mg/ml, 0.00082mg/ml, 8mg/ml and 0.049mg/ml respectively (see table 1.4a and b above); therefore, the total dissolution medium volume (34 ± 1ml) used would ensure that sink conditions was maintained.

The permeation efficacy of the lead formulations was compared with that of the commercial products, 1 tablet each of formulation 7 (used in section 5.3) was used while 2 g each of the commercial products were applied to the skin. Thus, with these parameters; it was known that each of the samples would contain approximately 20 – 23 mg of the active material except formulation 7, which contained 10 mg. However, for flux-solubility determination studies, saturated solutions (in PBS pH 7.4) of the selected drugs were used as donor formulations.

To validate the permeation method, 10ml of each drug (investigated) in solution was added to skin samples and diffusion studies was carried out as described above for a period of 24h. At least five repeats for each of the diffusion cells was carried out. The flux obtained for each drug after these repeats was similar (shown in table 2.6) and this indicates that the assay would be suitable for the permeation procedure.

Drug	Flux (µg/cm^2/hr)
Naproxen	24.05 ± 2.00
Flurbiprofen	106.61 ± 0.93
Ibuprofen	131.16 ± 1.17
Diclofenac	176.01 ± 2.98

Table 2.6. Validation data for skin permeation study. Mean ± s.d. N= 5.

Calculation for permeability coefficient and Enhancement ratio

Permeability coefficient is a parameter calculated by dividing the calculated flux of a drug by its loading concentration in the applied formulation. Enhancement ratio provides a better indication of any changes between two comparative parameters or conditions such as the effect of an excipient that was added to a formulation at different concentration, on the permeation of a drug through a membrane (Goodman and Barry, 1988).

$$\text{Enhancement ratio (ER)} = \frac{\text{Kp after addition of excipient}}{\text{Kp of control formulation}} \qquad \text{(Equation 14)}$$

In all cases, ER was calculated by dividing the Kp value of theformulation of interest by the Kp value of control formulation (1); which serves at the constant divisor in this case.

2.12. Determination of skin concentrations of drug following permeation experiments

The amount of drug retained within the skin following the permeation study was determined by modification of the method described by Touitou et al (1994). The full skin was removed from the Franz cell after 24 hours while the remaining portion of the applied formulation was rubbed off the skin with a dampened (in phosphate buffer, pH 7.4) blue roll before the skin surface was washed with phosphate buffer. The full dorsal skin was then cut into very small portions using a pair of surgical scissors and the resulting fragments introduced into a vial containing approximately 10 ml of phosphate buffer: methanol (50:50). The resulting suspension was then homogenized by stirring overnight (~12 h) and maintained at 37°C, after which samples were removed, filtered (Micropore syringe filter, 0.45μm) and analysed via appropriate HPLC method.

To validate this technique, saturated solution of each drug containing known amount of the drugs was applied to the skin for permeation, the amount of drug recovered after skin extraction studies was added to the amount that penetrated the skin and the amount left in

the applied formulation, the percentage recovery was more than 97% as shown in table 2.7.

Drug	% of drug flux	% skin extraction	% left over of applied formulation	Total %
Naproxen	25	27	46	98
Flurbiprofen	35	40	22	97
Ibuprofen	43	42	14	99
Diclofenac	57	28	12	97

Table 2.7. Validation data for skin extraction studies. Mean ± s.d. N = 5.

2.13. Determination of drug content for stability test

The drug stability in the matrix was determined by dissolving 1 tablet of each formulation in 10ml PBS in a glass vial stirred in a water bath maintained at 37 °C for 24 hours. The solution mixture was filtered and assayed using HPLC. At least five repeats of each formulation were analysed. For long-term stability test, a similar procedure was followed but the lead formulation samples of diclofenac were chosen at 0, 7 and 360 days after preparation and were analysed for comparison. Diclofenac was selected due to known stability issues (European Pharmacopeia, 2006).

2.14. Statistical analysis

For the statistical analysis, Primer biostatistics software (version 4.0) was used (Glantz, 1996). One-way anova (parametric statistical analysis method) and student- t- test methods were used throughout this thesis.

For comparison of values across a whole set of results, one way anova was applied but for statistical comparison between two values in a set, student t-test was employed. Bon- ferroni test was also employed as a non-parametric test. In each case, number of subjects in a set was at least 3 and standard deviation calculations were worked out accordingly using Microsoft Excel spreadsheet.

2.15. Validation of methodology

2. 15. 1. Validation of HPLC assays

The method assays (2.15.2, 2.15.3 and 2.15.4) were validated for repeatability, intra – and inter-day precision by analysis of the standard solutions prepared for each drug (at least three concentrations were chosen) within the expected range of the samples. At least five analyses of each samples was done.

The relative standard deviation at selected concentrations calculated for; diclofenac (1μg/ml, 10 μg/ml and 100 μg/ml), flurbiprofen (5 μg/ml, 50 μg/ml and 100 μg/ml), naproxen 0.01 μg/ml, 0.1 μg/ml and 1 μg/ml) and ibuprofen (1 μg/ml, 25 μg/ml and 100 μg/ml) was within 2% for each of these drugs and the lower limits of quantification was within 20% of the calculated concentrations.
The limit of detection of ibuprofen, naproxen, flurbiprofen and diclofenac calculated using the intercept from the calibration curve where three times the intercept value is a measure of the limit of detection and ten times provides the limit of quantification.

The assays were also tested for specificity by injection of blank samples of PBS containing no analyte. There was no peak observed in the chromatogram for these samples.

2.15.1. 1.Diclofenac assay

Fischer chemicals (UK) supplied the USP –BP grade sodium diclofenac that was used for this analysis. 1 – 100μg /ml standard solutions were prepared via serial dilution of a 1 .0 mg/ml stock solution. Diclofenac was analysed using a modified protocol published by Rouini et al (2004). The chromatographic parameters were:

Injection volume (µl)	100
Mobile phase	double distilled water, acetonitrile and acetic acid: 50:50:2
Wavelength (nm)	276
Flow rate(ml/minute)	1.60
Run time(minutes)	10
Column	Thermo-ODS-2 Hypersil 150mm x 4.6mm x 5µm

Table 2.8. Chromatographic conditions for analysis of diclofenac.

The mobile phase was run under isocratic conditions. An example of the typical chromatogram of diclofenac obtained from the analysis of samples of the drug derived from freshly prepared formulations is shown in figure 2.1. A similar chromatogram was obtained from analysis of samples 7 and 360 days old (section 2.13), although the area under the curve was much reduced. Investigations into the applicability of this assay on the stability of diclofenac will be carried out in further studies.

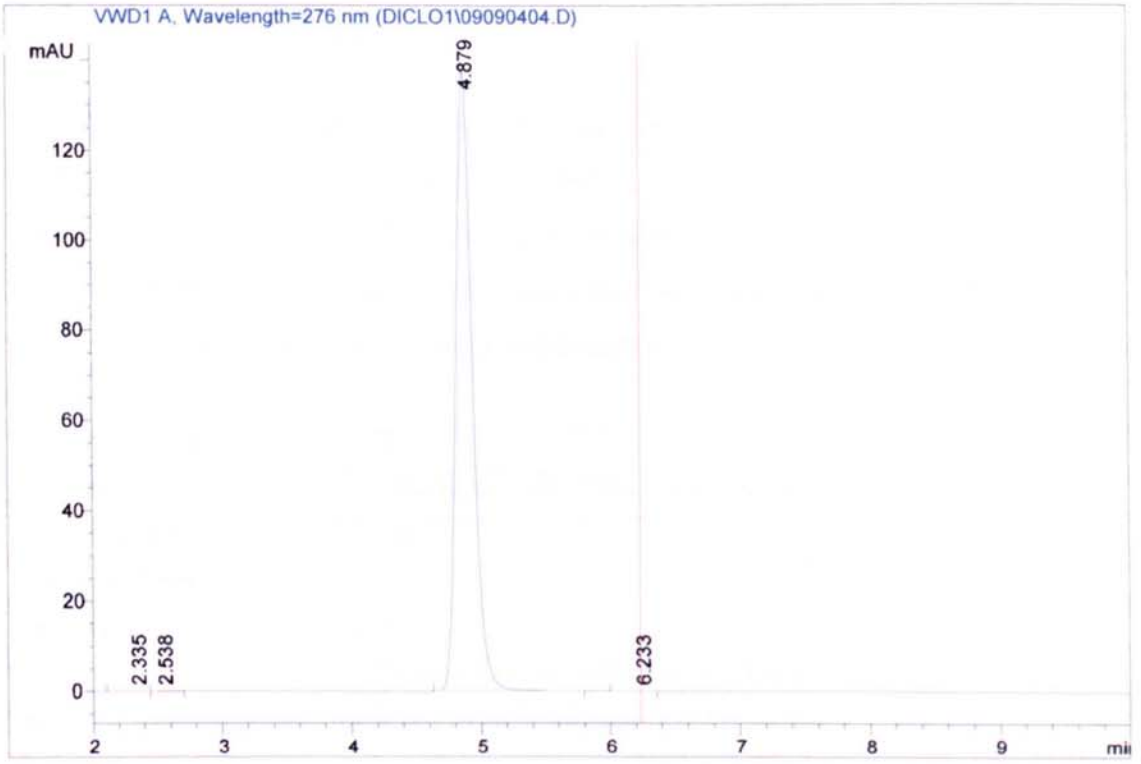


Figure 2.1: Typical HPLC chromatogram of diclofenac (concentration 0.1mg/ml at 276nm). Absorbance is on the y-axis and time (minutes) is on the x-axis.

An example of the calibration data for diclofenac is presented in below. The correlation coefficient, r^2 for this calibration was 0.998 with a limit of detection of 1.1µg/mL and the limit of quantification was 3.6µg/mL.

Concentration (µg/ml)	Area ± s.d	Relative standard deviation %	Relative Recovery (%)
0	0.00 ± 0.00	0.00	
1	0.36 ± 0.08	19.09	99
10	4.30 ± 0.04	1.05	98
25	11.05 ± 0.14	1.29	98
50	21.84 ± 0.04	0.22	
100	40.56 ± 0.21	0.52	

Table 2.9. Calibration and validation data of Diclofenac Sodium assay. n = 5. Data shows mean ± s.d.

2.15.1.2. Flurbiprofen assay

The USP grade flurbiprofen used during these studies was bought from Erregierre (Italy). Calibration samples in the range of 0.001, 0.005, 0.01, 0.025, 0.05, 0.075 and 0.1 mg/ml were made by serial dilution of the 1mg/ml stock solution in phosphate buffer pH 7.4. These concentration ranges were used because they were more appropriate. The method published by David (2006) with the chromatographic parameters was adapted.

Injection volume (µl)	20
Mobile phase	acetonitrile, double distilled water and orthophosphoric acid: 65:35:1
Wavelength (nm)	250
Flow rate(ml/minute)	1.00
Run time(minutes)	10
Column	Thermo-ODS-2 Hypersil 150mm x 4.6mm x 5µm

Table 2.10. Chromatographic (isocratic) conditions for analysis of flurbiprofen.

An example of the typical chromatogram of Flurbiprofen is presented in figure 2.2 below.

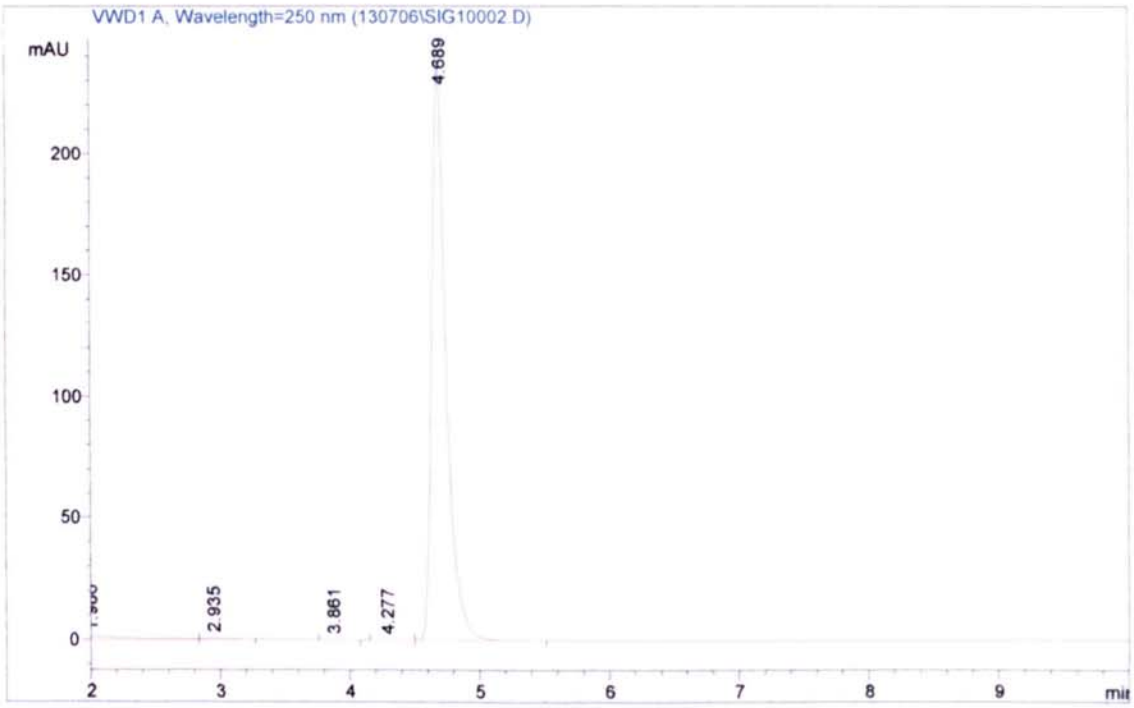


Figure 2.2: Typical HPLC chromatogram of flurbiprofen (concentration 0.1mg/ml at 225nm). Absorbance is on the y-axis and time (minutes) is on the x-axis.

An example of the calibration data for flurbiprofen is shown in the table below. The correlation coefficient, r^2 for this calibration was 0.999. The limit of detection for this was 1.14µg/mL and the limit of quantification was 5.11 µg/mL.

Concentration (µg/ml)	Area ± s.d	Relative standard deviation %	Relative Recovery (%)
0	0.00 ± 0.00	0.00	
5	463.31 ± 0.23	0.05	97.50
10	866.88. ± 3.10	3.57	
25	2111.31 ± 1.94	0.91	98.00
50	4145.49 ± 0.75	0.18	
75	6123.75 ± 3.63	0.59	
100	8182.54 ± 4.19	0.51	99.00

Table 2.11: Calibration and validation data of Flurbiprofen assay. N = 5. Data shows mean ± s.d

2.15.1.3. Ibuprofen assay

USP-BP grade ibuprofen used for these studies was a gift from GSK (Weybridge, UK). Calibration standards in the range of 0.001, 0.005, 0.01, 0.025, 0.05, 0.075 and 0.1 mg/ml were produced by serial dilution of the 1mg/ml stock solution in phosphate buffer pH 7.4. This media was chosen, as the drug is more soluble at this pH. Further details of the pH effect can be seen in section 3.5.1.

The chromatographic parameters were:

Injection volume (µl)	100
Mobile phase	acetonitrile, double distilled water and orthophosphoric acid: 65:35:1
Wavelength (nm)	225
Flow rate(ml/minute)	1.00
Run time(minutes)	10
Column	Thermo-ODS-2 Hypersil 150mm x 4.6mm x 5µm

Table 2.12. Chromatographic (isocratic) conditions for analysis of ibuprofen.

An example of the usual chromatogram of ibuprofen is shown in figure 2.3. Characteristic calibration data for ibuprofen is presented the table below. The correlation coefficient, r^2 for this calibration was 0.999. The peak seen at 5.069 min indicates ketoprofen while peak at 8.76 min is that of ibuprofen. Ketoprofen was used as an internal standard in this case due to the reported ability of ibuprofen to form interaction with menthol (Stott et al, 2004); an excipient to be used in these studies. The limit of detection for ibuprofen was calculated to be 0.39µg/mL and the limit of quantification was found to be 1.75µg/mL.

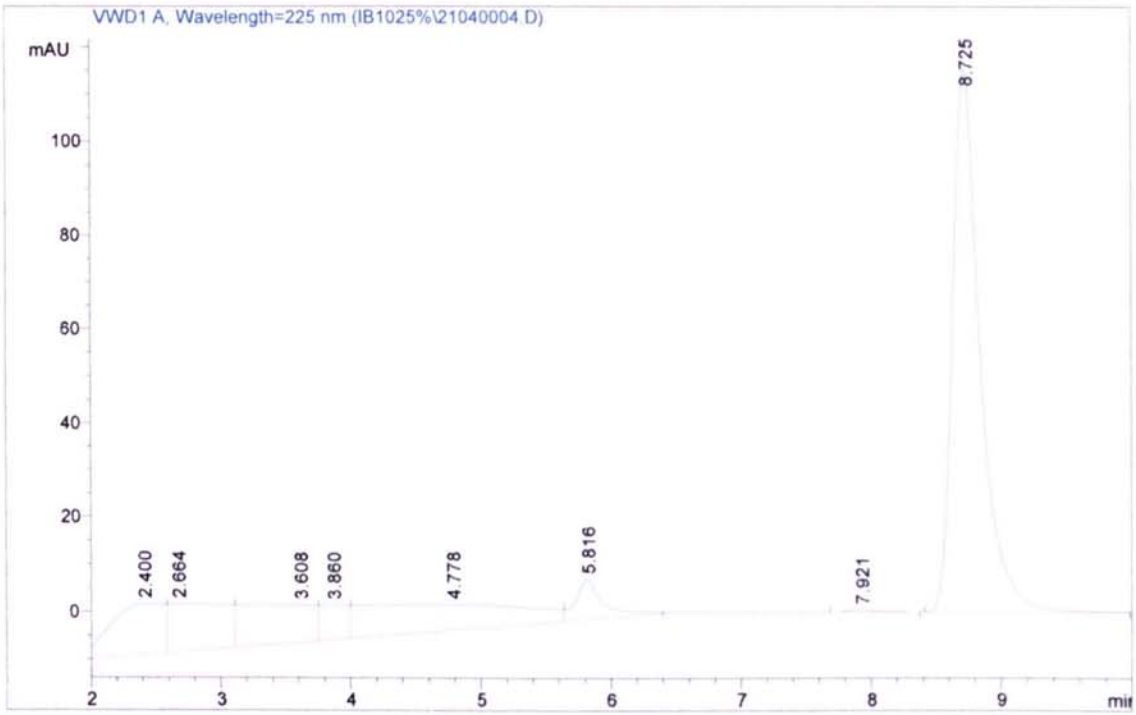


Figure 2.3: Typical HPLC chromatogram of ibuprofen (concentration 0.1mg/ml at 225nm). Absorbance is on the y-axis and time (minutes) is on the x-axis.

Concentration (µg/ml)	Area ± s.d	Relative standard deviation %	Relative Recovery (%)
0	0 ± 0.00	0.00	
1	171.37 ± 0.05	0.03	98
5	855.35 ± 0.10	0.12	
10	1678.14 ± 4.79	5.80	99
25	4012.98 ± 4.35	1.60	99
50	8057.20 ± 3.69	0.45	
100	15996.3 ± 4.58	0.28	

Table 2.13: Calibration and validation data of ibuprofen. N = 5. Data shows mean ± s.d

2.15.1.4. Naproxen assay

Sigma Aldrich (Poole, UK) supplied the USP-BP grade naproxen (USP; 100% purity) used for these studies. Working standards used for calibration were in the range of 0.001, 0.005, 0.01, 0.025, 0.05, 0.075 and 0.1 mg/ml and they were produced by serial dilution of the 1mg/ml stock solution in phosphate buffer pH 7.4. This media was chosen as the

drug was found to be more soluble at this pH compared to lower pHs (Shaw, 2005). The chromatograhpic parameters previously reported by David (2006) were applied.

Injection volume (µl)	20
Mobile phase	acetonitrile, double distilled water and orthophosphoric acid: 65:35:1
Wavelength (nm)	225
Flow rate(ml/minute)	1.00
Run time(minutes)	10
Column	Thermo-ODS-2 Hypersil 150mm x 4.6mm x 5µm

Table 2.14. Chromatographic (isocratic) conditions for analysis of naproxen

An example of a typical chromatogram of naproxen is shown in figure 2.4 below.

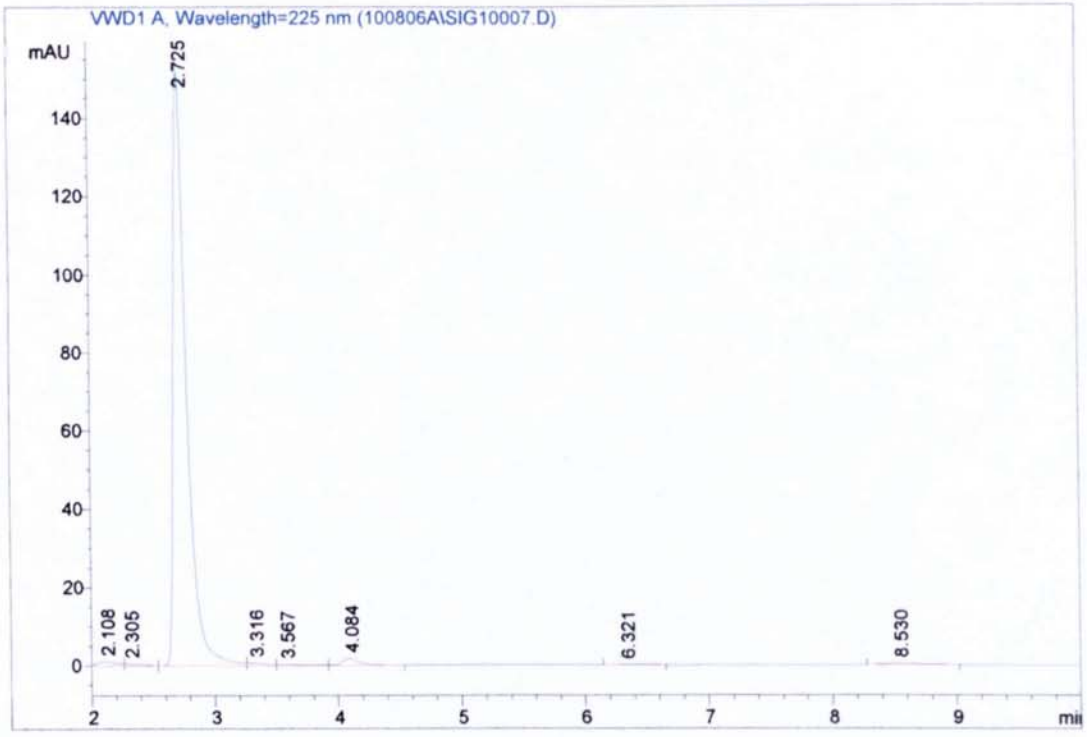


Figure 2.4: Typical HPLC chromatogram of naproxen (concentration 0.1mg/ml at 225nm). Absorbance is on the y-axis and Time (minutes) is on the x-axis.

Characteristic calibration data for naproxen is shown below. The correlation coefficient, r^2 for this calibration was 0.999. The limit of detection for naproxen was calculated to be 0.006µg/mL and the limit of quantification was found to be 0.030µg/mL.

Concentration (μg/ml)	Area ± s.d	Relative standard deviation %	Relative Recovery (%)
0.01	2.48 ± 0.18	7.41	
0.05	7.07 ± 0.07	1.09	
0.1	14.60 ± 0.18	1.25	99
0.25	36.66 ± 0.05	0.15	
0.5	74.28 ± 2.99	4.03	98
0.75	109.25 ± 1.90	1.74	
1	145.37 ± 1.37	0.94	99

Table 2.15: Calibration and validation data of naproxen. N = 5. Data shows mean ± s.d.

2.15.2. Validation for Hyper-DSC assay

The method assays were validated for repeatability, intra – and inter-day precision by performing a calibration using indium as a standard according to the manufacturer's specifications. This calibration was repeated for at least five times daily on various days prior to analysis of samples. The calibration was saved, retrieved and applied to each of the analysis carried out for the prepared drug formulations. This method was used as recommended by the manufacturer.

The validation parameters covered the experimental conditions specified in table 2.2 set for this study and it is reported in table 2.16

The relative standard deviation calculated for indium was within 2% for each analysis and the lower limits of quantification (indicated by the relative standard deviation) were also within 2%. Data representation of these analyses is shown below.

Average Heat of fusion of indium (J/g)	Relative standard deviation %
6.80 ± 0.00	0.00
6.85 ± 0.07	1.03
6.77 ± 0.03	0.52
6.85 ± 0.07	1.03
6.75 ± 0.07	1.04

Table 2.16. Validation and calibration data for indium standard. N = 5. Data shows mean ± s.d

The assays were also tested for specificity by analysis of blank samples of Witepsol® H15containing no analyte. There was no peak observed in the thermogram within the melting points of the drugs for these samples. However the melting endotherm for Witepsol® H15 was measured to be 11.02 J/g.

Average Heat of fusion of witepsol H-15 (J/g)	Relative standard deviation
11.02 ± 0.09	0.59
11.14 ± 0.03	0.19
11.08 ± 0.03	0.19
11.04 ± 0.07	0.46
11.27 ± 0.16	1.05

Table 2.17. Validation data for Witepsol® H-15. N = 5. Data shows mean ± s.d.

2.16. Hildebrand Theoretical solubility method

Predicting the solubility behaviour of ibuprofen, naproxen, flurbiprofen and diclofenac in Witepsol H15 was carried was carried out using the Fedors group (Fedor, 1974) contribution method as described in section 1.12.1.8.The solubility parameter of these drugs was drawn from an example used by (Greenhalgh et al, 1999) to demonstrate the use of Fedors group contribution method.

The Hildebrand solubility parameter for each drug and the Witepsol®H-15 base was calculated using equation 9 and 10 (section 1.12.1.8). The solubility parameter for the drugs was worked out following the example from a study (Fedor, 1974) that used the group contribution method based on structural formula. Calculation of the molar vaporisation energy (ΔEv) for the drugs involved the summation of the molar vaporisation enthalpies of their structural fragments. The molecular volume (Vm) was calculated using the volume of the molecular fragment present in each drug in an additive fashion. These figures were then put into equation 12 (section 1.12.1.8) to find the solubility parameter.

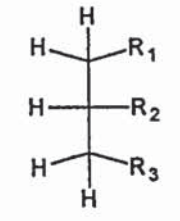
In these investigations, to utilize this approach, the established structures for diclofenac, ibuprofen, naproxen and flurbiprofen were used (table 1.4 a, b) while the structure of Witepsol® H15 was constructed (figure 2.5b). The structure of diclofenac free acid was employed because this model cannot be used for prediction of metal salts of model drugs (Greenhalgh et al, 1999).

The structure for Witepsol® H15 has proved difficult to establish, however, it is known that Witepsol® H-15 is composed of the following substances (http://www.sciencelab.com/page/S/PVAR/23053/SLW1078):

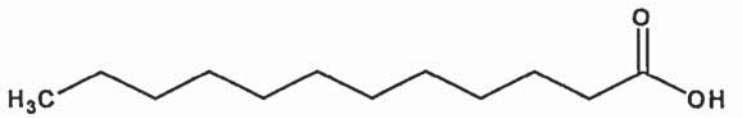
Composition	% by weight
Glycerides (C10-C18)	98
1,2,3-Propanetriol (Glycerol)	1
Fatty Acids (C10-C18)	1

Table 2.18: The composition of Witepsol

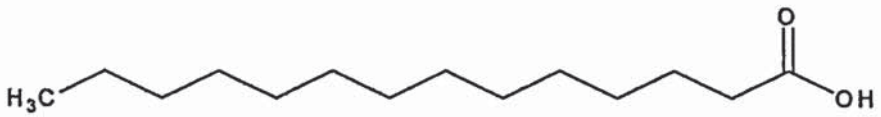
From this information obtained, it was proposed that Witepsol® H15 was a triglyceride, which existed in a mixture of varying combinations of fatty acid chain length. The proposed structure of Witepsol® H-15 with possible combinations of fatty acids is shown in figures 2.5a & b, below. Lauric acid, myristic acid and stearic acid have been chosen as the possible fatty acids, their structures were found at http://ecb.jrc.it/esis/index.php.



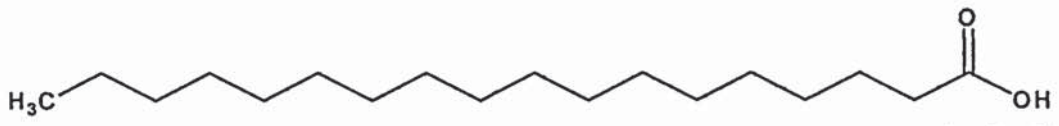
Triglyceride with 3 R-groups attached



Lauric acid



Myristic acid



Stearic acid

* Where R can be any or a combination of the following acids

Figure 2.5a: Proposed combination of chemical structures for Witepsol® H15

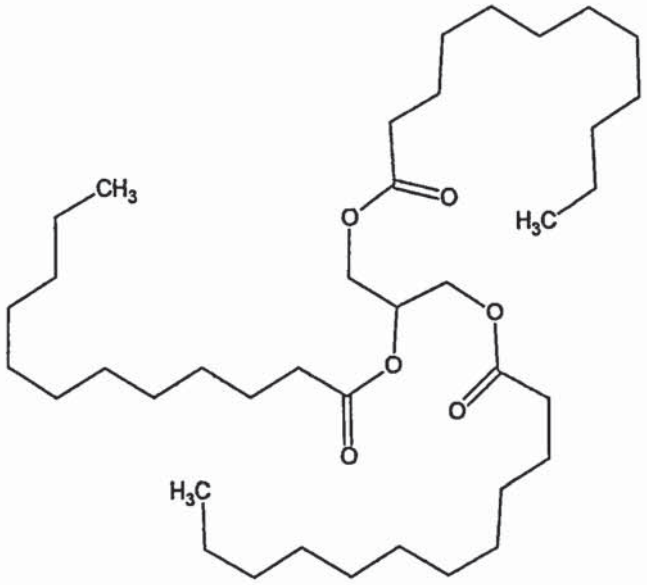


Figure 2.5b: Proposed chemical structure of Witepsol® H-15

Molecular Formula = $C_{39}H_{74}O_6$

Molecular Weight = 638

Once the structures of the investigated molecules were established, their molar cohesive energy (ΔEv) and the molar volume (Vm) were calculated using standard values reported by Greenhalgh et al (1999) and Fedor (1974). ΔEv and Vm were calculated based on the structural formula of these molecules.

Chapter Three

Microscopy, Higuchi release, hyper-DSC, Skin permeation and Hildebrand parameter as techniques for the determination of the solubility of drug in wax

3.1. Introduction

In chapter one (section 1.12.1.1), visible microscopy was discussed as a technique that can be used to measure solubility of drug crystals in a drug delivery vehicle. In this chapter, the simple technique of microscopy was applied as a technique to estimate the solubility of the selected drugs in Witepsol® H15. The advantages of this technique for solubility determination were detailed in table 1.2.

Furthermore, in this chapter the release of the chosen NSAID agents from the wax was measured as drug loading increased, covering a wide range of the drugs' concentration to ensure the drugs were present both above and below the solubility within the wax. The Higuchi approach was then used to determine the solubility of the drugs in the wax as explained in chapter one (section 1.12.1.6).

Moreover, the results obtained when hyper-DSC (discussed in section 1.12.1.5) was used as an alternative novel approach to study the solubility of the chosen drugs in the same semi-solid wax based formulations were reported.

In addition, in this chapter, the effect of varying concentration of the selected drugs in the prepared formulation on their flux through full thickness dorsal porcine skin was measured in order to correlate the calculated flux values with the drug's solubility in the wax. It was assumed that the flux would be greatest at the saturated solubility of the drugs in the wax (detailed in section 1.12.1.7).

Methods of quantifying solvent–drug and solvent–membrane interactions using solubility parameters (δ) have been suggested as useful in predicting drug flux (J). Hildebrand and

co-workers first developed the theory of the solubility parameter. This theory was based on regular solution theory and was described in some detail in section 1.12.1.8 (Hildebrand and Scott, 1950). The general theory is based upon solubility within the vehicle being a driver for drug permeation due to an increase in the concentration gradient. However, there is a delicate balance between a drug's solubility and its leaving potential from a vehicle. In addition, it is interesting to examine the solubility of a drug within skin as an indicator of drug flux. In this chapter, mathematical modelling (Hildebrand solubility parameter) was investigated as another choice technique to determine the solubility of the drugs in prepared formulations.

Finally, the results obtained from the use of these five techniques was reported and disucussed. Correlation and differences was highlighted and appropriate conclusions were drawn.

3.2. Aims and Objectives

One of the main aims of this study was to derive qualitative data of the solubility of naproxen, flurbiprofen and ibuprofen in Witepsol® H15 using visible microscopy as an investigative tool.
In addition, quantitative data would be generated from release experiments and these data would be used to predict the solubility of the selected drugs by applying Higuchi release kinetics.

Besides, one more key objective of this work was to investigate the suitability of hyper-DSC, as a novel technique to determine solubility of the drugs in the prepared formulations. It is aimed that the calculated solubility value for each of the drugs would be used to draw conclusions on the effect of the intrinsic physicochemical factors of each drug on their solubility in the wax.

Another chief aim of this chapter was to also apply skin permeation as a useful approach for solubility determination. As well, the Hildebrand theoretical solubility parameter

value of Witepsol® H15, diclofenac, naproxen, ibuprofen and flurbiprofen would be calculated. It is hoped that this will help further clarify the influence of physicochemical parameters on the drugs' solubility in the wax.

Finally, it is aimed that a detailed discussion provided in this chapter would draw together all the results obtained from the various techniques employed and the suitable conclusions could be reached.

3.3. Methods

For visible microscopy experiments, the method described in section 2.6 was employed in this study. For Higuchi release kinetics, the method employed was detailed in section 2.9. How the data obtained from hyper-DSC could be used to determine solubility was explained in section 2.5 while section 2.11 highlights the skin permeation procedure. Hildebrand theoretical solubility parameter methodology was described in section 2.16.

3.4. Results and discussions

3.4.1. Visible Microscopy

Microscopic examination provided visual evidence of the presence or absence of solid drug in each formulation with unique shaped crystals evident at higher drug loadings, indicating that insoluble (solid) was drug present but absence of these crystals indicates a complete solubility in the wax. All images were taken at 4x magnification.

Observation of ibuprofen solubility

Ibuprofen loaded Witepsol® H15 was observed under a microscope at loadings of 1, 5, 10, 15, 20, 25 and 30 % w/w. Figure 3.1.a depicts picture of Witepsol® H-15 alone and the small bubbles seen represents the wax as was placed on the microscope slide. Solid crystals were first observed in the 20% loaded ibuprofen with no solid material being observed at 10 or 15 % w/w. As the loading increased, greater numbers of solid crystals

were observed (Figures 3.1.a, b, c and d below). This suggested that the solubility of ibuprofen is between 15 and 20 % w/w.

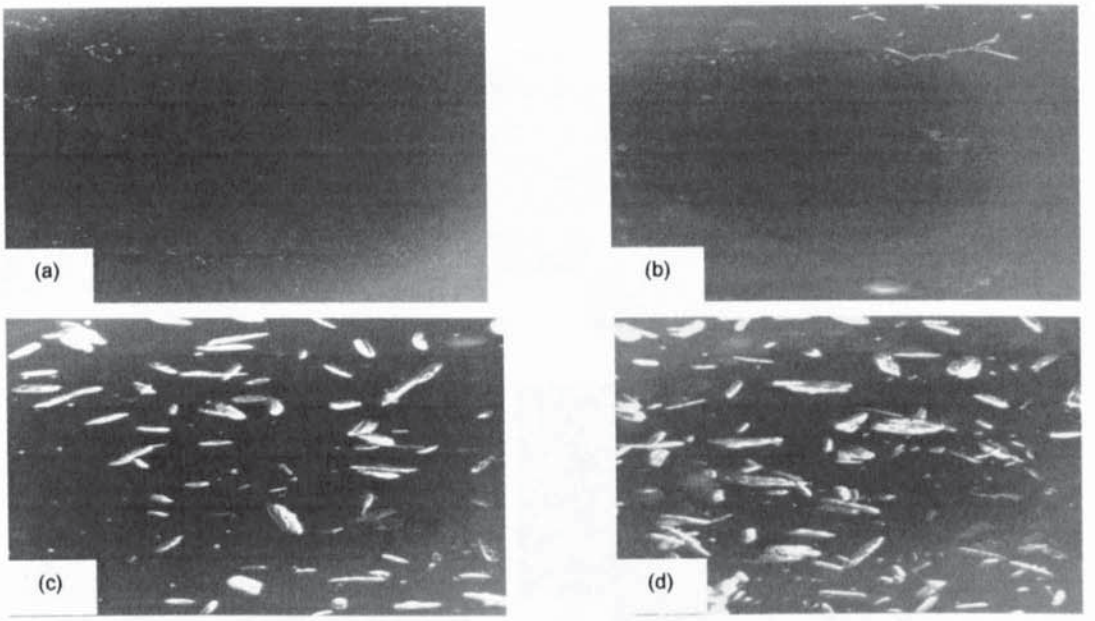


Figure 3.1. Microscopy images of Witepsol® H-15 alone (a) and Witepsol® H-15 plus ibuprofen at 15% w/w loading (b); Witepsol® H-15 plus ibuprofen at 20% w/w loading (c) and Witepsol® H-15 plus ibuprofen at 25% w/w loading (d).

Visible microscopy was a simple technique that demonstrated the solubility of ibuprofen to be between 15 and 20% w/w.

Microscopy offered an uncomplicated technique that provided qualitative data on the solubility of a drug in a semi-solid and also demonstrated the homogeneity of drug distribution within the wax matrix.

Observation of flurbiprofen solubility

As with ibuprofen above, as flurbiprofen loading increased the presence of crystals was observed, but this time, initially at 25 %w/w loading while at loadings of 40 % w/w many crystals were observed (Figures 3.2.a, b, c and d). This result suggested that the solubility of flurbiprofen in Witepsol® H15 is between 20 and 25% w/w. At 30%w/w loading, a

similar image to figure 3.2.c (30%w/w) was observed but with even more crystals observed (image not shown).

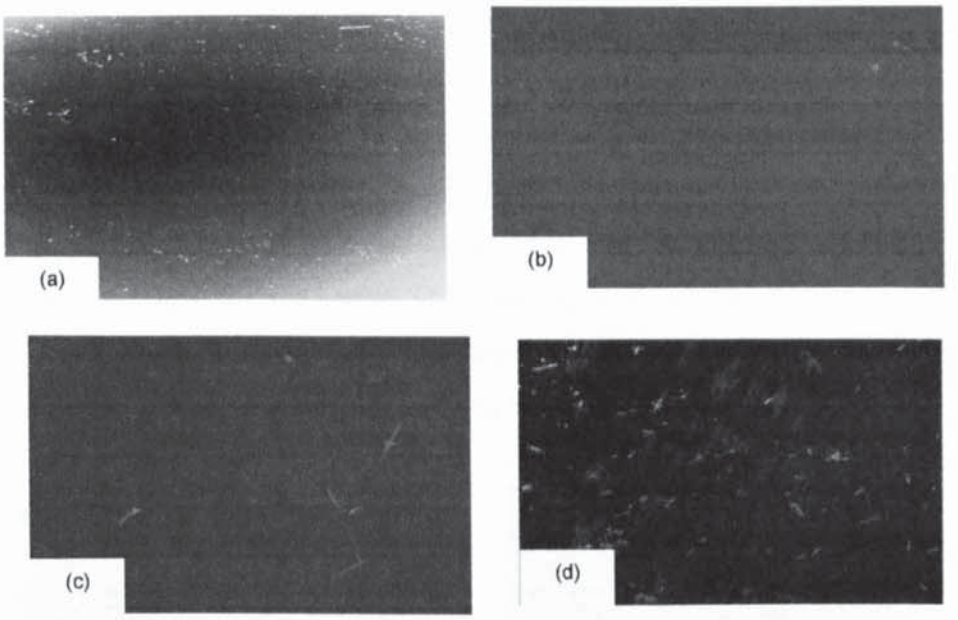


Figure 3.2. Microscopy images of Witepsol® H-15 alone (a); Witepsol® H-15 plus flurbiprofen at 20% w/w loading (b); Witepsol® H-15 plus flurbiprofen at 25% w/w loading (c) and Witepsol® H-15 plus flurbiprofen at 40% w/w loading (d).

Visual macroscopic observation of flurbiprofen-wax blends

In addition to the visible microscopy technique, visual macroscopic observation of the formulated preparations was also recorded during manufacture. In the molten formulation, drug loadings above 25 % w/w were observed to be cloudy indicating a heterogeneous mixture while at lower concentrations up to 20 % w/w, a clear homogenous mixture was observed which suggested that the drug molecules were dissolved in the wax. However, this approach provides only give qualitative data as reported by Ahmed et al (2004) and could be best used for preliminary solubility studies. This result suggested that drug solubility is between 20 and 25 % w/w.

Observation of naproxen solubility

Examination of naproxen formulations was also carried out under the microscope. The essence of which was to gather visual evidence for the presence or absence of solid naproxen molecules suspended in each formulation represented via unique shaped crystals. At higher drug loadings, these crystals were expected, indicating that insoluble (solid) drug molecules are present. As with ibuprofen and flurbiprofen reported in sections 3.3.1 and 3.3.2 above the presence of crystals observed increased with naproxen loading. The crystals were first seen at 20% w/w loading, and they became more prominent at 25 %w/w loading while at 30 % w/w loading many crystals were further observed (Figures 3.3. b and c). There was no crystal observed at 15 % w/w loading (figure 3.3.d). This result suggested that the solubility of naproxen in Witepsol® H15 is less than 20 % w/w. All images were similarly taken at 4x magnification.

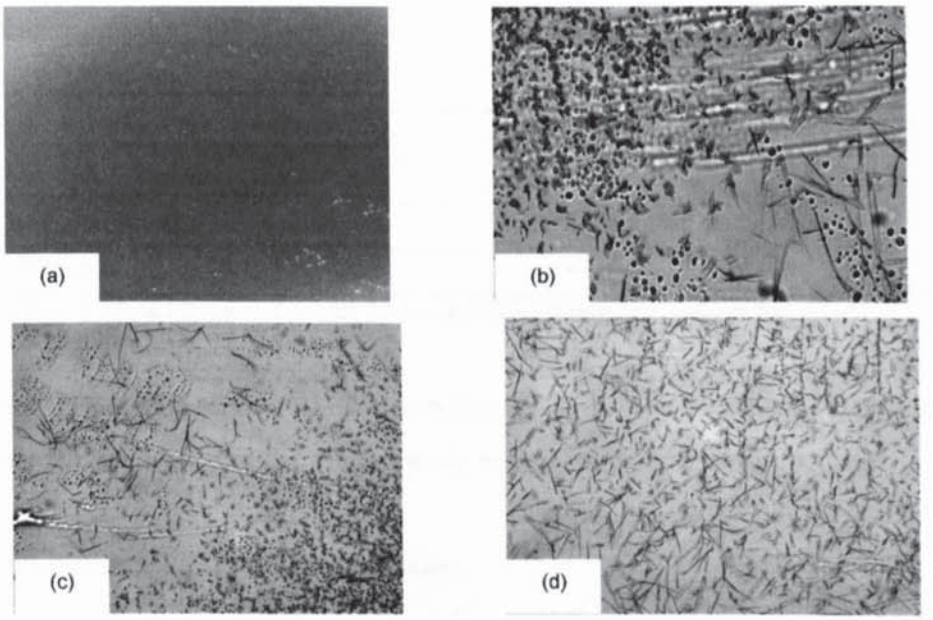


Figure 3.3.a. Witepsol® H-15 plus naproxen at 15 % w/w loadings (a); Microscopy images of Witepsol® H-15 plus naproxen at 20% w/w loading (b); Witepsol® H-15 plus naproxen at 25% w/w loading (c) and Witepsol® H-15 plus naproxen at 30% w/w loading (d).

The advantages of this technique above others include; ease of use, short time required in obtaining data, non-destructive in nature and highly sensitive. Furthermore, crystal shapes

of drugs can be observed with the distribution and homogeneity clearly seen. These crystal shapes and distribution can give an idea about which drug is present in a formulation, the stability of the active compound as well as provide other information such as release kinetics. A further advantage of visible miscroscopy is that good estimation of drug solubility can be done and active does not have to be crystalline. The result of the study will be compared (section 3.5) with the data obtained from other techniques.

3.4.2. Higuchi release

Uniformity of weight of tablets

The uniformity in weight of the tablets used for this study is shown in table 3.1 below. All measured tablets were within 2.0 % of the mean tablet mass. Statistical analysis was performed on the obtained data to ascertain statistical similarity between within the three batches of tablet formulated.

Drug	Mass (mg)
Ibuprofen	97.97 ± 0.72
Flurbiprofen	97.54 ± 0.64
Naproxen	97.65 ± 0.84

Table 3.1: Mass of prepared tablets for ibuprofen, naproxen and flurbiprofen. Mean ± s.d. N = 20.

The data shown represents the mean mass (mg ± s.d) of 20 tablets taken at random from formulations (used in the release study below) containing different loadings of each drug.

Statistical comparison of tablet mass

Application of one-way anova and t-test comparison statistical tests on the data of the weight study revealed that there was no significant difference ($p>0.05$) between and within the batches of the tablets used. Hence, there should not be any effect of the mass of the tablets on the drug release.

Release study: Effect of drug loading

The release of each drug at different loading concentrations (shown in table 3.2 below) from the wax-based matrix was investigated using phosphate buffer solution at pH 7.4 as the dissolution media. Higher drug loadings (>45% w/w) were excluded from this study, as the release rates were not reproducible, probably due to inhomogeneous distribution of drug within the tablet and the solubility limitations in the release buffer.

A plot of the cumulative amount released for each drug versus the square root of time was carried out for all the drugs. All the plots for each of the drug loadings versus the square root of time investigated were linear (with a linear regression value; $r^2 \geq 0.90$ in all cases). See Figures 3.4, 3.5 and 3.6 for typical representation of the plots. This was an indication that drug release followed Higuchi kinetics. The gradient was calculated and represented K_H.

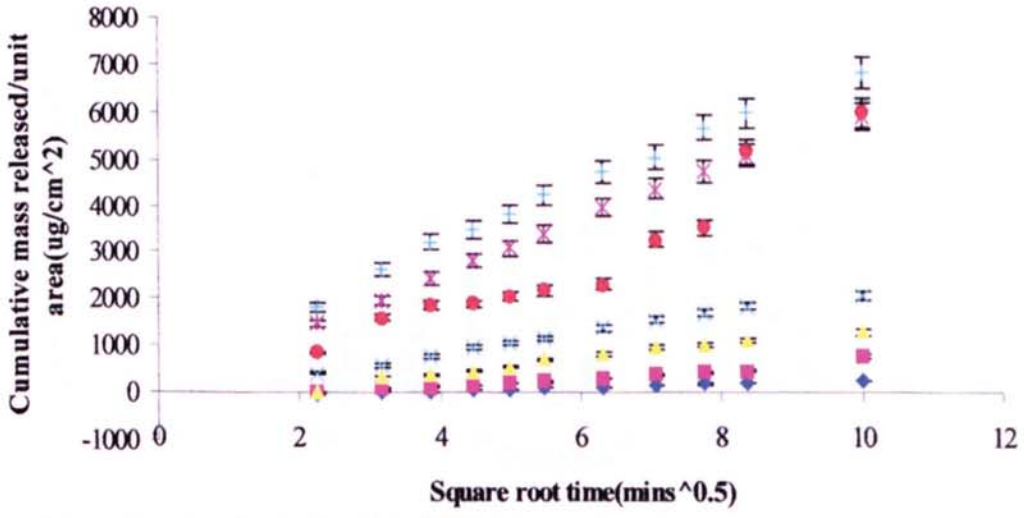


Figure 3.4. Higuchi square root of time plots for ibuprofen release from wax matrices at (♦) 1 % w/w, (■) 2% w/w, (▲) 5 % w/w, (×) 7.5 % w/w, (-)10 % w/w, ( )15% w/w, (●) 25 % w/w, (x) 30% w/w and (+) 35 % w/w ibuprofen loading. Data show mean ± standard deviation. N=3.

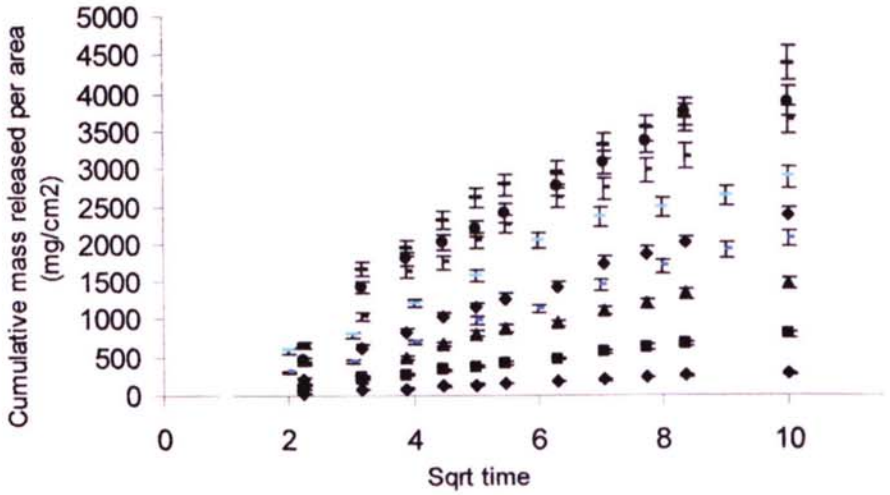


Figure 3.5. Higuchi square root of time plots for flurbiprofen release from wax matrices at (♦)1 % w/w, (■) 2% w/w, (▲)5 % w/w, (-) 7.5 % w/w, (■)15% w/w, (—) 10 % w/w, (*) 25 % w/w, (●) 30% w/w and (+) 40 % w/w flurbiprofen loading. Data show mean ± standard deviation. n=3.

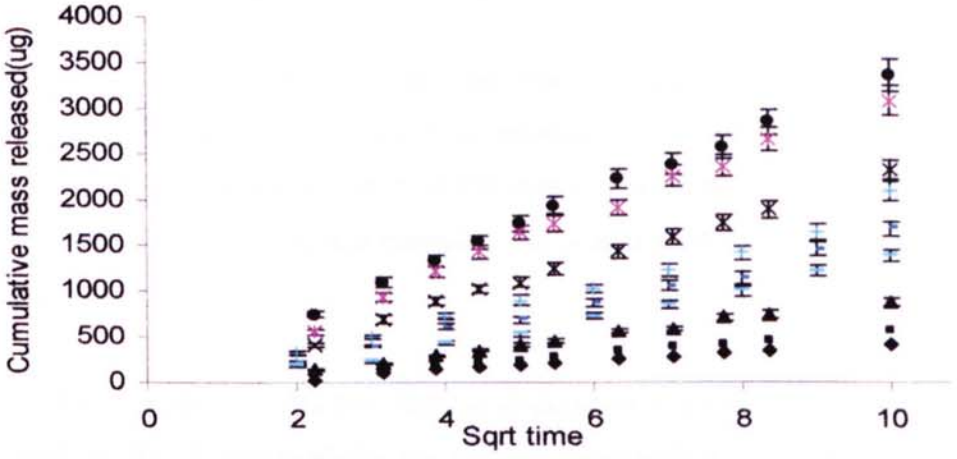


Figure 3.6. Higuchi square root of time plots for naproxen release from wax matrices at (♦)1 % w/w, (■)2% w/w, (▲)5 % w/w, (¯) 7.5 % w/w, (-) 10% w/w, (+) 15% w/w, (x) 30 % w/w, (*) 40% w/w and (●) 45% w/w naproxen loading. Data show mean ± standard deviation. n=3.

A summary of the release rate data obtained is shown in table 3.2.

	Ibuprofen	Flurbiprofen	Naproxen
Loading (%w/w)	Release rate; KH ± s.d ($\mu g/cm^2/min^{1/2}$)	Release rate; K_H ± s.d ($\mu g/cm^2/min^{1/2}$)	Release rate; K_H ± s.d ($\mu g/cm^2/min^{1/2}$)
1	36.39 ± 0.50	34.04 ± 0.29	47.27 ± 0.30
2	89.32 ± 0.37	91.81 ± 0.20	61.69 ± 0.41
5	164.49 ± 1.64	178.7 ± 0.40	96.71 ± 0.36
7.5	228.69 ± 1.61	216.26 ± 4.86	127.53 ± 3.02
10	296.31 ± 0.38	267.8 ± 1.01	158.53 ± 1.46
15	459.01 ± 0.01	381.68 ± 3.96	213.09 ± 3.70
20	559.68 ± 0.25	390.77 ± 4.21	280.18 ± 2.30
25	589.99 ± 0.56	398.20 ± 1.84	303.32 ± 0.68
30	629.34 ± 0.44	422.9 ± 0.24	311.89 ± 0.37
35	633.19 ± 1.41	--	--
40	--	433.7 ± 0.72	321.08 ± 0.76
45	--	--	334.96 ± 0.45

Table 3.2: Summary of the release studies data showing loading concentration (w/w), calculated release rate; K_H. - - Represents data not available. Mean ± s.d. n=4.

Employing statistical analysis on the release data obtained using one-way anova (across a whole set of data), Bonferroni and Student t-test multiple comparisons (between two chosen data in a whole set) indicated that the difference between the release rates of any two loadings for each of the drugs was considered to be statistically significant with a p value of 0.000.

The release rate constant; K_H, obtained from the slope of the Higuchi plots increased as the concentration of each drug loaded in the matrices increased for each of the drug investigated(Table 3.2). This increase was expected as the concentration gradient; which is the driving force that facilitates the diffusion process from the drug-matrix mixture to the release medium, will increase with increasing loading concentration of the drug in a matrix; hence the increase in the release rate.

This is linked with the thermodynamic activity; which is the leaving potential, of each drug in the matrix. As the drug loading concentration approached the solubility value large increases in release rate were expected as the concentration gradient between the

formulations and surrounding dissolution medium was maximised. However, beyond saturation, when excess drug was present further increases were not expected to be as large as before although more drug is present, the concentration gradient remains the same as no further drug can be solubilised within the matrix.

For example, from table 3.2, there was a big leap in the K_H value as the concentration of ibuprofen was increased from 1 % w/w (36.39 ± 0.5027 $\mu g/cm^2/min^{1/2}$) to 2% w/w (89.32 ± 0.3742 $\mu g/cm^2/min^{1/2}$). However, the increase in the calculated K_H values at all higher drug loading concentrations for ibuprofen was less as seen with K_H data at 30% w/w (629.34 ± 0.4422 $\mu g/cm^2/min^{1/2}$) and at 35% w/w (633.19 ± 1.41 $\mu g/cm^2/min^{1/2}$). Thus, the change in K_H values between drug loadings was greater at low loading concentrations with an increase by a factor of 2.5 from 1% w/w to 2%w/w loadings, however, increase in K_H values was very small at higher concentrations, for example, between loadings at 30% w/w and 35% w/w with less than 1 % w/w increase.
A similar trend was seen with flurbiprofen and naproxen.

The data in table 3.2 shows that the increase in K_H value as the concentration of naproxen loaded in the wax was increased from 1 to 2 % was lower compared to ibuprofen and flurbiprofen. This order of increase corresponds with the respective Log P value of the drugs.

Log P is a measure of the differential solubility (hydrophobicity and hydrophilicity) of a substance in two solvents; commonly water and octanol. A higher Log P denotes higher affinity for the hydrophobic environment of the wax; hence, lower leaving potential, therefore it was expected that flurbiprofen, with the highest log P should demonstrate the lowest change in release at low concentrations but it showed the biggest change. This could be as a result of the lower pKa value of flurbiprofen 4.13; which is less than the other the pKa value of the other two drugs (naproxen and ibuprofen: 4.15 and 4.91). Higher pH (7.4) of the dissolution media would thus promote more flurbiprofen molecules to be ionised to come into solution than molecules of the other two drugs.

Another common observation with all three-drug cases considered was the reduced r^2 value of the regression line for release data of drug at higher drug loading in the matrix. This was due to the close values obtained for the release rate constants at higher drug loading. The close values of release rate constants was as a result of reduced thermodynamic activity at higher drug loading in formulations with drug suspended in the matrix. For example, figures 3.7, 3.8 and 3.9 shows that r^2 value for soluble portion of the plot of calculated K_H against respective concentrations of naproxen loaded into the investigated formulations was 0.99 while the r^2 value of the suspended portion of the graph gave a value of 0.90. The initial values shows good fit to the model while the latter shows a slight deviation from the mathematical representation of release.

In summary, the relatively high increase in K_H with loading when the drug is dissolved is a function of the concentration gradient (linked with thermodynamic potential or activity) between the matrix and the dissolution medium. As the drug loading reaches saturation the suspended drug does not contribute to the concentration gradient directly but provides a reservoir from which drug can be dissolved into the surrounding liquid. At higher loadings there may also be drug present on the exterior of the tablets for immediate dissolution.
However, to validate this assumption, it is important to calculate the solubility of each drug within the matrix.

Solubility determination

Using the data obtained from the release studies (table 3.2); the Higuchi approach was employed to determine the solubility of ibuprofen, flurbiprofen and naproxen within the matrix. According to Higuchi (1960, 1961), in principle, a plot of release rate constant; K_H versus drug loading in a matrix; would show two distinct linear relationships; one relating to solubilised drug as described by equation 9 and the other for suspended drug as described by equation 8. And extrapolation of the point where the two linear graphs meet to the x- axis is expected to give the derived solubility value. Thus, the solubility was determined as the inflection from a plot of k_H versus drug loading.

Using this approach, calculated K_H was plotted against ibuprofen, flurbiprofen and naproxen loading concentration. For example, Figure 3.7 below shows the linear regression analysis of data for ibuprofen dissolved in the wax gave a straight line and regression of data for the drug suspended in the wax (when excess drug was present) also gave a straight line. Intersection of the two extrapolated lines provides an estimation of drug solubility, the value calculated from Figure 3.7 below to be 18.6 % w/w.

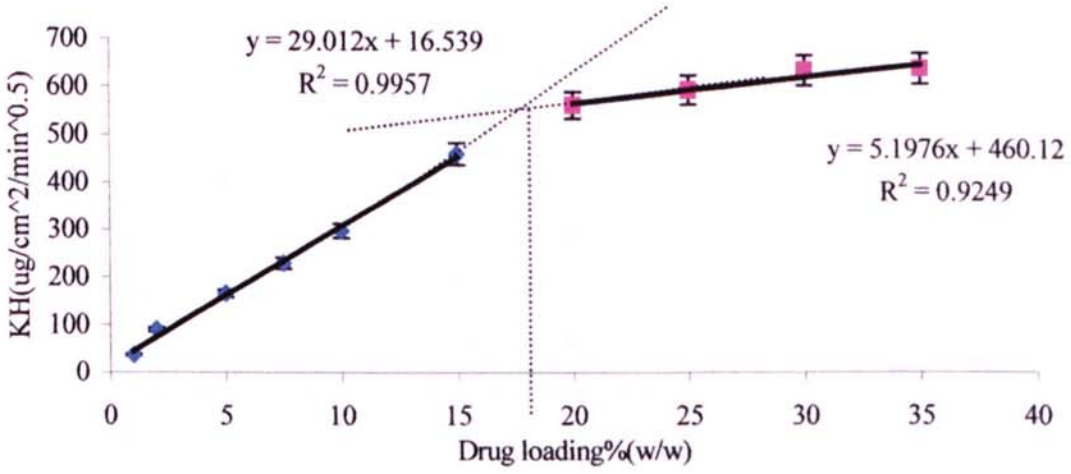


Figure 3.7. Plot of release rate constant, K_H versus ibuprofen drug loading.(♦) drug dissolved in wax with dashed line extrapolation of best fit line, (■) drug suspended in wax with dashed line extrapolation of best fit line. Data show mean ± standard deviation (n=4).

Figure 3.8 below shows that the linear regression analysis of data for flurbiprofen; when dissolved in the wax gave a straight line, $r^2 = 0.96$ ($y = 24.72x + 31.646$) and regression of data for the and when suspended in the wax (when excess drug was present) also gave a straight line, $r^2 = 0.94$ ($y = 2.2208x + 347.71$). Intersection of the two extrapolated lines provides an estimation of drug solubility; the value calculated from Figure 3.8 below was 14.0 % w/w.

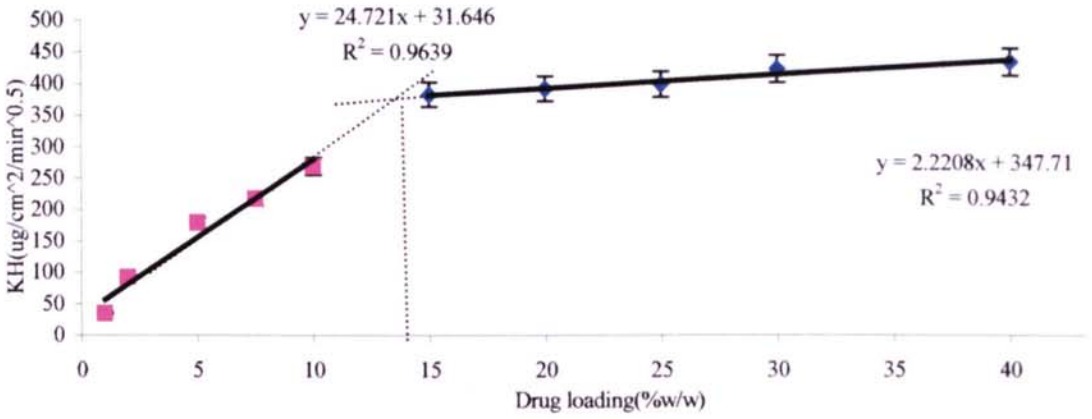


Figure 3.8. A plot of release rate constant, k_H versus flurbiprofen drug loading, drug dissolved (■) in wax with dashed line extrapolation of best fit line, drug suspended (♦) in wax with dashed line extrapolation of best fit line. Data show mean ± standard deviation, n = 4.

The predicted solubility value of naproxen in the wax is shown in figure 3.9 below. The linear regression analysis of data for the drug dissolved in the wax gave a straight line, r^2 = 0.99 (y = 12.202x + 36.019and regression of data for the drug suspended in the wax (when excess drug was present) also gave a straight line, r^2 = 0.90 (y = 1.4496x + 267.08). Intersection of the two extrapolated lines provides an estimation of drug solubility, the value calculated from Figure 3.9 below to be 21.4 % w/w.

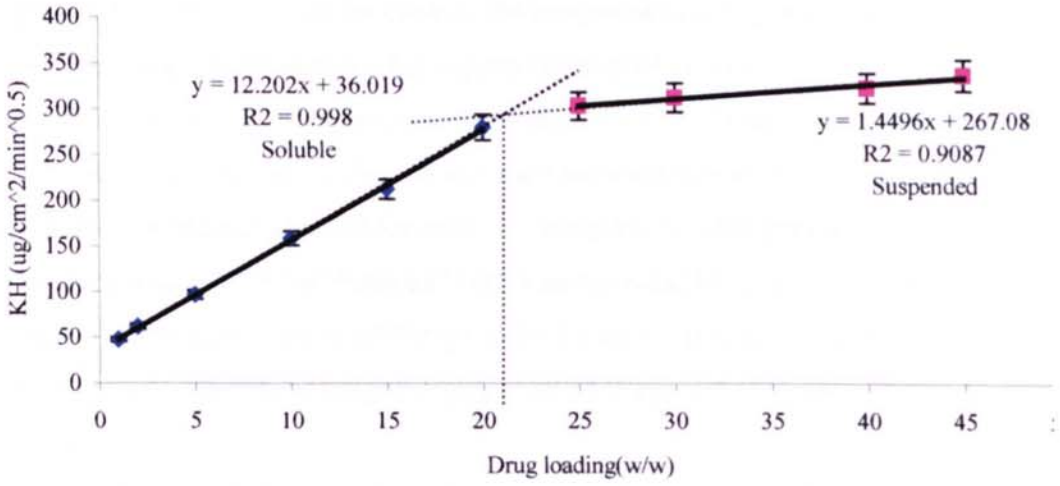


Figure 3.9. A plot of release rate constant, k_H versus naproxen drug loading. (♦) drug dissolved in wax with dashed line extrapolation of best fit line, (■) drug suspended in wax with dashed line extrapolation of best fit line. Data show mean ± standard deviation, n = 4.

To conclude, it can be seen from the release study that the release rate constant was concentration dependent with increased release rate achievable as concentration of loaded drugs increased in the matrix. This was attributed to effect of thermodynamic activity. Furthermore, this study demonstrates that the calculated drug solubility in the wax was in the reverse order as the log P value of the drugs (table 1.4a and b). The order of increase in log P values is flurbiprofen > ibuprofen > naproxen while the calculated solubility in the wax was in the order flurbiprofen < ibuprofen < naproxen: 14.0% (w/w) < 18.6 % (w/w) < 21.4% (w/w). This was not expected as the previous literature reports that higher log P denotes stronger affinity for the hydrophobic environment (of the wax in this case); hence, lowers leaving potential for the aqueous environment of the dissolution media (Sloan, 1989; Majumdar et al, 2007).

Further discussions on the factors that might have contributed to these findings will be discussed in section 3.5below.

3.4.3. Hyper-DSC

The area under the phase transition endotherm; heat of fusion ΔHf was integrated and recorded. The inputs for the start and end of the peak were manual but the software integrated the area. The onset for each of the integrated thermograms was used as a single value for melting temperature as this depicts the start of melting (Gabbot, 2005).

Although the Witepsol® H-15 peak shown in figure 3.10, 3.11 and 3.12 appeared to be different due to the adjustment by the software and even though the software takes into account the sample mass loaded for each run, integration of the peak area shows that the heat of fusion calculated for Witepsol® H15 was reproducible (t-test, p = 1, n= 5). The calculated melting point value of Witepsol® H15 was not statistically different from one another in each case. The average melting point of Witepsol® H15 was noted to be 33±1° C (n= 5).

Drug solubility was determined from the intercept of the plot of enthalpy of fusion (J/g) versus drug loading (% w/w). Numerical data used for construction of the graphs represented in this chapter are presented in the appendix.

Ibuprofen solubility

Ibuprofen gave a sharp single endothermic peak at 75°C and enthalpy of fusion of 150 ± 2 J/g (Figure 3.10). This represents the melting temperature of ibuprofen (75°C, Hadgraft, 2000). This result corresponds well to previous studies reported in the literature that showed the enthalpy of fusion of ibuprofen to be 150 J/g (Manish et al, 2005). Witepsol® H15 alone showed a single well-defined endothermic peak with an onset of 33°C (Figure 5.1). This corresponds to the reported melting point of Witepsol® H15 (Takatori, 2004).

Figure 3.10 shows the example of the thermogram of ibuprofen and the Witepsol® H-15.

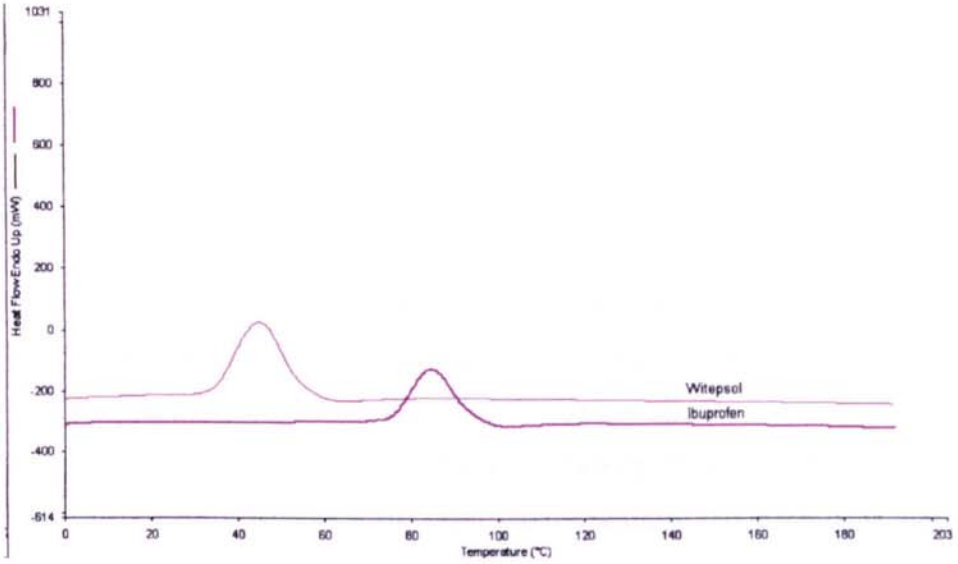


Figure 3.10. An example of thermogram of Ibuprofen and Witepsol® H15

The profile labelled Witepsol® H15 shows the melting endotherm peak of this matrix while the other profile indicates ibuprofen with its melting endotherm.

Broad endotherms were observed for all formulations of drug in wax between 30°C and 45°C corresponding to the melting of the wax (Figure 3.11). However, as ibuprofen concentration increased to 30% w/w, a second, smaller endothermic peak with the onset at 68 ± 1°C was observed which became more prominent as the drug concentration increased to 50% w/w ibuprofen loading (Figure 3.12). This second peak is related to the

dispersed drug within the formulation as it occurred at the melting temperature of ibuprofen.

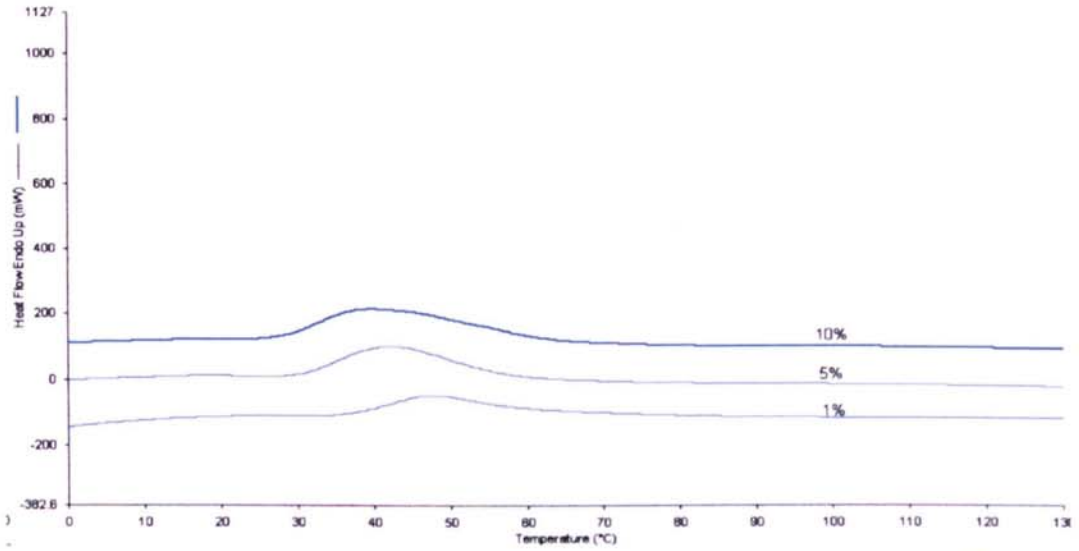


Figure 3.11. Hyper differential scanning calorimetry thermogram of ibuprofen loaded Witepsol® H15 base formulations at drug concentrations 1, 5 and 10 % (w/w).

Figure 3.12 shows the effect of increasing the drug loading concentrations from 30% to 50% (w/w). Further, it indicated that there was a decrease in the observed onset of melting of the drug (68 ± 1°C) compared to that calculated when pure ibuprofen alone was analysed (75°C). Furthermore, the onset melting point of the base was also lowered from 33 ± 1°C to around 25 ± 1°C; thus the formulation can easily melt at body temperature.

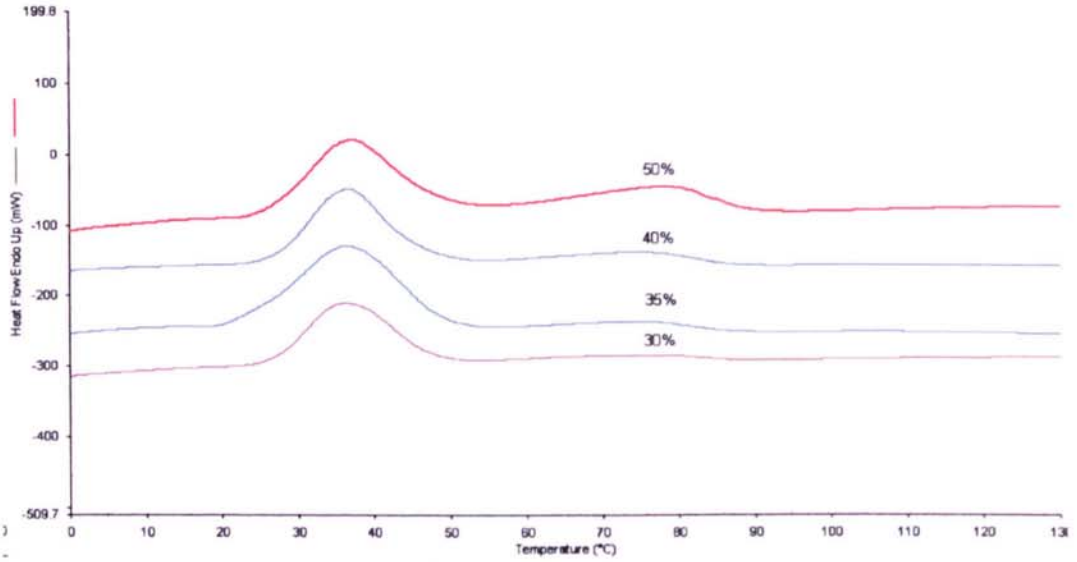


Figure 3.12. Hyper differential scanning calorimetry thermograms of ibuprofen loaded wax matrices at increasing drug loadings.

According to the concept first described by Theeuwes et al (1974) that thermodynamic effects (represented by endothermic peaks at the melting temperature of the drug) seen during thermal analysis of a drug in a matrix result from the un-dissolved and/or dispersed part of a drug (Jenquin, 1994).

Using the approach explained briefly above and in more detail in section 1.12.1.5, figure 3.13 shows the extrapolated value of the drug solubility within the matrix. The data points were fitted to a linear best-fit trend-line and extrapolated to the x- intercept and this value measured to be the solubility. The solubility value of ibuprofen in the wax matrix as calculated by this method was 12.7% w/w. The error associated with this method was calculated using the standard deviations to produce a data set of positive and negative deviations and following the procedure as with the mean values; the solubility range was determined to be 10.4–15.0% w/w. Figure 3.13 shows the plot of the mean values with standard deviations and the line of best fit through the mean data set with a regression value of 0.995.

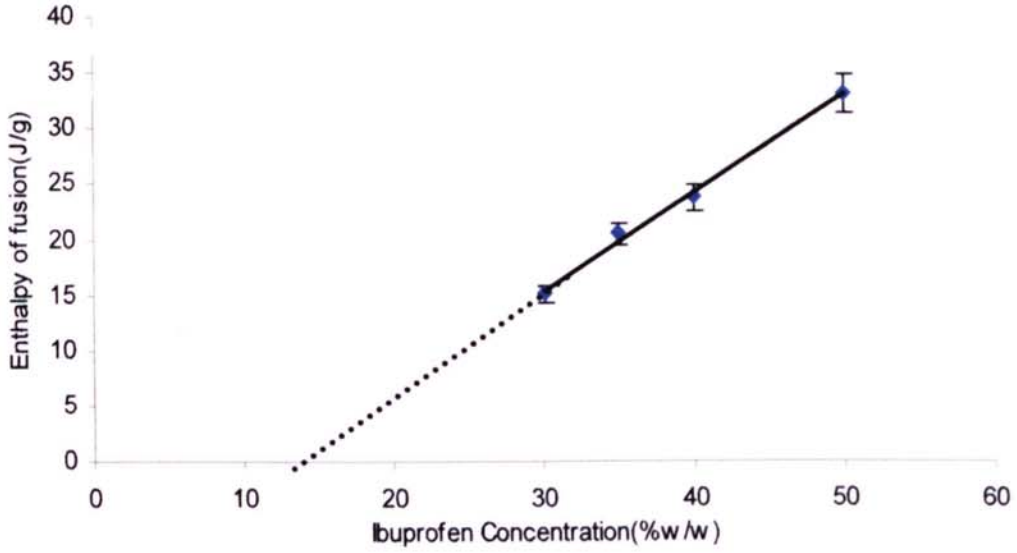


Figure 3. 13. A plot of enthalpy of fusion versus ibuprofen loading, the extrapolated line intersects the x-axis at the solubility of drug in the wax. Data show means ± standard deviation. N = 4

It was interesting to see that there was no peak visible for ibuprofen between 12.7% w/w and 30% w/w loading on the thermograms represented in figures 3.11 and 3.12 above. This would be investigated in future studies.

Flurbiprofen solubility

Analytical grade flurbiprofen gave a well-defined endothermic curve with an onset of 110°C, the melting temperature of the drug (Oladiran and Batchelor, 2007). This single melting peak demonstrated that no impure polymorph of flurbiprofen was present in the formulation and that no inter-conversion from one form to the other took place during the heating process (Figure 3.14). Furthermore, a thermal scan of the wax was carried out and a single well defined endothermic peak with an onset of 33°C and the enthalpy of fusion was 11.04 ± 0.07 J/g (n=5) was also observed at the melting temperature of the wax (Figure 3.14).

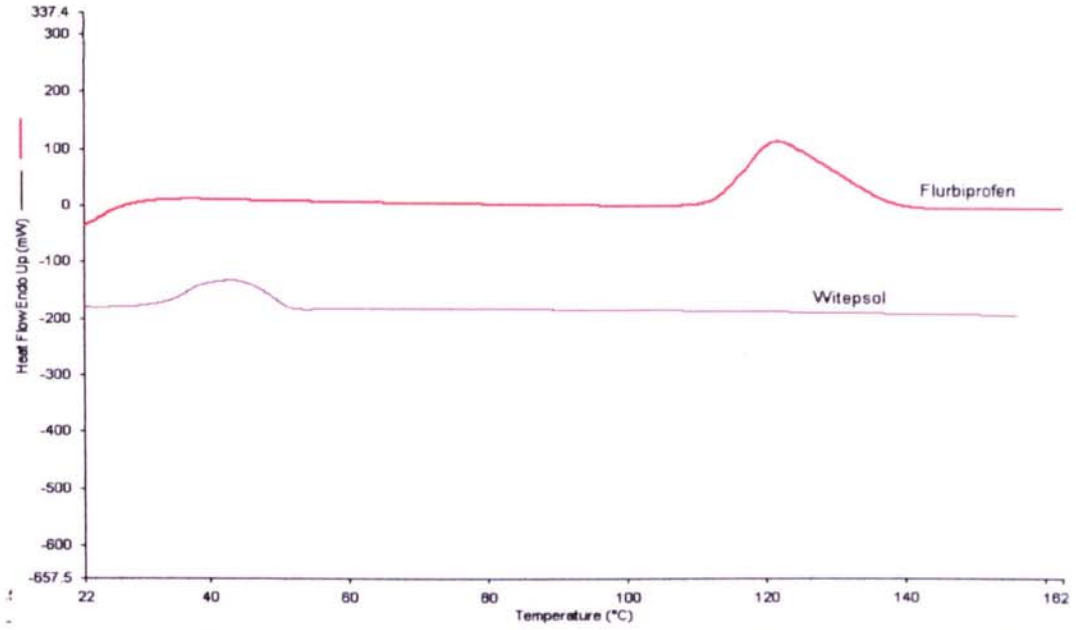


Figure 3.14. Hyper differential scanning calorimetry thermograms of pure flurbiprofen and pure Witepsol® H15.

Similar to the above study on ibuprofen samples, all the formulations of flurbiprofen-loaded wax were investigated under identical conditions using HyperDSC. At flurbiprofen loadings of 20% w/w and below, only one well-defined endothermic peak in the region of the wax melting point (25-30 °C) was observed. Figure 3.15 shows thermograms of formulations with loadings of 0.5,1,10 and 25% w/w.

It can be seen that at 0.5, 1 and 10 % (w/w) drug loadings, no visible endothermic transition was seen within the melting temperature range of the drug. Hence, this indicated that no un-dissolved drug is present suggesting that the drug was completely soluble within the base matrix at these concentrations.

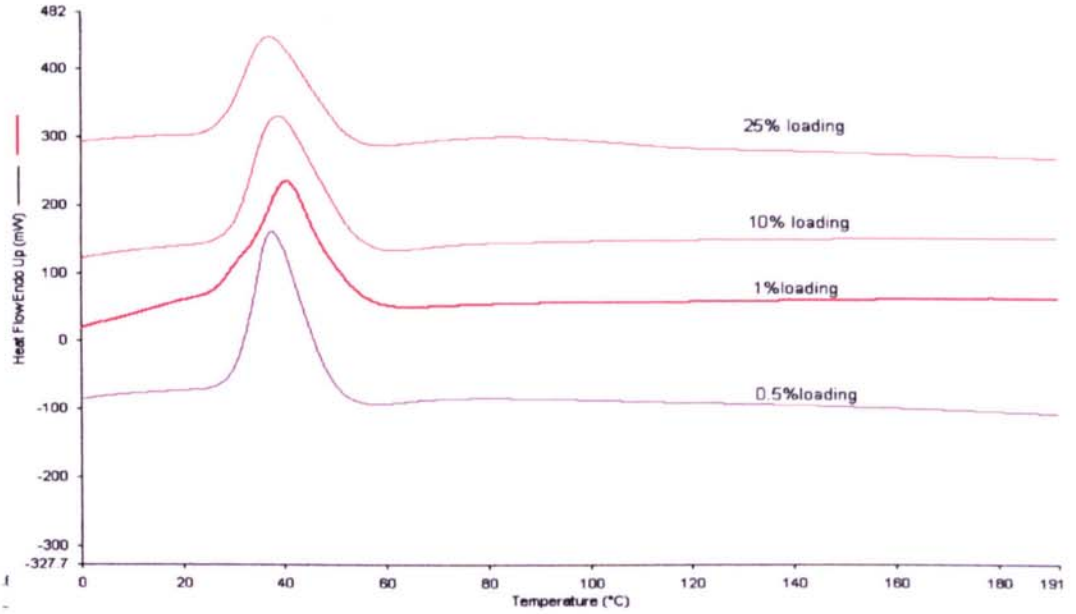


Figure 3.15. Hyper differential scanning calorimetry thermograms of flurbiprofen loaded wax matrices at increasing drug loadings.

However, as the loading concentration of flurbiprofen was increased to 25%w/w, another smaller (and much broader) endothermic peak was observed which became more prominent as the drug concentration was increased gradually to 45%w/w flurbiprofen loading (Figure 3.16). This second peak related to drug dispersed within the formulation.

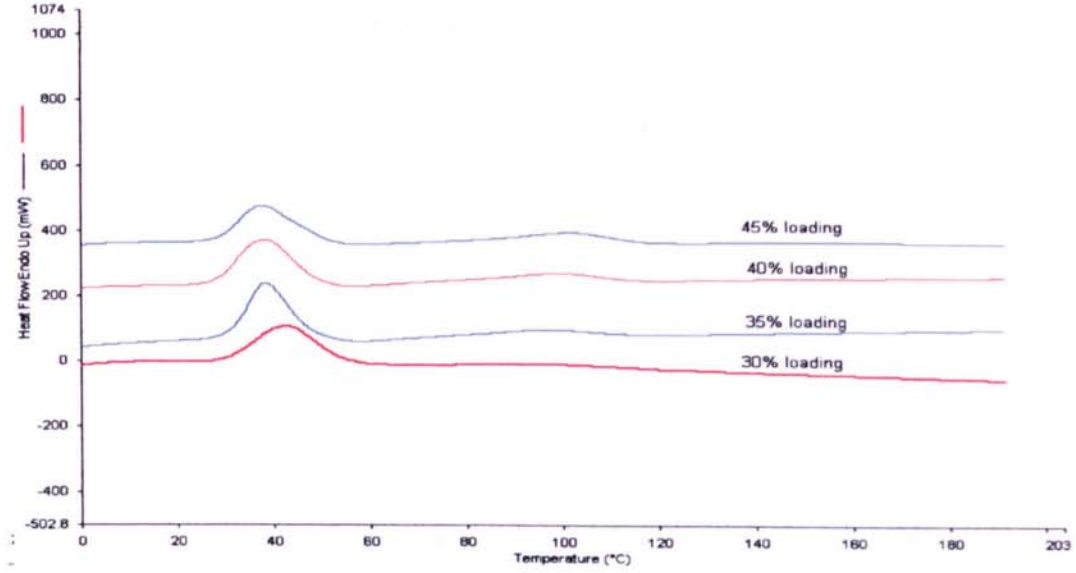


Figure 3.16. Hyper differential scanning calorimetry thermograms of flurbiprofen loaded wax matrices at drug loadings of 30, 35, 40 and 45 % (w/w).

Using the Theeuwes et al (1974) concept described above, the solubility value of flurbiprofen in the wax matrix as calculated by this method was 20.13 ± 0.26 %(w/w) under the stated conditions. Figure 3.17 shows the plot and the line of best fit with a regression value (R^2) of 0.97.

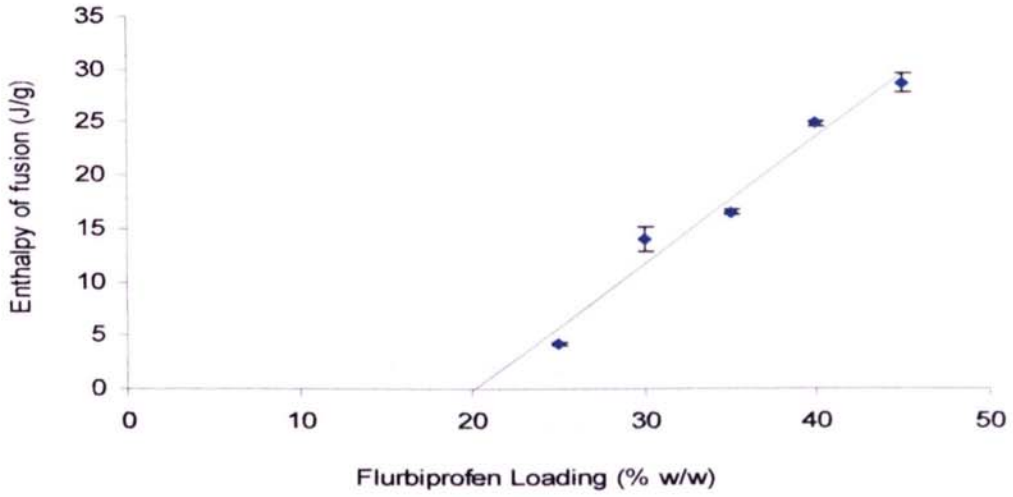


Figure 3.17. A plot of enthalpy of fusion versus flurbiprofen loading, the extrapolated line intersects the x-axis at the solubility of drug in the wax. Data show mean ± standard deviation. N = 4.

Naproxen solubility

Figure 3.18 shows an example of the thermogram of naproxen and Witepsol® H-15.

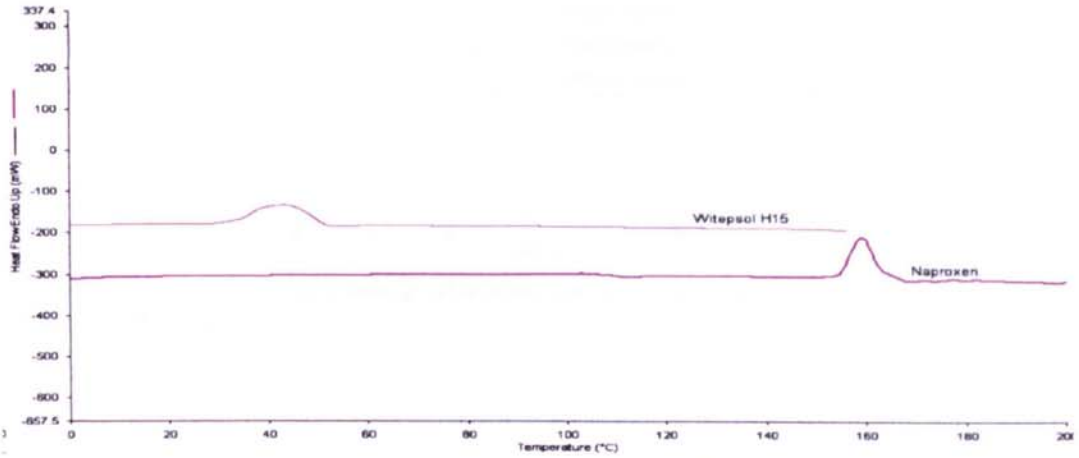


Figure 3.18. An example of thermogram of naproxen and Witepsol® H-15

The melting point and enthalpy of fusion of naproxen was found to be 152 ± 1°C and 140 ± 1 J/g. This relates to literature values of 153°C and 137 J/g (Bettinetti et al, 1996).

Using similar experimental conditions, samples of naproxen in the wax matrix were also analysed using Hyper-DSC. Figure 3.19 show that a single well-defined peak within the region of the base (25-30 °C) was observed at the low drug loadings: 0.5% (w/w), 1% (w/w), 2% (w/w) and 10% (w/w). At higher drug loadings, from 20% w/w and above; up to 45% (w/w), the second peak, which also became more prominent as the drug concentration increased was noticeable. This peak was that of the drug at around 153°C as shown in the thermograms in figure 3.18.

As expected, these results are similar to those discussed above. Hence, it can be suggested that the drug was completely soluble within the base matrix at the lower loadings while it was dispersed at the higher loadings.

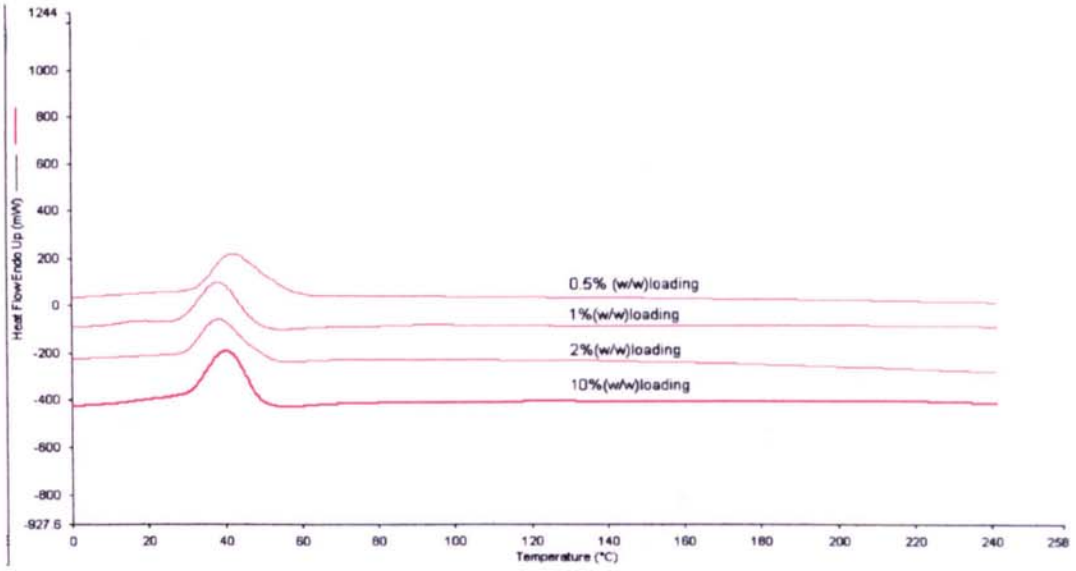


Figure 3.19. Hyper differential scanning calorimetry thermograms of naproxen loaded wax matrices at 0.5,1,2 & 10 % drug loadings.

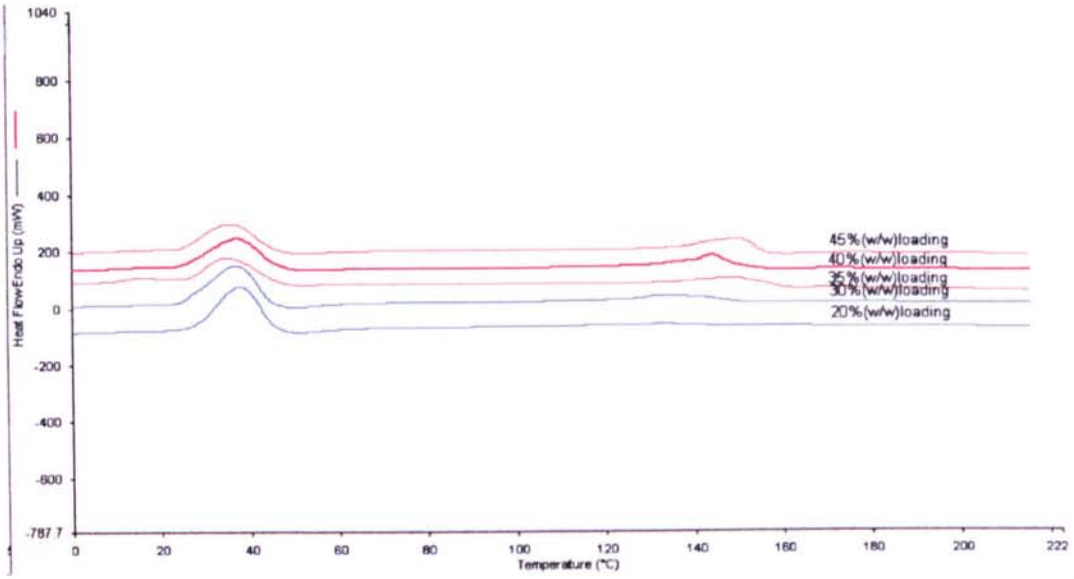


Figure 3.20. Hyper differential scanning calorimetry thermograms of naproxen loaded wax matrices at 20, 30, 35, 40 and 45% drug loadings

Employing a similar technique as discussed above for ibuprofen and flurbiprofen, the solubility value of naproxen in the wax matrix as calculated by this method was 22.10 ± 0.10 %(w/w) under the stated conditions. Figure 3.21 shows the plot and the line of best fit with a regression value (R^2) of 0.95.

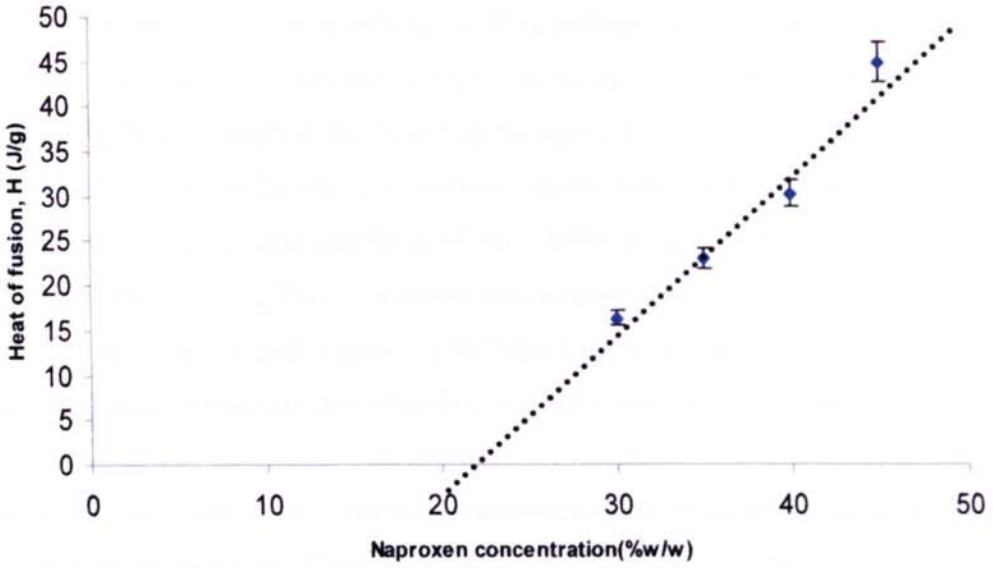


Figure 3.21. A plot of enthalpy of fusion versus naproxen loading, the extrapolated line intersects the x-axis at the solubility of drug in the wax. Data show mean ± standard deviation. N = 4.

A decrease in the melting onset of each of the drugs investigated when formulated with the wax was observed when compared to the melting point of the pure drugs alone. The reduction in the onset melting temperature (85 ± 1°C) of the flurbiprofen in the matrix compared to that of the pure drug (110 ± 1°C), was probably due to the dissociation effect caused by the melting method used during the formulation process as well as the distribution effect of the drug molecules within the matrix, these factors have been reported previously by Gramaglia et al (2005). This same reason could account for the observed onset of melting of the ibuprofen (68 ± 1°C) compared to that calculated when pure ibuprofen alone was analysed (75 ± 1°C) as well as for the reduction in onset of melting of the base from 33 ± 1°C to 25 ± 1°C in both cases. Surprisingly, there was no change in the melting onset value of naproxen when analysed alone or when it was formulated in the wax matrix (153 ± 1°C or 152 ± 1°C respectively). A good reason for this might be due to interactions that occurred between the molecules of ibuprofen and flurbiprofen with the molecules of the wax. Such interactions might not have taken place with naproxen molecules, though it does not mean that there was no relationship between the molecules of the wax and naproxen. These interactions have been reported to produce mixtures that have lower melting onsets than the constituents of the mixture (Stott et al, 1998, 2001).

The reduced melting temperature of both the drug and the wax could also be an indication that there was an interaction between the two, which can occur via hydrogen bonding between the hydroxyl groups of the base and the reactive functional groups of the drug. This has been reported in the literature that fat-soluble medication such as chloral hydrate depressed the melting point of wax base (Rowe, 2005). In another study, Stott et al (2001), demonstrated using DSC technique, that a eutectic mixture was formed between propanolol hydrochloride and both lauric and capric acids via the hydrogen bonding interaction between amino group of the drug and the carbonyl group of the fatty acids.

However, this is contrary to the result reported when indomethacin was added to witepsol H-15 where an increase in melting temperature was observed (De Muynck, 1992).

Interestingly, the time taken for the molten mixture of the drug and wax to solidify during preparation decreased with increased drug loading. This is likely to be due to the interaction between the molten matrix (solvent) and the drug although it may simply relate to the volume of wax present to solidify decreased as the drug loading increased. At low drug loadings when the drugs were observed to be soluble within the matrix, these formulations took longer to set than in formulations where the drug was dispersed. This is likely to be due to the interaction between the molten matrix (solvent) and the drug molecules. At lower drug loadings, the drug molecules break away from their crystals and are inserted into the 'solubilising cavity' of the molten matrix thereby disrupting the bond between the molecules of the matrix. But at the higher drug loadings; when drug is dispersed, more molecular drug interactions enhance formation of a more stable and organised crystal structure to outweigh the solubilising effect seen when drug molecules are completely soluble in the molten matrix, with drug crystals acting as seeds to promote wax-hardening.

Furthermore, the observed lowered melting point of both the wax and the drug could be associated with the re-arrangement of the structure of the wax molecules by the drug molecules and the disruption of the molecular lattice of the drug molecules on interaction with the wax functional groups; resulting in a mixture with less organised structural arrangement when compared with the pure drug and the pure matrix.

In summary, using Hyper-DSC, it has been shown that the solubility range value of ibuprofen, flurbiprofen and naproxen in the wax-based matrix was calculated to be 12.7 % (w/w), 20.13 ± 0.26 %(w/w) and 22.10 ± 0.10 %(w/w) respectively.
In addition, another notable conclusion from this study was the decreased melting onset of the drugs when they were formulated with the wax. The reasons adduced for the results obtained can be attributed to the physicochemical properties of these drugs. Further details will be highlighted later on in section 3.5.

3.4.4. Skin Permeation

Effect of drug loading on flux

The Flux; J, a parameter that determines the performance of a formulation based on the penetrating efficiency of the drug across a membrane was first derived. It is a factor that is commonly used to compare the efficacy of formulation with similar compositions.

The flux is taken as the slope of the linear portion of the plot of cumulative mass of the drug, which penetrated through the skin against time. An example is shown in figure 3.22 below.

For the purpose of these investigations, the samples analysed contained only a mixture of each drug and the wax with no additional excipients.

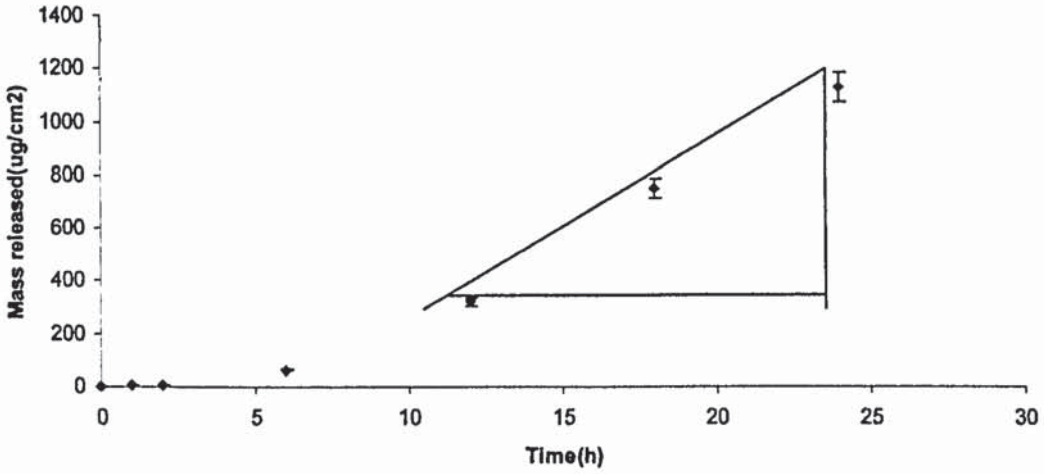


Figure 3.22: Example plot of mass released (ug/cm^2) against time (hours) for diclofenac sodium 5 % (w/w) through porcine skin. Mean ± s.d. N = 3.

To determine solubility via this approach, initially the fluxes of formulations that contain different drug loadings were worked out as shown in table 3.3.

In a different experiment, the flux of the saturated solution of each drug was determined by taking the slope of the plot between the cumulative amounts of drug which permeated through the full dorsal porcine skin versus set time points on application of the saturated solution.

Formulation (%w/w)	Naproxen; J(μg/cm^2/ h)	Flurbiprofen, J (μg/cm^2/ h)	Ibuprofen, J (μg/cm^2/ h)
0.5	19.00 ± 0.28	--	--
1	38.83 ± 1.88	21.52 ± 2.00	20.96 ± 0.63
2	71.59 ± 0.76	50.21 ± 1.14	42.13 ± 0.29
5	202.54 ± 0.97	79.01 ± 3.01	122.25 ± 1.40
10	316.12 ± 2.10	179.15 ± 5.30	222.75 ± 3.61
20	--	338.07 ± 3.09	352.26 ± 24.07
30	--	414.40 ±1.44	416.93 ± 8.89
Saturated solution	170.62 ± 6.74	165. 85 ± 8.34	110.01 ± 9.12

Table 3.3: Calculated flux values for formulations and saturated solution of each drug investigated. N = 3. – Data not available.

Thereafter, a plot of the calculated flux against the drug loadings was drawn. Figures 3.23, 3.24 and 3.25 below, show graphical example of such plot.

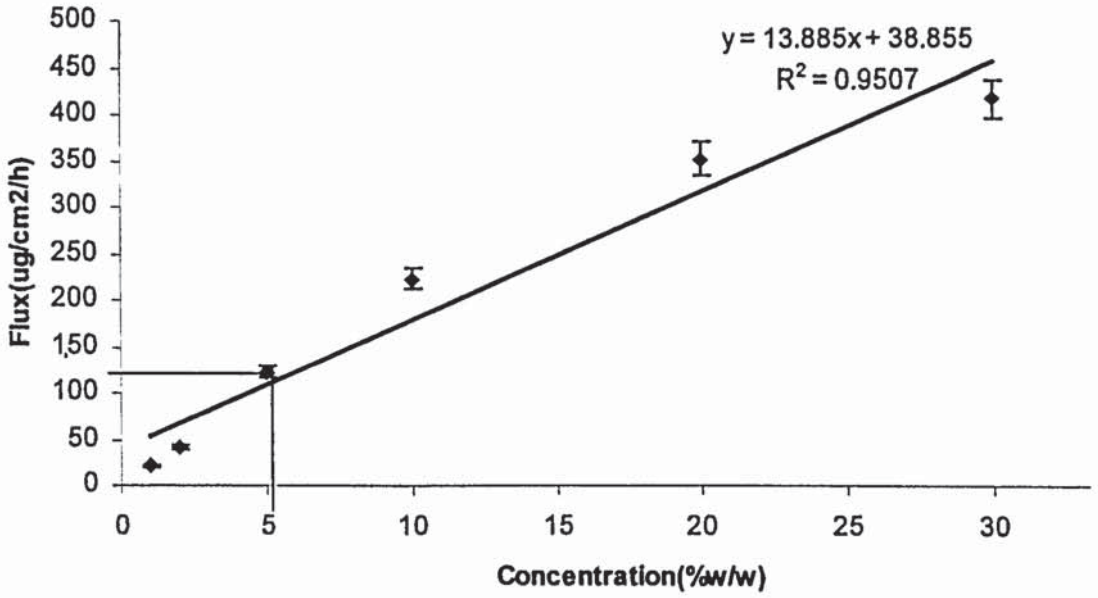


Figure 3.23: Plot of flux of ibuprofen against drug loading. N = 3

Then, the experimental flux calculated for the saturated solution of the drug was applied as y to the equation of the plot shown in figure 3.23(y =13.885x + 38.855). The value of x

was worked out to be 5.12% w/w (table 3.4) and taken as the solubility value for the drug in the matrix.

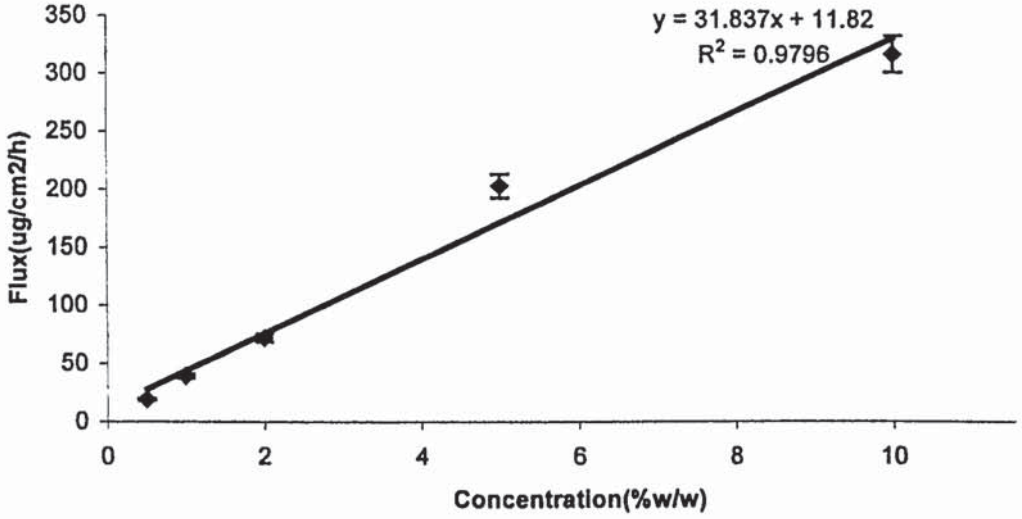


Figure 3.24: Plot of the flux of naproxen against drug loading. N = 3

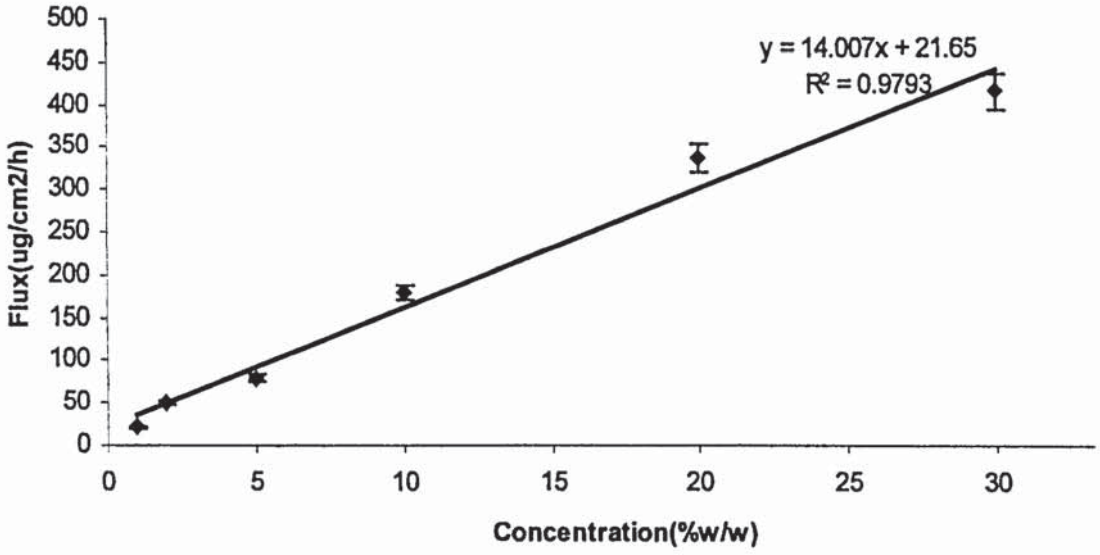


Figure 3.25: Plot of the Flux of flurbiprofen against drug loading. N = 3.

Using a similar approach, the experimental fluxes calculated (y) for naproxen and flurbiprofen were applied to equation of the graph shown in figures 3.24 and 3.25

respectively. The value of x was worked out to be 4.96% w/w and 10.23%w/w for naproxen and flurbiprofen respectively.
Table 3.4 shows the calculated solubility value for each drug.

Drug	Naproxen	Flurbiprofen	Ibuprofen
Solubility value (%w/w)	4.96	10.23	5.12

Table 3.4: Calculated solubility values for naproxen, ibuprofen and flurbiprofen.

In summary, the plot of flux of ibuprofen, naproxen and flurbiprofen against their investigated concentrations in the wax was used as a calibration standard as represented in figure 3.23, 3.24 and 3.25.

The calculated flux of the saturated solution of each of these drugs (shown in table 3.3) in a media (PBS pH 7.4) was then applied to the calibration curve and x value (taken as solubility) was worked out accordingly (shown in table 3.4).
This result will be compared with other data obtained via application of the other techniques employed for determination of drug solubility in the semi-solid wax in section 3.5.

3.4.5. Hildebrand Theoretical solubility model

The tables below (3.5, 3.6, 3.7, 3.8 and 3.9) show the manner in which the values for ΔEv and Vm for the investigated drugs and Witepsol® H-15 were calculated (as described in section 1.12.1.8 and 2.16).

(i) Ibuprofen

Group	ΔEv /kJ mol^{-1}	∑ ΔEv /kJ mol^{-1}	Vm/cm^3 mol^{-1}	∑ Vm/cm^3 mol^{-1}
1 (COOH)	27.61	27.61	28.5	28.5
1 (Phenylene)	31.9	31.9	52.4	52.4
2 (CH)	3.43	6.86	-1.0	-2.00
3 (CH_3)	4.71	14.13	33.5	100.5
1 (CH_2)	4.94	4.94	16.1	16.10
	Total	85.44	Total	195.5

Table 3.5: Calculation of molar volume (Vm) and molar cohesive energy (U) of ibuprofen.

Group	ΔEv /kJ mol^{-1}	∑ ΔEv /kJ mol^{-1}	Vm/cm^3 mol^{-1}	∑ Vm/cm^3 mol^{-1}
1 (COOH)	27.61	27.61	28.5	28.5
9CH=	4.30	38.70	13.5	121.50
1 (O)	3.34	3.34	3.80	3.80
5(Con.d.bond)	1.67	8.36	-2.2	-10.10
2(6-MR)	1.04	2.08	16.0	32.0
1C=	4.30	4.30	-5.50	-5.5
2(CH_3)	4.71	9.42	33.5	67.0
2(CO)	17.36	34.72	10.8	21.6
3(CH)	3.43	10.29	-1.0	-3.0
	Total	138.82	Total	255.8

Table 3.6: Calculation of molar volume (Vm) and molar cohesive energy (ΔEv) of naproxen.

Group	ΔEv /kJ mol^{-1}	∑ ΔEv /kJ mol^{-1}	Vm/cm^3 mol^{-1}	∑ Vm/cm^3 mol^{-1}
1 (COOH)	27.61	27.61	28.5	28.5
1 (Phenylene)	31.9	31.9	33.4	33.4
1C=	4.30	4.30	-5.50	-5.50
1 (CH_3)	4.71	4.71	33.5	33.5
4(CH)	3.43	13.72	-1.0	-4.0
1 (F)	4.18	4.18	18.00	18.00
11(CH=)	4.30	47.3	13.5	148.5
1(6-Mem.ring)	1.04	1.04	16.0	16.0
6(Conj.d.b)	1.67	10.02	-2.2	-13.2
	Total	144.78	Total	255.2

Table 3.7: Calculation of molar volume (Vm) and molar cohesive energy (ΔEv) of flurbiprofen.

Group	ΔEv /kJ mol^{-1}	∑ ΔEv /kJ mol^{-1}	Vm/cm^3 mol^{-1}	∑ Vm/cm^3 mol^{-1}
1 (COOH)	27.61	27.61	28.5	28.5
1 (Phenylene)	31.9	31.9	52.4	52.4
9 (CH=)	4.30	38.70	13.50	121.50
2(Cl)	9.62	19.24	26.00	52.00
1(NH)	8.36	8.36	4.50	4.50
5(CH)	3.43	17.15	-1.0	-5.0
1(6 Mem.ring)	1.04	1.04	16.0	16.0
6(Conj.d.b)	1.67	10.02	-2.2	-13.2
1(C=C)	4.30	4.30	-5.5	-5.5
2(C-Cl)	7.69	7.69	4.0	8.0
1(CH_2)	4.94	4.94	16.10	16.10
	Total	170.95	Total	275.3

Table 3.8: Calculation of molar volume (Vm) and molar cohesive energy (ΔEv) of diclofenac.

Group	ΔEv /kJ mol^{-1}	∑ ΔEv /kJ mol^{-1}	Vm/cm^3 mol^{-1}	∑ Vm/cm^3 mol^{-1}
3 (COOH)	27.61	82.83	28.5	85.5
32 (CH_2)	4.94	158.08	16.1	515.2
3(CH_3)	4.71	14.13	33.5	100.5
1(CH)	3.43	3.43	-1.0	-1.0
	Total	258.47	Total	700.2

Table 3.9 Calculation of molar volume (Vm) and molar cohesive energy (ΔEv) of Witepsol® H15.

Using this method allowed a solubility parameter for each molecule to be calculated, these were then used to predict the behaviour of the drugs when combined with the matrix.

For ibuprofen,

$$\delta = \sqrt{\frac{\Delta Ev}{V_m}} = \sqrt{\frac{85440}{195.5}} = 20.9\sqrt{J/cm^3}$$

δ calculated for ibuprofen was 20.9J/cm^3.

For naproxen $$\delta = \sqrt{\frac{\Delta Ev}{V_m}} = \sqrt{\frac{138820}{255.8}} = 23.29\sqrt{J/cm^3}$$

δ calculated for naproxen was 23.29 J/cm^3.

For flurbiprofen $$\delta = \sqrt{\frac{\Delta Ev}{V_m}} = \sqrt{\frac{144780}{255.2}} = 23.81\sqrt{J/cm^3}$$

δ calculated for flurbiprofen was 23.81 J/cm^3.

For diclofenac $$\delta = \sqrt{\frac{\Delta Ev}{V_m}} = \sqrt{\frac{170950}{275.3}} = 24.91\sqrt{J/cm^3}$$

δ calculated for diclofenac was 24.91 J/cm^3.

For Witepsol H15 $$\delta = \sqrt{\frac{\Delta Ev}{V_m}} = \sqrt{\frac{258470}{700.2}} = 19.21\sqrt{J/cm^3}$$

δ calculated for witepsol H15 was 19.21 J/cm^3.

Once combinations of the possible structures for Witepsol® H15 had been established, they were sketched into a JAVA applet online programme at www.pirika.com, which calculated the solubility parameters. This allowed a range of Hildebrand solubility values for Witepsol® H15 to be calculated. Values in the range 15-22√ (J/cm^3) were derived. These values are similar to the solubility parameter for ibuprofen, naproxen, diclofenac and flurbiprofen suggesting that the drugs are likely to be soluble in Witepsol®H15 since interactions in the former and the latter are similar. Therefore the overall energy needed to facilitate mixing of the constituents will be small, as the energy required to break the interactions within the components will be equally compensated for by the energy released due to interactions between unlike molecules.

Drug / Carrier	δ $(J/cm^3)^{1/2}$	$\Delta\delta$ (solubility parameter)
ibuprofen	20.9	1.69
naproxen	23.29	4.08
flurbiprofen	23.81	4.60
diclofenac	24.91	5.70
Witepsol H-15	19.21	--

Table 3.10: Drug/ carrier system and their solubility parameter values.

Table 3.10 above shows that differences between the solubility parameter of the drugs and that of Witepsol® H15 was less than $7\sqrt{J/cm^3}$. Hence, according to Greenhalgh et al (1999), the systems produced by the combination of any of these drugs with Witepsol® H-15 indicate a high degree of interactions (miscibility) between these components.

The smallest difference in δ values between the drug and vehicle indicate the most soluble compound. Therefore ibuprofen should demonstrate the greatest solubility followed by naproxen, then flurbiprofen and finally diclofenac. Interestingly the Hildebrand solubility parameter of porcine skin has been reported to be 9.7-10 $(J/cm^3)^{½}$ which converts to 19.85-20.46 $(J/cm^3)^{1/2}$ (Hatzenbuhler et al, 1987). Similarity in solubility parameter is known to indicate higher possibility of interaction (Greenhalgh et al, 1999), which might influence the permeation of drugs delivered by the wax.

According to equation 10 from the introduction the mole fraction solubility can be calculated using the solubility parameters of both the drug and the solvent.

$$-\ln X_2 = \frac{\Delta Hf}{RT}\left(\frac{T_o - T}{T_o}\right) + \frac{V_2\phi_1^2}{RT}(\delta_1 - \delta_2)^2$$

Equation 14

where X_2: molar fraction solubility; Φ_1: volume fraction of solvent; V_2: molar volume of solute; R: gas law constant; T: temperature in degrees Kelvin; T_0: melting point of the solid; ΔHf: molar heat of fusion; δ_1: the solubility parameter of the solvent; δ_2: the solubility parameter of drug.

The volume fraction of the solvent is not known and most calculations involve using the mole fraction or some other fractional concentration to calculate this value. As the wax is

present in larger proportions compared to the drug a value of 1 was used as an assumption within the equation above when calculating the mole fraction solubility of the drugs within the wax.

The enthalpy of fusion for ibuprofen and naproxen was reported in the HyperDSC chapter as 150 and 140 J/g respectively; which on conversion denotes 30.95 kJ/mol (ibuprofen) and 32.24 kJ/mol (naproxen). The enthalpy of fusion for flurbiprofen was reported to be 27.9 kJ/mol by Henck et al (1999), while the literature value for sodium diclofenac was 39.77 kJ/mol (Zilnik et al, 2007). The melting temperatures as recorded in table 1.4 in the introduction was used as To the temperature used here was 293K for T and the solubility parameters used were those calculated in the previous work.

Using the values here the molar fraction solubility and percentage solubility of this range of drugs in wax were calculated and the results presented in table 3.11.

	X_2 molar fraction solubility	% w/w solubility
Ibuprofen	0.1005	3.5
Naproxen	0.0027	0.101
Flurbiprofen	0.0073	0.281
Diclofenac	1.11×10^{-5}	0.00055

Table 3.11. The calculated solubility values of the NSAID drugs in Witepsol H15 using the Hildebrand solubility parameters.

The defining characteristic of the solubility as calculated was the difference in solubility parameter between the drug and the vehicle; however the melting temperature of the drug also had a large effect. The values calculated here are much lower than anticipated when compared with the data obtained from other previous techniques (section 3.4.1, 3.4.2, 3.4.3 and 3.4.4). This may be due to assumptions made during the calculation that all the chemical bonds in the structural matrix of a drug are involved in its solubility in a vehicle. In reality, this may not be the case.

Nevertheless, the order of solubility should hold true with ibuprofen > flurbiprofen > naproxen > diclofenac. This order matches the order of melting temperature of the four drugs and the solubility parameters of ibuprofen and diclofenac are at either end of the scale. The solubility parameters calculated for naproxen and flurbiprofen were similar and therefore the values for solubility of these drugs are of the same order.

The result obtained from using this technique also corroborates the facts highlighted in the previous study (section 3.4.4) that the structural composition of each molecule of the drug investigated plays a vital role in prediction of their solubility within a wax.

So far from this chapter, the solubility of ibuprofen, sodium diclofenac, flurbiprofen and naproxen was calculated using a range of techniques. The results are similar in some cases while it was greatly different in others. However, one common indication observed was the influence of physical properties of the selected drugs on their solubility within the wax.

In the next section (3.5) the limitations and merits of each technique will be highlighted. Comparison will be made on the solubility results reported in the preceeding sections of this chapter and the related with the physicochemical proerties of the drugs.

3.5. Discussions

The solubility of drug in wax was measured using five different techniques and a summary of the results study reported in sections 3.4.1, 3.4.2, 3.4.3 and 3.4.4, is shown in the table 3.12 below.

	Drug Solubility (%w/w)			
Method	Ibuprofen	Naproxen	Flurbiprofen	Sodium Diclofenac
Higuchi Release kinetics	18.6	21.4	14.0	--
Saturated solution/permeation	5.12	4.96	10.23	--
HyperDSC	12.7	22.1	20.13	
Hildebrand	3.5	0.101	0.281	0.00055 (Diclofenac)
Microscopy	15-20	~20	20-25	--
Drug Properties				
Log P	3.481	3.313	4.078	0.70
pKa	4.91	4.15	4.13	4.15
Molecular weight	206.3	230.26	244.26	318.13
Melting point (°C)	75-77	153	110-111	283-285
Water solubility (mg/mL)	0.049	15.9	8	0.00082

Table 3.12. Summary of the predicted solubility values and the physicochemical properties of selected drugs. Drug properties are taken from table 1.4a and b.

From table 3.12, the solubility of ibuprofen in the wax matrix as measured using the Higuchi release studies and hyper-DSC approaches was 18.6 % w/w and 12.7%w/w; these values are well above the value obtained using saturation flux (5.12 %w/w) and Hildebrand solubility parameter (3.5%w/w). A similar trend was observed for all other drugs.

For example, the value of flurbiprofen solubility was observed to be 20 % – 25 % w/w via qualitative techniques of microscopic observation of crystals and macroscopic observations during the formulation process. Hyper-DSC provided a quantitative estimation of 20.1% w/w in table 3.12 while Higuchi kinetics provided a solubility estimate of 14.0% w/w in chapter three. The calculated solubility via Hildebrand method suggested a value of 0.28% w/w while flux saturation predicted the solubility of flurbiprofen to be 10.23% w/w.

In the case of naproxen, solubility value of less than 20% w/w (but around this value) was determined on microscopic observation of naproxen crystals. Hyper-DSC provided a

quantitative estimation of 22.1 % w/w for the solubility of this drug in the wax as summarised in table 3.12 and Hildebrand solubility suggested 0.101% w/w as highlighted in chapter seven. The Higuchi release and flux saturation indicated that naproxen solubility was 21.4% w/w and 4.96% w/w respectively.

3.5.1. Effect of physicochemical factors on predicted solubility values

Physicochemical factors such as pKa, log P, melting point, molecular weight, aqueous solubility and structural formula play an important role in the efficacy of transdermal product as these factors affect the amount of solubilised molecules of the active that may be available for permeation from the donor formulation (Williams, 2003).

Amongst the most vital factors which determine the solubility of a drug in a medium is the lipophilicity / hydrophilicity nature; log P. This is a parameter that measures how much a drug partitions either in octanol or water in a mixture of both phases. It serves as a quantitative descriptor of lipophilicity. A study, (Beetge et al, 2000) identified log P as an optimum parameter that could be used to predict the *in vivo* bioavailability of selected NSAIDs via the influence of this parameter on other physicochemical factors such as solubility. The log P values of drugs considered in this study vary from 0.7 to 4.0. From table 3.12, theoretically, it can be predicted that the solubility of the selected drugs should be in the order of sodium diclofenac < naproxen < ibuprofen < flurbiprofen; provided that log P alone was considered as the main determinant of solubility and the wax was taken to be a lipophilic vehicle. In an aqueous environment such as the dissolution media, Witepsol® H15 would not be expected to act completely as a lipophilic vehicle.

Witepsol® H15 has a hydroxyl value of 5-15 mg KOH/g (Sasol Product information sheet, 2007); the hydroxyl value is a measure of the degree of esterification. It is defined as the milligrams of potassium hydroxide required to neutralise the free hydroxyl groups in 1g of sample. A high number of hydroxyl groups increase the polarity and therefore the water miscibility. The acid value of Witepsol® H15 is reported to be 0.2 mg KOH/g (Sasol Product Information sheet)(maximum value); the acid value is the milligrams of

potassium hydroxide required to neutralise the free carboxyl groups in 1g of sample, usually expressed as mg/KOH per g. The acid value is determined in the cold and measures the free fatty acid content.

These two properties of Witepsol® H15 indicate that as well as having long chain fatty acids that are lipophilic there is a significant amount of more polar hydroxyl groups present that enable miscibility with water as well as solubilisation of a range of drug molecules.

From the results of the studies conducted on solubility using various techniques and summarised in table 3.12; both microscopy and the saturated solution flux study suggested that flurbiprofen demonstrated the greatest solubility and this may be due, in part to the higher log P of this drug compared to the others.

Furthermore, as shown in table 3.12, both microscopy and saturation solution flux techniques showed that increased log P corresponded with increased solubility of all the drugs in the wax in the order of flurbiprofen > ibuprofen > naproxen. This was expected as the previous literature reports that higher log P denotes stronger affinity for the hydrophobic environment (of the wax in this case); hence, lowers leaving potential for the aqueous environment of the dissolution media (Sloan, 1989; Majumdar et al, 2007).

However, the calculated solubility values were close for these techniques and did not follow the log P trend. It should be noted that the log P values of ibuprofen and naproxen are relatively similar and therefore the technique may be unable to differentiate these drugs based on log P alone. Therefore log P alone cannot be used as a direct indicator of solubility for Witepsol®H15.

Surprisingly, Higuchi kinetics, hyper-DSC and Hildebrand solubility techniques demonstrated a mixed picture of the relationship between log P and the calculated drug solubility in the wax. For example, table 3.12 showed the order of log P value for the drugs to be flurbiprofen > ibuprofen > naproxen while the calculated solubility in the wax

via Higuchi release was in the order flurbiprofen < ibuprofen < naproxen: 14.0% (w/w) < 18.6% (w/w) < 21.4% (w/w).

One possible explanation for the order of solubility observed could be the pH (7.4) at which the dissolution studies were carried out favoured more release of the drugs in this order; naproxen > ibuprofen > flurbiprofen and this mirrored the calculated solubility values. The investigated drugs are acidic in nature; hence, dissolution in pH media above their pKa will enhance their ionisation. It is known that only ionised species of this class of drug are soluble. Although, both naproxen and flurbiprofen have similar pKa values (table 3.12), reduced molecular weight and lower log P values might have compensated for the increased release rate; hence increased solubility value calculated for naproxen. On the other hand, lower log P value (3.48), higher pKa (4.91) and a much lower molecular weight would have favoured the release rate and hence solubility of ibuprofen when compared with flurbiprofen.

The pKa of the drugs was also considered to be important, as this was also likely to influence release rates when solubility was calculated using Higuchi release kinetics. The pKa value is an indication of the ionisation of a drug at a given pH. At pH values higher than the pKa value a greater fraction will be ionised and therefore soluble in a polar medium (e.g. the release medium used in the Higuchi experiments). As flurbiprofen and naproxen have lower pKa values compared to ibuprofen it was anticipated that more molecules of these two drugs would be soluble in water, this is also suggested by the aqueous solubility values noted in table 3.12. However, the wax also has some polarity as indicated by the hydroxyl value of 5-15 mg KOH/g; therefore the wax may also enable dissociation of the drug and therefore induce greater solubilisation.

However, if aqueous solubility values alone were to be considered as inversely related to the wax solubility (table 8.1), the order of solubility ought to have been sodium diclofenac < ibuprofen < flurbiprofen < naproxen. Both HyperDSC and Higuchi release kinetic solubility methods suggested that naproxen had the highest solubility (sodium diclofenac was not measured using these techniques) therefore there may be some merit

in inverse water solubility influencing wax solubility. For HyperDSC this trend carried through the three drugs tested, with naproxen>flurbiprofen>ibuprofen.

The melting point of a drug is a measure of the strength of the internal bonds as a higher melting temperature is required to break strongly bound structures; often solubility can be linked to melting temperature where lower melting points are thought to have higher solubility as they are more easily disrupted and solubilised. This theory suggests that ibuprofen should demonstrate the greatest solubility followed by flurbiprofen then naproxen and finally sodium diclofenac. This order was followed exactly when looking at the theoretical solubility as calculated using the Hildebrand solubility method (table 3.12). This is unsurprising as this technique uses the intermolecular properties of the drug as a basis for the calculations.

The structural formulae of these drugs (table 1.4a and b) reveals that naproxen has a highly reactive hydroxyl group (yet it have same pKa as flurbiprofen and sodium diclofenac) located at the para-position of its structure, which will be easily available to interact with reactive groups (such as carbonyl or hydroxyl functional groups) of the saturated fatty acids within the wax. Ibuprofen does not have any reactive group in its structure aside from the common carboxylic acid group. In a reported study, Stott et al (2001) found that propanolol interacted with the carboxylic acid groups of capric and lauric fatty acids. They concluded the interactions led to formation of the eutectic mixture between the acids and the drug; and this mixture had a lower melting point when compared with the two constituents.

Thus, the high solubility values (via hyper-DSC approach and microscopic observation for both drugs, Higuchi release for naproxen and saturated solution flux for flurbiprofen) recorded for both naproxen and flurbiprofen may be due to more interactions (caused by the reactive hydroxyl and fluorine group present in the structure of these drugs) between the molecules of these drugs and the wax compared to ibuprofen. This greater number of interactions might have enabled these drugs to be better solubilised within the wax matrix. However, Hildebrand solubility showed the highest solubility value for ibuprofen.

In summary, it is possible to conclude that interplay of physicochemical factors influenced the solubility of the selected drugs in Witepsol®H15. There was also a limited correlation between the different techniques in determining solubility of the drugs examined. This lack of defined correlation may be due to the inherent similarity of the compounds under test or the differences in technique used to determine the solubility. This reflects the complications associated with determination of the solubility of a drug within a semi-solid. The main advantages and disadvantages of each technique are summarised in table 1.2. Here, a brief summary of when each technique should be used according to the level of information required and from the results obtained from various studies reported in preceding chapters of this thesis was discussed.

3.5.2. Microscopy approach: Limitation and Usefulness

Microscopy was described in chapter three. Comparing the result obtained via this method with that from other techniques (table 3.12), this approach correlated more with the Higuchi kinetics and hyper-DSC techniques than with the saturation flux and Hildebrand solubility parameter approaches. A difficulty encountered in using this method was maintaining the wax formulation in a liquid opaque state for easier visibility of the drug crystals under white light.

However, the method demonstrates that a qualitative technique such as this may be used to roughly determine the solubility limit to allow better design of a Hyper-DSC protocol to quantitatively measure drug solubility in a semi-solid.

A comparison of the solubility values obtained from each technique is shown in table 3.12. From this table, it can be seen that the microscopy and hyper-DSC approaches are very comparable. The additional benefit of this method is its simplicity.

3.5.3. Higuchi release: Limitation and Usefulness

In section 3.4.2, Higuchi release approach was employed, the calculated solubility in the wax was in the order flurbiprofen < ibuprofen < naproxen: 14.0% (w/w) < 18.6 % (w/w)

< 21.4% (w/w) (table 3.12). This result was similar to the results obtained from the studies where both microscopy and hyper-DSC were used.

The difficulty of making a valid conclusion based on the result of this technique can be associated with the several difficulties encountered in the course of the study. This technique was based on the assumption that only the solubilised molecules of a drug will dissolute from a matrix into the surrounding medium. Hence, it may not mirror typically the exact amount of drug dissolved in a base matrix. Furthermore, it was assumed that the wax or vehicle does not dissolute or melt and this assumption would be invalid in this case. Although, the large proportion of the wax was intact during the study, it will be difficult to prove that none of it melts.

Another problem for lower estimation of solubility may be also be explained by the drug being present in the more complicated system of a melting wax compared to creams or ointments from which the mathematical derivations are derived.

Moreover, this approach assumed that drug release was a function of drug concentration within the matrix. However, as seen in this study (section 3.4.2), a linear relationship was only observed at lower concentration drug loadings while there were a lot of mathematical manipulations for the release data especially at higher drug loadings for suspended formulations.

Also, release experiments such as this, are time-consuming to perform and require additional analysis via HPLC (or a similar analytical technique to measure drug concentration). In addition, data gathered from concentrations distant to the region of interest are useful in determining the solubility within a semi-solid.
However, the advantage of this technique is that it also provides interesting information about the release profiles of the drug from the formulation, which is of importance in formulation design (Ahmed et al, 2004).

3.5.4. Saturation solution flux: Limitations and Usefulness

In section 3.4.4, a relatively uncommon technique was applied for prediction of the solubility of the investigated drugs. This approach was mainly based on the assumptions that a saturated solution of a drug has the same thermodynamic activity as a semi-solid formulation, which contains equal saturated amount of the drug.

The calculated solubility value for naproxen, ibuprofen and flurbiprofen was 4.96 %(w/w), 5.12 % (w/w) and 10.23 % (w/w) respectively. These values are quite low in comparison with the results obtained from the previous study that involved use of Higuchi release kinetics. This result was not surprising because it also demonstrates the problems associated with the use of this method in determination of the solubility of drugs in semi-solid formulations such as the wax employed in this case.

The assumptions made using the permeation approach to determine solubility include; no interaction between the drugs and the wax, no effect of the wax on permeation and that there was a simple relationship between the flux and drug solubility, might have being too ambiguous and invalid. For example, the wax is a complex system made up of lipophilic fatty acids that have reactive groups that can not only interact with the drugs but also influence their permeation through the porcine skin. One possibility for future experiment would be to look at using a different membrane such as dialysis tubing that will not be affected by the wax, for this study.

Furthermore, reduced fluxes might have been caused by the retention of drugs in the porcine skin, thereby; questions could be raised about the validity of the calibration curve from which the calculated solubility was estimated.

In addition to these limitations, there is a possibility that this method measures the solubility of the drugs in the skin and possible not in the wax. Also, the approach is time consuming and involves a series of experimentation that are simple but yet demanding.

Besides, the Higuchi kinetics calculations applied was based on the assumption that only the solubilised molecules of a drug will dissolute from the matrix into the skin membrane. This cannot be true in this case as part of the wax might have permeated through the skin. The similar Hildebrand solubility calculated in section 3.4.5 supported this statement. Thus, once flux was affected, the calculated drug solubility would be affected. The use of silastic membrane might be useful to overcome this problem.

The data obtained from this study showed the complexity and difficulty involved in the use of this approach for determination of drugs solubility in semi-solids. However, the information provided about the kinetics of drug release could be valuable as such data could be used to predict the *in-vivo* kinetics of these drugs.

3.5.5. Hyper-DSC technique: Limitations and Usefulness

In section 3.4.3, hyper-DSC was used as a relatively new technique as another approach for this investigation. The result of this technique shows that the solubility of ibuprofen, flurbiprofen and naproxen in the wax-based matrix was calculated to be 12.7 % w/w, 20.1 % w/w and 22.1% w/w respectively.

In comparison with the Higuchi approach discussed in chapter four, where reduced solubility values were calculated for both flurbiprofen (14.0 %w/w) and ibuprofen (18.6 % w/w) when compared with naproxen (21.4 % w/w); the solubility results from this approach indicated that both ibuprofen (12.7 % w/w) and flurbiprofen (20.1 % w/w) also had lower solubility values compared with naproxen (22.1 % w/w).

If there was an interaction between flurbiprofen and naproxen with the wax molecules (section 5.5), indicated by the lower melting point of these drugs, then, the solubility of both drugs would be increased in the wax and in similar lipids such as the skin. Thus, the permeation of both drugs through the porcine skin will be better than naproxen and this will occur via a similar mechanism used by eutectic systems (Williams, 2003).

Furthermore, one factor to consider might be the lower melting point of ibuprofen, thus, making it difficult to distinguish a distinctly separate melt from the wax-drug blend peak which might have compromised the results.

Accordingly, this order of calculated solubility values determined by the hyper-DSC approach corresponded with the physicochemical properties of the drugs, in which the structural functional groups and the pKa value seem to be the determinant factors. In addition, the lowered melting point recorded from the various thermograms are evidences that proves the earlier speculations that there was interaction between the functional groups of the drugs (aside from the carboxyl group) and the carbonyl groups of the fatty acids that makes up the wax.

The advantages of this technique as an analytical tool is that it is rapid (at least, once the calibration has been done and the formulations prepared), requires very small sample sizes (less than 2 mg), data is quantitative and provides an estimation of drug solubility within semi-solids. Furthermore, it provides additional information on interactions that might have occurred between the drug and excipients present within a formulation compared with other techniques such as release studies and visible microscopy.

However, the main disadvantages of this technique are; it requires expert personnel, the instrumentation is expensive, requires crystalline samples and validation of data is an issue. But in comparison with the earlier methodologies reported above, hyper-DSC is a high throughput technique that is extremely sensitive and the principle did not involve ambiguous assumptions (Oladiran and Batchelor, 2007).

3.5.6. Hildebrand solubility method: Limitation and Usefulness

In section 3.4.5, mathematical approach was used to determine the solubility parameter of each of the drugs as well as the Witepsol® H15 base. The calculated values where then used to discuss how physicochemical properties of the drugs affected their solubility in the base. This method involves the prediction of solubility based on Fedor's group

contribution. A set of standard values were analysed after determination of the functional groups of the investigated compound that will affect its solubility. This method requires the user to have a sound knowledge of chemistry. Another limitation to this technique is the fact that it could not be applied for metal salts. Hence, in this case, prediction was made for solubility of free salt of diclofenac.

Thus, because of these limitations, a reduced calculated solubility values (0.00055% w/w, 0.281% w/w, 0.101% w/w and 3.5% w/w) for diclofenac sodium > flurbiprofen > naproxen > ibuprofen respectively. However, the result provided basis for explanation on how the physicochemical factors of these drugs might affect their solubility (Zilnik et al, 2007).

3.6. Conclusion

From this chapter, the results of the determination of the solubility of selected drugs in Witepsol® H15 was reported by employing a range of techniques. Visible microscopy was observed to give qualitative data that was comparable to other time consuming and complex method such as Higuchi release and hyper-DSC respectively. The simplicity of this technique makes it a proposed first point of contact for estimating drug (crystallised) in semi-solid formulations prior to exploitation of other approaches.
On the one hand, Higuchi release proved to provide enormous amount of quantitative data thst might be useful in kinetic studies. However, the use of this approach should be restricted to matrices that will not melt or dissolute when drug formulated with them are released. Also, the choice of dissolution method applied is a matter that requires consideration.

On the other hand, in situations where further information such as interaction between the matrix and the drug is required alongside the solubility determination of the latter in the former, hyper-DSC should be the method of choice. In this chapter, mixture with lower melting point than constituting drug and matrix was formed between ibuprofen or naproxen and the wax. Also, in cases where there is an established validation assay and

available prepared formulations, the rapidity of hyper-DSC could be very useful for solubility determination.

In another vein, for studies with strict time restrictions and where the qualitative solubility data is required, visible microscopy technique should be a method of choice. However, should there arise a necessity to examine how constituent molecular constituents of a drug influences its solubility in a semi-solid, Hildebrand solubility parameter methodology should be employed.

Moreover, the skin permeation method should be used when kinetic data and solubility value are of paramount importance. The choice of membrane to be used in permeation studies should be a matter of importance.

Overall, each of the methods examined in this chapter have their limitations and usefulness. The choice of method is a function of the investigations to be conducted. It is strongly advisable that a combination of methods in determining drug solubility within a semi-solid formulation will be a better option.

Finally, it is possible to conclude that many physicochemical factors such as log P and pKa are vital intrinsic properties amongst others that affected the calculated solubility of the selected drugs in Witepsol® H15.

Chapter Four

Formulation manipulation to optimise drug release

4.1. Introduction.

In vitro drug release testing is one important formulation performance efficiency test for transdermal dosage forms (Dohner, 1987). Hence, the effect of chosen excipients on the release of the selected drugs is of utmost importance.

The suitability of Tween 20, menthol and Carbopol® 971 as excipients in transdermal drug delivery system was discussed in sections 1.20.1, 1.20.2 and 1.20.3. Thus, to reduce brittleness, increase the 'tabletability' and enhance the release rate of the selected drugs from the chosen Witepsol H15 base, Tween 20, menthol and Carbopol® 971 (incorporated at loadings less than or equal to 2% w/w) were included in the formulation. In addition, to avoid other problems commonly encountered during use of Witepsol H15 as a suppository base, cold tableting technology was employed for the preparation of all formulations as described in section 2.2.

4.2. Aims and objectives

The main aim of this study was to investigate the effect of incorporation of a range of concentrations of hydrophilic non-ionic surfactant; Tween 20 (HLB=16.7), menthol and hydrophilic polymer; Carbopol® 971 as known acceptable additional excipients on the release of the selected drugs as a pre-formulation process to develop a thermodynamically efficient delivery system. The results from this study are expected to provide insights about the possible interactions between the drug, excipients and the vehicle. The method employed for this study can be found in section 2.9. Student t-test was used for statistical analysis of the release rate data to determine if addition of excipients or change in their concentration has any influence on the release of the actives.

4.3. Results and Discussion

4.3.1. Formulation manipulation

Manipulation of formulations required incorporation of additional excipients; these manipulations were listed in table 2.4. Release and permeation studies were performed on

these manipulated formulations in comparison with the formulations where only the drug in wax was measured. In each case, for the manipulation study the concentration of drug remained constant; ibuprofen and naproxen were loaded at 5% w/w while flurbiprofen and diclofenac were loaded at 10 % w/w; the drug loadings varied due to the results from the investigations of saturated solubility flux studies on the permeability of each drug as reported in table 3.4. Tween 20, Carbopol® 971 and menthol were sourced from suppliers as listed in table 2.1.

In all cases, Higuchi plots of the cumulative mass released per unit area against the square root of time were applied and the release rate constant; K_H, was taken as the slope of the plot. One-way ANOVA statistical analysis was done to determine differences between release data sets while Student t –tests statistical analysis was employed to compare data pairs between the control and those formulations containing the excipients. All the statistical analysis results are shown in the appendix.
Table 4.1 below shows the release rate constant values calculated for each formulation prepared using ibuprofen, naproxen, flurbiprofen and diclofenac as model drugs.

%(w/w)of excipients added							
F	T	C	M	Diclofenac; K_H $\mu gcm^{-2}h^{-1/2} \pm$ s.d	Ibuprofen;K_H $\mu gcm^{-2}h^{-1/2} \pm$ s.d	Flurbiprofen; K_H $\mu gcm^{-2}h^{-1/2} \pm$ s.d	Naproxen; K_H $\mu gcm^{-2}h^{-1/2} \pm$ s.d
1	0	0	0	31.70 ± 2.20	57.19± 0.42	251.10 ± 1.16	30.20 ± 0.79
2	1	0	0	41.30 ± 0.40	58.64 ± 0.51	307.42 ± 2.40	33.45 ± 0.31
3	2	0	0	65.20 ± 2.30	66.52 ± 0.34	350.27 ± 2.91	45.25 ± 1.07
4	1	1	0	86.10 ± 1.70	69.20± 0.26	345.32 ± 2.38	35.43 ± 0.71
5	1	2	0	53.80 ± 0.60	67.81± 0.53	330.22 ± 3.19	37.76 ± 0.62
6	2	0	5	382.60 ± 4.10	59.34 ±0.83	288.85 ± 2.68	31.75 ± 0.66
7	1	1	5	468.30 ± 7.50	59.10 ± 0.29	267.97 ±2.32	33.13 ± 0.74
8	0	0	5	641.77 ± 3.84	53.75 ± 0.46	315.59 ± 3.61	29.89 ± 0.75
9	0	1	0	648.97 ± 0.76	58.54 ± 0.01	245.05 ± 1.22	34.29 ± 0.55
10	0	2	0	326.66 ± 4.61	52.19 ± 0.18	247.73 ± 1.48	34.39 ± 0.53

Table 4.1: Release rate constant data for ibuprofen, naproxen, flurbiprofen and diclofenac from F= formulations (1-10) containing different concentrations of Tween 20 (T), menthol (M) and Carbopol® 971(C). N= 3; data show mean ± standard deviation. All lines had $r^2 > 0.97$.

The effect of incorporating the chosen excipients on the release potential of each drug from the wax matrix was discussed individually below.

4.3.2. The effect of Tween 20

Table 4.1 shows that there was a proportional increase in the release rate constant K_H value of diclofenac with the increase in the concentration of Tween 20 added to the formulations. For example, the release rate of diclofenac increased from 31.7 $\mu gcm^{-2}h^{-1/2}$ to 41.3 $\mu gcm^{-2}h^{-1/2}$ and further to 65.2 $\mu gcm^{-2}h^{-1/2}$ when the concentration of Tween 20 was increased from 0% to 1% and then to 2% (w/w) respectively demonstrating a significant effect ($p = 0.00$) of Tween 20 on diclofenac release from the wax based matrix (formulations 1, 2 and 3). There was also a significant difference ($p = 0.00$) between the release rate of diclofenac in the other formulations (4, 5, 6 and 7) containing Tween 20 either as the 1 or 2 % (w/w) loading and the control batch (formulation 1).

In the same vein, Student t-test statistical tests revealed that the release rate constant value for ibuprofen also increased as the concentration of Tween 20 added to the formulation was increased from 1 to 2% (w/w). Although, formulations 1 (control) and 2 (control +1% Tween 20) respectively for this drug were not statistically different ($p = 0.01$) when their K_H values was compared.

In addition, the data (table 4.1 above) for the release of naproxen from the wax matrix in the presence of Tween 20 show that there was an agreement with previous studies highlighted above. There was an increase in K_H value compared to the control batch (formulation 1) as Tween 20 concentration was increased from 0 %(w/w); the control to 1%(w/w) loading and to 2%(w/w). In fact, the highest K_H value recorded for all the naproxen formulations was achieved with the 2 %(w/w) loading of Tween 20 (formulation 3). Furthermore, a detailed look at the results (table 4.1) above indicated that incorporation of Tween 20 significantly ($p = 0.00$) increased the release of naproxen in formulations 3, 4 and 5 when compared with the control while the release rate data for all other batches investigated was either not statistically different ($p > 0.00$), or has a reduced

release rate such as formulation 6 that has additional 5% (w/w) menthol as a further excipient (control + 2% Tween 20 + 5% menthol).

The results also indicate that the release of flurbiprofen from the wax matrix was increased in the presence of Tween 20 in conformity with previous studies highlighted above. There was a significant ($p = 0.00$) increase in K_H value compared to the control batch (formulation 1) when Tween concentration was increased from 0 % (w/w) to 1% (w/w) and then to 2 % (w/w).

The high K_H value obtained for flurbiprofen formulations containing Tween 20 when compared to ibuprofen and naproxen formulations with the same excipient could be attributed to the increased chemical gradient due to higher drug loading of flurbiprofen (as stated in section 4.3.1; table 3.4). The reduced release for diclofenac can be attributed to increased thermodynamic activity of the drug within the vehicle and this might have been brought about by increased solubility (Hanaee et al, 2004).

The highest K_H value recorded amongst all the flurbiprofen loaded formulations investigated was that of the 2 %(w/w) loading of Tween 20 in the absence of any other excipient (formulation 3). There is a strong possibility that increasing the concentration of Tween 20 further beyond 2 % (w/w) loading might increase the release rate further.

The reasons for the increase in release rate with incorporation of Tween 20 in all the formulations investigated could be best described by the study carried out by Buckton et al (1991) where they proposed three mechanisms by which surfactants increase drug release from hydrophobic matrices. These mechanisms are: improved wetting of the matrix surface; dissolution of the surfactants; and reduced cohesion between the hydrophobic molecules of the wax; each of these complements one another to make it easier for the dissolution of the drugs into the release medium (Buckton et al, 1991). These mechanisms would complement one another in effect to enhance the release rate of the drug from the matrix. Furthermore, the stated mechanisms would possibly enhance

the thermodynamic activity or leaving potential of the drugs from the fatty base by reducing the latter's attraction for them (Hanaee et al, 2004).

Another possible explanation could be that solubilisation of the Tween 20 in the mixture by the dissolution media resulted in improved wetting of the drugs in the Tween 20 rich microenvironment formed at the surface of the drug crystals, and this may promote the dissolution of the drugs. This will could also lead to enhanced solubility of the drugs in the matrix; ultimately promoting its thermodynamic activity.

In summary, incorporation of Tween 20 in all the formulations investigated resulted in a significant increase in the release rate of diclofenac, ibuprofen, naproxen and flurbiprofen from some formulations while there was no real change in some cases when compared to the control formulations.

4.3.3. The effect of Carbopol® 971

Table 4.1 equally shows the release rate data of diclofenac from the lipophilic base containing Carbopol® 971 as an additional excipient. Evidently, K_H was significantly increased to 86.1 $\mu gcm^{-2}h^{-1/2}$ in the presence of 1% (w/w) Carbopol® 971 with 1% Tween 20; this was almost three times the calculated value for the control (formulation 1). This increase was statistically significant (p= 0.00) as revealed by the Student t-test when compared with formulation 1(control).

In the presence of Tween 20 at 1% (w/w), there was a significant increase in K_H value as the concentration of Carbopol® was increased from 0%(w/w) to 1%(w/w) and then to 2%(w/w) loadings (formulations 1, 4 and 5). A further look at formulations 9 and 10 (with only wax and Carbopol® present at 1 and 2 % (w/w)) indicated that the K_H value was almost halved from $648.97 \pm 0.76 \mu gcm^{-2}h^{-1/2}$ to $326.66 \pm 4.61 \mu gcm^{-2}h^{-1/2}$ ($p = 0.00$) with the increase of Carbopol® 971 concentration from 1 to 2 % (w/w) in the absence of any other excipients. However, both of these values were significantly higher than the control at $31.7 \pm 2.2 \mu gcm^{-2}h^{-1/2}$. The sharp increase in release of diclofenac by almost a

factor of 20 (when compared to the control; formulation 1) as the concentration of Carbopol® 971 was increased to 1 % (w/w) loading (formulation 9) is an indication that the leaving potential of the drug was significantly enhanced in the presence of Carbopol® 971. However, this was reduced when Carbopol® 971 concentration was further increased to 2 % (w/w). These results further confirm the earlier prediction that there was an interaction between the molecules of the wax and the drugs; in this case diclofenac sodium and Witepsol® H-15 (section 8.3). The presence of Carbopol® 971 in these formulations may have reduced this interaction, thus increasing the concentration gradient; which is the driving force for drug release. Infrared microscopy could be employed as a technique of choice to investigate the interactions and this could be looked at during future studies.

The release rate of ibuprofen from the wax base was also increased significantly ($p = 0.00$) to 69.20± 0.26 $\mu gcm^{-2}h^{-1/2}$ in formulation 4 containing 1% (w/w) Carbopol® 971 and 1% Tween 20 compared to the control ($57.19 \pm 0.42 \mu gcm^{-2}h^{-1/2}$). However, in the absence of any other excipients, the release rate of the drug from the formulation containing 1% Carbopol® was not different statistically ($p > 0.00$) to that of the control. In addition, an increase in Carbopol® to 2 % (w/w) of the formulation significantly reduced the release rate compared to the control ($p=0.00$).

The effect of Carbopol® 971 on flurbiprofen release was similar to that for ibuprofen. Carbopol® 971 in the presence of Tween 20 increased the rate of release, with the greatest release achieved with 1 % (w/w) of Carbopol® rather than 2 % (w/w). However, when wax and Carbopol® were the only excipients present the K_H value for both formulations 9 and 10 were significantly below the calculated value for the control (formulation 1). The K_H value was significantly ($p = 0.00$) decreased as concentration of Carbopol® 971 was increased from 1% (w/w) to 2 %(w/w) in the presence of 1% (w/w) Tween 20; the calculated K_H value of formulation 4 was decreased from $345.32 \pm 2.38 \mu gcm^{-2}h^{-1/2}$ to $330.22 \pm 3.19 \mu gcm^{-2}h^{-1/2}$ in formulation 5.

As seen from the results in Table 4.1 above, the effect of adding Carbopol® to all formulations loaded with 5% (w/w) naproxen was quite clear. In all cases, there was a notable increase in the release rate of the drug as compared to the control (formulation 1). For example, K_H increased from 30.2 ± 0.79μgcm^{-2}h$^{-1/2}$ to 35.43± 0.71μgcm^{-2}h$^{-1/2}$ and then to 37.76 ± 0.62μgcm^{-2}h$^{-1/2}$ when the concentration of added Carbopol® increased from the control to 1% and finally to 2 % (w/w) loading in the presence of 1 % (w/w) Tween 20. In addition, significantly ($p = 0.00$) higher K_H values were calculated for each of formulations 7, 9 and 10 (table 4.1) which contained at least 1 %(w/w) loading of Carbopol® (Formulation 7 contained 1% (w/w) Tween 20; 1% (w/w) Carbopol® and 5 % (w/w) menthol; formulation 9 contained wax with 1 % (w/w) Carbopol®; formulation 10 contained wax with 2 % (w/w) Carbopol®). These results are in line with the release data observed from the investigations on the release of diclofenac as explained above. The calculated K_H value for formulations 9 and 10 (table 4.1); in this case, were neither statistically different from each other, nor was each value significantly ($p > 0.00$) higher than the control as revealed by t- test.

These results also complement the earlier prediction about possible interaction between naproxen and Witepsol® H-15 due to the hydroxyl functional group of the drug. The presence of Carbopol®) interfered with this interaction, hence, promoting the release of the drug from the wax, evidenced by K_H value of formulations 2, 4 and 5.

In summary, incorporation of Carbopol® at a concentration of 1% (w/w) resulted in an increase in release rate of each of the drugs considered in the presence of Tween 20 at 1 % (w/w). However, when the concentration of Carbopol® 971 was further increased to 2% (w/w) (again in the presence of Tween 20), a further increase in K_H value was only observed for naproxen, although again all formulations showed a higher release rate constant compared to the control. In formulations where Carbopol® (at 1 % (w/w)) and wax were the only two excipients present; the release rates were increased above the controls for diclofenac and naproxen to a large extent, ibuprofen only slightly and the release rate was reduced with flurbiprofen.

This provides further evidence that there was a possibility that there was interaction between Witepsol® H-15 and the investigated drugs. Specifically, the extent of the

interaction as denoted by calculated solubility values (table 3.12) corresponded with the extent of release of the investigated drugs in the presence of Carbopol®. Hence, Carbopol® might have reduced these interactions to enhance the drugs release.

A further increase in Carbopol® loading to 2 % (w/w) reduced the release rate constant for ibuprofen, yet statistically; the results obtained were similar for flurbiprofen and naproxen when compared with the control. Only diclofenac still maintained a release rate constant greater than the control at the higher loading of Carbopol®.

The increase in K_H value due to the incorporation of Carbopol® was possibly due to the ability of Carbopol® to enhance water absorbability of the matrix and thus, the aqueous solubility of the drugs, hence, improving their release (Yahagi; 1999, 2000).
Another possibility by which this increase might have occurred is through the swelling of the Carbopol® molecules when they come in contact with the aqueous environment and this will result in disruption of the inter- and intra- molecular arrangement between the molecules of the wax matrix thereby creating channels that will promote drug release (Buckton et al, 1991). This increase in the disintegration time of tablets provides a greater surface area for release and may be a physical reason for Carbopol® 971 to enhance the release of drugs from modified formulations.

As not all formulations followed the same trend in drug release rate there must be additional factors that relate to the physico-chemical properties of the drug that contribute to the release rate constants observed. For example, both naproxen and diclofenac have similar pKa values (4.15,table 1.4) while ibuprofen has a higher pKa (4.91,table 1.4). This might have play an important role in the higher release rate data (table 4.1) recorded for diclofenac from the wax matrix in the presence of Carbopol® 971 as compared to ibuprofen. Naproxen release might have been caused by possible interaction between the molecules of the wax and the drug. Such interactions might be too strong for Carbopol® 971 to interfere. Within the release media, more of the diclofenac/ naproxen will be soluble therefore this will favour their release compared to ibuprofen. These results only corroborate earlier findings by Beetge et al (2000) that physicochemical factors such as

pKa plays an important role in the solubility and therefore, release of drugs from a matrix.

On the other hand, the decrease in K_H value seen when Carbopol® 971 concentration in all flurbiprofen and diclofenac formulations was increased to 2% (w/w) could be attributed to the increase in the viscosity (as observed visually during formulation preparation) of these formulations and this is likely to cause impediment for the drug molecules to move from within the matrix to dissolute into the surrounding medium (Yahagi; 1999, 2000).

Yahagi et al (1999, 2000) reported a similar decrease in K_H value when 10% (w/w) Carbopol® was incorporated into a wax based suppository formulation. The effect of the increase in viscosity to overcome the wetting effect Carbopol® molecules had on these formulations can be best seen when formulations 9 and 10 with diclofenac are compared; an increase in Carbopol® 971 loading to 2% almost halved the calculated K_H value from $648.97 \pm 0.76 \mu g cm^{-2} h^{-1/2}$ to $326.66 \pm 4.61 \mu g cm^{-2} h^{-1/2}$. Another possible explanation for this sharp decrease in release rate observed for diclofenac could be due to reduced thermodynamic activity of the drug brought about by an interaction between the wax, Carbopol® at 2%(w/w) and the drug.

4.3.4. The effect of menthol

A summary of the effect of menthol on the release rate of diclofenac, ibuprofen, naproxen and flurbiprofen is also summarised in table 4.1. In summary, the following formulations were investigated:

Formulation 8 contained wax with 5 % (w/w) menthol

Formulation 6 contained wax with 2 % (w/w) Tween and 5 % (w/w) menthol

Formulation 7 contained wax with 1 % (w/w) Tween, 1% (w/w) Carbopol® and 5 % (w/w) menthol

An increase in K_H value was observed in all cases when 5% (w/w) menthol was added to diclofenac formulations. K_H value for this drug was amplified by almost six times in the presence of 5% (w/w) menthol in formulations 6 and 7 as compared to those without menthol (formulations 3 and 4). The release rate constant of formulation 8 was increased by a factor of twenty, when compared to formulation 1 without menthol.

On a contrary note, a decrease in release rate of ibuprofen was observed in all formulations containing 5% (w/w) menthol, compared to the equivalent formulations without menthol present. This decrease could be attributed to formation of a eutectic system caused by interaction between the molecules of the drug and menthol.

Like ibuprofen, all naproxen formulations that contained 5% (w/w) menthol resulted in a release rate lower than those of the equivalent formulations that did not contain menthol. For flurbiprofen, a significantly ($p<0.05$) higher release rate constant was seen when 5% (w/w) menthol was added to formulation 8; compared to the control, formulation 1. Formulations 6 and 7 with flurbiprofen showed lower release rate constants that the equivalent formulations that did not contain menthol.

In summary, 5% (w/w) menthol resulted in an increase in K_H value of diclofenac and flurbiprofen (provided no other excipients were present in the latter cases) but K_H value was decreased for all ibuprofen and naproxen formulations.

The increase in K_H value observed for all formulations containing menthol was probably due to the action of menthol on some physicochemical concepts; such as solubility, partition coefficient and thermodynamic activity, which affects the release of these drugs. The most likely factor to be affected could be aqueous solubility.

In the same vein, the sharp increase in the release rate of diclofenac might be due to interference of menthol with the interaction between the drug and Witepsol® H-15, thus, enhancing the leaving potential of the drug. Also, a similar explanation could be given to

the observed result for flurbiprofen. This result is not surprising as it clarifies the earlier prediction that there was interaction between these drugs and the wax.

In the presence of menthol, there is a possibility that the hydroxyl group of menthol will interact with the carbonyl group of the fatty acids that constitute Witepsol® H-15, thereby displacing more molecules of the drugs (flurbiprofen and diclofenac) for dissolution.

However, the reduced release rate calculated for both ibuprofen and naproxen might have been as a result of direct interaction of menthol with these drugs thereby making them more soluble in the wax. Thus, their leaving potential was reduced.

These interactions are expected due to variability in the structural composition of the investigated drugs. Similar studies have being reported in the literature, Mills et al (2005) have reported that menthol can increase the release kinetics of drugs in wax-based formulations. Also, Yong et al (2004) reported from a similar study, that menthol increased the release of ibuprofen; another lipophilic drug, which consequently enhanced the bioavailability of the drug. Hence, the action of menthol to enhance the release of diclofenac and flurbiprofen could be attributed to its activity as a co-solvent.

Menthol has also been reported to increase blood flow to the skin's surface (Brian et al, 2006) and this action would enhance the permeation of drugs through live skin; this mechanism of action is not mimicked in these studies.

The release rate of ibuprofen was decreased in all formulations of the drug containing menthol; this was expected as some similar findings were reported in the literature. For example, Stott et al (1998) and Yong (2004) identified that ibuprofen and menthol form a eutectic system only when drug: excipient loading ratio is 6:4, at which point thermodynamic activity is greatest. However, in all ibuprofen formulations investigated within this report, the drug: menthol-loading ratio was 1:1. This significant ($p < 0.05$) decrease in release rates for ibuprofen release rate could then be explained in the context of thermodynamic activity. As the drug solubility reaches the maximum in the matrix, the

driving force is the greatest at saturation; however, any shift to the right or left of this equilibrium state will result in a decrease in the thermodynamic activity (Williams, 2003). In future studies, the effect of varying concentration of menthol in the wax, with or without other excipients will be investigated.

In addition to these theories, another possibility to explain the effect of menthol on the release of these drugs could be the influence of the physicochemical properties of the investigated drugs. For example, a critical look at table 1.4 revealed that the pKa properties might have contributed to the effect of menthol on the release of these drugs. El-Kattan et al (2001) have shown non–polar terpenes, such as menthol, to increase the bioavailability of non–polar drugs. Hence, in decreasing release rate order; flurbiprofen > diclofenac sodium > naproxen > ibuprofen, according to their pKa values (table 1.4); however, the reduced release of naproxen might be due to its interaction with menthol as explained above, though it has a similar pKa value to sodium diclofenac. The limited release rate of ibuprofen could possibly be attributed to the reduced thermodynamic activity caused by increased solubility of the drug in the matrix.

4.4. Conclusion

It is a generally accepted concept that improving the release rate of a poorly soluble drug will not only enhance its dissolution but also its *in-vivo* performance via enhanced penetration when applied to the skin. From these investigations, it can be seen that to optimize the release of these drugs; naproxen, ibuprofen, diclofenac and flurbiprofen from a lipophilic matrix, their physicochemical properties play an important role. The important characteristics are, pKa, log P and aqueous solubility. Incorporation of commonly available excipients (the hydrophilic non-ionic surfactant Tween 20, menthol and the hydrophilic polymer Carbopol® 971) in different concentrations enhanced the drugs' dissolution in some cases while release rate values were decreased for other drugs. The effect depended on the solubility of the drugs within the wax matrix as well as how conducive the dissolution media environment was. Striking a balance between increasing the solubility of these poorly soluble drugs within a lipophilic fatty base as well as

promoting release is a challenge that was overcome by a thorough understanding of the relationship between the drugs and added excipients with the matrix.

Verheyen (2002) explained that the mechanisms involved for the increased dissolution of diazepam and temazepam from polyethylene glycol 6000 solid dispersions were solubilisation and improved wetting of the drug in the polyethylene glycol rich micro-environment formed at the surface of drug crystals after dissolution of the polymer. A similar mechanism is believed to occur via the incorporation of Tween 20 into the formulations tested within this thesis.

This study reveals that Tween 20 increased the release rate of all the drugs investigated while Carbopol® 971 only increased the dissolution of diclofenac significantly, naproxen and ibuprofen slightly but reduced the release rate of flurbiprofen.

Incorporation of Carbopol® 971 at a concentration of 1% (w/w) resulted in an increase in release rate of each of the drugs considered in the presence of Tween 20 at 1 % (w/w) but at 2 % (w/w) and in the presence of Tween 20, increase in K_H value was only observed for naproxen. In formulations where Carbopol® (at 1 % (w/w)) and wax were the only two excipients present; the release rates were significantly increased above the controls for diclofenac and naproxen to a large extent, ibuprofen only slightly and the release rate was reduced with flurbiprofen. This provided further evidence that there was interaction between Witepsol® H-15 and the investigated drugs. Specifically, the extent of the interaction as denoted by calculated solubility values (section 6.2.1) corresponded with the extent of release of the investigated drugs in the presence of Carbopol® 971. Hence, it is possible to postulate that Carbopol® 971 reduced these interactions to enhance the drugs release.

Menthol on the other hand, increased the release of diclofenac and flurbiprofen and reduced the release of ibuprofen and naproxen. This could be as a result of the direct interaction of the latter drugs with menthol which increased their solubility in the wax, thus reduced their thermodynamic activity while the former drugs interaction with the

matrix as speculated earlier, and was disturbed by menthol displacing these drugs to interact with the wax molecules. This difference was due to the physicochemical characteristics of the investigated drugs. There was a possibility that eutectic system was formed between ibuprofen and naproxen with the molecules of menthol.

The release rate of the drugs investigated was affected by the composition of the vehicles under test; the maximum release rates for each drug were found from the following formulations diclofenac in wax plus 1% Carbopol® 971P; ibuprofen in wax plus 1% Tween and 1% Carbopol® 971P; and both flurbiprofen and naproxen in wax plus 2% Tween.

Chapter Five

Effect of excipients on permeation

5.1. Introduction.

In chapter four, the effect of the added excipients on the release of ibuprofen, naproxen, diclofenac and flurbiprofen from the wax was reported.

To improve the bioavailability in topical treatments, one approach is to increase the release of drugs by manipulating vehicular composition and ultimately increases skin permeation. Hence, the formulations reported in the previous chapter were investigated for efficiency on the permeation of the selected drugs through full dorsal porcine skin.

5.2. Aims and objectives

The main aim of this study was to investigate the effect of excipients (menthol, Tween 20 and Carbopol® 971) on the skin penetration of drugs (ibuprofen, naproxen, flurbiprofen and diclofenac).

5.3. Results and Discussions

The method used in this study can be found in section 2.11.
The formulations contained 5 % (w/w) loadings of ibuprofen and naproxen and 10 % (w/w) loadings for diclofenac and flurbiprofen, as determined from the permeation studies discussed in section 3.5.4. In addition, each formulation also contained varying concentrations of the excipients (Tween 20, Carbopol ® and menthol) as shown in table 4.1. The actual drug loading present in the donor formulations was the same, however, the thermodynamic activity may have changed due to the presence of these excipients as discussed in section 4.3. Nevertheless, the effect of the various excipients on the permeation of each drug through the full thickness dorsal porcine skin was reported.

The permeability coefficient; Kp and enhancement ratio (ER) values of ibuprofen, naproxen, diclofenac and flurbiprofen were determined after the application of formulations 1 – 10 (table 4.1) of each drug to full dorsal porcine skin.
Table 5.1.a and 5.1.b below show the summary data for ibuprofen, flurbiprofen, naproxen and diclofenac for each formulation investigated. Student t-test was employed as a statistical analytical method.

	Ibuprofen			Flurbiprofen		ER
Form	Flux ($\mu g/cm^2$) ± s.d	Kp ($cm/h \times 10^{-3}$)	ER	Flux ($\mu g/cm^2$) ± s.d	Kp ($cm/h \times 10^{-3}$)	
1	41.73 ± 6.17	8.34	1.00	33.92 ± 0.93	3.39	1.00
2	43.47 ± 2.84	8.69	1.04	42.61 ± 1.48	4.26	1.25
3	38.05 ± 3.58	7.61	0.91	39.88 ± 0.74	3.98	1.17
4	41.00 ± 4.95	8.20	0.98	40.20 ± 1.24	4.02	1.18
5	39.49 ± 0.84	7.89	0.94	31.72 ± 1.37	3.17	0.93
6	30.91 ± 1.05	6.18	0.74	34.76 ± 1.77	3.47	1.02
7	33.03 ± 1.30	6.60	0.79	37.47 ± 1.44	3.74	1.10
8	40.33 ± 0.55	8.06	0.96	26.79 ± 0.59	2.67	0.78
9	37.23 ± 2.49	7.44	0.89	38.54 ± 0.80	3.85	1.13
10	41.14 ± 1.74	8.22	0.98	38.01 ± 1.20	3.80	1.11

Table 5.1a: Flux; F, permeability coefficient; Kp, and enhancement ratio; ER, values of ibuprofen and flurbiprofen through full thickness dorsal porcine skin. Data show mean ± standard deviation. N = 3

	Naproxen			Diclofenac		
Form	Flux (μg/cm^2) ± s.d	Kp (cm/h × 10^{-3})	ER	Flux (μg/cm^2) ± s.d	Kp (cm/h × 10^{-3})	ER
1	7.79 ± 0.20	1.55	1.01	56.00 ± 1.66	5.60	1.00
2	8.22 ± 0.34	1.64	1.05	14.64 ± 1.17	1.46	0.82
3	8.42 ± 0.20	1.68	1.08	23.60 ± 0.29	2.36	1.32
4	9.15 ± 0.09	1.83	1.17	97.54 ± 1.56	9.75	5.47
5	8.33 ± 0.15	1.66	1.06	59.78 ± 1.92	5.97	3.35
6	6.26 ± 0.21	1.25	0.80	41.81 ± 0.33	4.18	2.34
7	6.08 ± 0.11	1.21	0.77	123.18 ±2.64	12.31	6.91
8	6.45 ± 0.02	1.29	0.82	43.37 ± 0.94	4.33	0.77
9	7.91 ± 0.18	1.58	1.01	48.28 ± 0.99	4.82	0.86
10	7.20 ± 0.18	1.44	0.92	38.31 ± 0.46	3.83	0.68

Table 5.1b: Permeability coefficient; Kp, and enhancement ratio; ER, values of naproxen and diclofenac through full dorsal porcine skin. Data show mean ± standard deviation. N = 3

Comparing formulations 1 that had no additional excipient added, a noticeable observation from the results above was that the higher flux calculated was for diclofenac and ibuprofen as compared to flurbiprofen and naproxen respectively. This might be due to a possible interaction between the molecules of these former drugs and the wax; in which the interaction favoured increased diffusion and partitioning of these drugs into the skin compared to flurbiprofen and naproxen.

5.3.1. The effect of Tween 20 on permeation

As shown in Tables 5.1a and 5.1b above, the addition of Tween 20 at 1% (w/w) (formulation 2) compared to formulation 1 (the control), increased the permeation of only flurbiprofen while the flux for ibuprofen and naproxen were not statistically different from the control, however the flux of diclofenac was reduced in the presence of Tween 20.

A further increase in concentration of Tween 20 from 1 % w/w (formulation 2) to 2 % w/w (formulation 3) led to a reduction in flux and Kp for both ibuprofen and flurbiprofen whereas flux of naproxen was still statistically the same as for formulation 2 and the

observed flux for diclofenac was slightly increased yet still much reduced compared to the control.

The observed increase in permeation of flurbiprofen amongst the formulations investigated containing Tween 20 could be attributed to the alteration of the thermodynamics of this donor formulation as well as the solubilisation of the membrane lipids of the skin by Tween 20; a non-ionic hydrophilic surfactant (Rigg and Barry, 1990; Williams and Barry, 2004).

In the same vein, the reduced permeation observed for all the drugs could be attributed to the action of Tween 20 as a surfactant not only to aid the solubilisation of these drugs in the wax matrix to promote their released from the vehicle (as seen in table 4.1) but also to increase the solubility of these drugs in the skin, thus, reducing the thermodynamic activity and hence, their permeation (Williams and Barry, 2004).

In comparison with the release study data (table 4.1), Tween 20 led to reduced permeation (table 5.1a and b) of the drugs although it increased their release from the wax.
This study shows the enhancing effect of a non-ionic surfactant on the permeation of selected drugs.

5.3.2. The effect of Carbopol ® 971 on permeation

Formulations 9 and 10 contained 1 and 2 % w/w loadings of Carbopol ® respectively. Carbopol ® was added to increase the tablet forming ability of the wax. However, as seen in table 4.1, it promoted the release of sodium diclofenac and naproxen from the matrix via a mechanism of aided water ingress into the formulation but the dissolution of ibuprofen and flurbiprofen were not affected or reduced.

In the permeation study, the flux of ibuprofen and naproxen (formulations 9 and 10) was not statistically different (student t-test, n= 3) when compared to the control (formulation

1). This was similar to the results achieved from the release study where the release of these two drugs was slightly increased (table 4.1). The flux for flurbiprofen was improved in the presence of Carbopol® compared to the control at both concentrations whereas the flux of diclofenac was decreased at 1% (w/w) Carbopol® loading and decreased further at the 2 % (w/w) Carbopol® loading. However, for diclofenac and flurbiprofen, a reverse of the release study result was noticed (table 4.1). In fact, it was quite surprising that at 1 % w/w loading, the permeation of diclofenac was reduced when compared with the control formulation despite the highly significant release data obtained for the former (table 4.1 and 5. 1b). This shows that Carbopol® 971 actually decreased the permeation of this drug. A possible explanation could be that increased release due to swelling of the polymer in the presence of aqueous media while there was retention of the drug released within the skin. The viscosity of Carbopol® 971 will be investigated in future studies.

The increased flux observed for flurbiprofen might have been caused by the high log P value (log P= 4.07; table 1.4). However, diclofenac sodium has a lower log P value (log P = 0.70,table 1.4), the reduced flux observed despite the increased release achieved in the presence of Carbopol® 971can be attributed to higher retention the molecules of this drug in the skin. Furthermore, it was anticipated that the lowest flux would be observed for naproxen due to its log P value (log P=3.3, table 1.4).

Table 5.1.a and b further shows that increased concentration of Carbopol® from 1 % (w/w) to 2 % (w/w) loading produced no effect on the permeation of naproxen, ibuprofen and flurbiprofen when the flux of formulation 1, 9 and 10 were compared. On the other hand, the flux of diclofenac was reduced and this reduction can be attributed to reduce release of this drug from the formulation (see table 4.1).

The decrease in permeation observed with increased concentration of Carbopol® in the formulations either in the presence or absence of other excipients could be associated with the increased viscosity of the drug-matrix formulation as reported by Yahagi (1999, 2000). This will reduce the release of the drugs as seen in table 4.1, which is an important step prior to permeation. In addition, it is quite possible that Carbopol® 971 might have

increase the viscosity of formulations 9 and 10, as the concentration of Carbopol® was increased from 1 % to 2 % (w/w), when both formulations were compared with the control (formulation 1). Similar results, where a decreased in permeation was observed with an increase in viscosity, have been reported in the literature (Yahagi, 1999, 2000).

In summary, it is possible to conclude from these results that Carbopol® did not enhance the permeation of any of the drugs investigated in this study.

5.3.3. The effect of menthol on permeation

Formulation 8 contained menthol at 5%w/w and can be compared directly to formulation 1 to examine the effect of menthol on permeation. Menthol is used in transdermal formulations for many reasons; these include product appearance, its effects on the blood flow to the skin *in vivo*, its permeation enhancing properties and also as a co-solvent to improve solubility of hydrophobic agents (Williams, 2003).

From table 5.1.a and b, the flux for each of the drugs was reduced compared to the control in the presence of menthol (compare formulations 1 and 8 for all drugs). In comparison to the release studies results (table 4.1), menthol enhanced the release of diclofenac and flurbiprofen but reduced the release of ibuprofen while the release of naproxen was not affected.

The decreased flux of ibuprofen and naproxen can be justified by a reduced release of both drugs due to increased solubility in the wax while decreased permeation observed for flurbiprofen and diclofenac can be attributed to increased retention of these drugs in the formulation due to the non-polar skin. Menthol has been reported to act as a co-solvent depending on the polarity of the environment (Williams, 2003). In an aqueous environment, menthol can increase the release of drugs from a lipophilic vehicle but it can further solubilise drugs for the same vehicle in a lipophilic environment, therefore, reducing their leaving potential (Wagner et al, 1994 and Yong et al, 2004). This could be the reason for the poor permeation of ibuprofen, naproxen, flurbiprofen and diclofenac in

the presence of menthol (with or without any other excipients for the first two drugs and alone for the latter two). However, if the concentration of menthol was decreased, there is a possibility that this effect might be reduced and permeation of these drugs could be enhanced.

The action of menthol is due to ability of this excipient to interact with the molecules of the drug and possibly the vehicle. Wagner et al (1994) and Yong et al (2004) reported that menthol interacted via hydrogen bonding with ibuprofen molecules to form a eutectic system when formulated at a ratio 6:4 (ibuprofen: menthol).

Many of the permeation enhancing effects of menthol are only valid on live skin where its effects on blood flow can be utilised (Li et al, 2001). However, in this case *in vitro* experiments were conducted and this effect was therefore not monitored.

In the same vein, the enhanced permeation of flurbiprofen and diclofenac when menthol with Carbopol® 971 or Tween was present as excipients could also be attributed to the action of these excipients in either improving the release of these drugs by increasing their leaving potential via disruption of the interaction between menthol and the drugs' molecules or by directly affecting the barrier ability of the skin to permeation.

The present studies have shown that the effect of menthol in the investigated formulations depends largely on the interaction between menthol, the other excipients, the vehicle and the skin.

5.3.4. Effect of Tween 20 in combination with Carbopol® on permeation

Formulations 4 and 5 contained 1 % w/w Tween 20 with 1 and 2 % w/w loadings of Carbopol® respectively. These formulations can be compared to formulation 2 to look at the effect of the addition of Carbopol® to Tween containing formulations. For naproxen and diclofenac the presence of Carbopol® in addition to Tween 20 increased the flux; however, the opposite was true for ibuprofen and flurbiprofen. In all cases a further

increase in concentration of Carbopol® reduced the flux; this is likely to be due to decreased dissolution effects as discussed previously.

Likewise formulations 4 and 5 can be directly compared to formulations 9 and 10 where the only difference is the presence of Tween 20 at 1% in formulations 4 and 5. In all cases where Carbopol® is present at 1 % loading the presence of Tween improves the flux of these formulations. Where Carbopol® is present at 2 % loadings only flurbiprofen show a statistically significant increased flux in the absence of Tween 20 whereas ibuprofen, naproxen and diclofenac show decreased flux. The decreased flux of naproxen and diclofenac in spite of increased release rate (table 4.1) can be linked with increased retention of these drugs in the skin while reduced flux of ibuprofen was as a result of reduced release of this drug (table 4.1).

5.3.5. The effect of Tween 20 in combination with menthol on permeation

Formulation 6 contained 2 % Tween 20 and 5 % menthol and can be compared to formulation 3, which contained only 2 % w/w Tween 20, only diclofenac showed an increase in flux for the combination of Tween and menthol whereas all other drugs showed greater flux in the presence of Tween 20 alone. Formulation 8 contained menthol at 5% as the only additional excipient to the wax, this showed, slight increased flux compared to formulation 6 for naproxen and diclofenac, a large increase for ibuprofen and a decrease for flurbiprofen. The significant increase observed for ibuprofen could be associated with the effect of menthol in increasing the partitioning of the drug into the skin (both ibuprofen and menthol have similar value log P = 3.4 and 3.3 respectively, Williams, 2003). The slight increase and decrease in flux observed for diclofenac and flurbiprofen respectively, was possibly caused by high retention in the skin while that of naproxen was due to reduced release.

5.3.6. Effect of Tween 20 and Carbopol® in combination with menthol on permeation

Formulation 7 contained 1% w/w Tween 20, 1 % w/w Carbopol® 971 and 5% w/w menthol. This can be compared to formulation 4 that was identical except that menthol

was absent from this formulation. A decrease in flux was observed for ibuprofen, flurbiprofen and naproxen when menthol was present in this formulation; the opposite was observed for diclofenac. The increased flux for diclofenac was due to increased release (table 4.1) while the reduced flux observed for the other drugs was due to decreased release from the wax.

5.3.7. Preferred formulation for each drug for permeation

In conclusion, this study has shown that optimum flux from a wax-based matrix could be obtained by the preparation of formulations outlined in the table 5.2 below.

Drug	Preferred Formulation
Ibuprofen	Formulation 2: wax + 1% w/w Tween 20
Flurbiprofen	Formulation 2: wax + 1% w/w Tween 20
Naproxen	Formulation 4: wax + 1 % w/w Tween 20 + 1% w/w Carbopol®
Diclofenac	Formulation 7: wax + 1 % w/w Tween 20 + 1% w/w Carbopol® + 5% w/w menthol

Table 5.2: Table of preferred formulations of ibuprofen, flurbiprofen, naproxen and diclofenac.

Further to all the above-mentioned mechanisms by which excipients incorporated into our formulations have affected the permeation of the selected drugs, suffice it to say that the role of the vehicle to influence the flux of the drug is a vital point to mention.

5.4. Skin saturation studies

The method used for this study can be found in section 2.12.After each permeation study, the skin that was used, as a membrane was further treated as explained in section 2.12 to measure the skin level of drug.

This section details the results of the saturation studies after investigation of the samples 1 to 10 mentioned in table 5.1a and b.

The detailed statistical results are shown in the appendix. One-way anova was used to determine the difference between amount of drug left on application of formulation 1 –10

while linear regression and correlation was employed to examine the relationship between the amount that permeated the skin and the amount left in it.

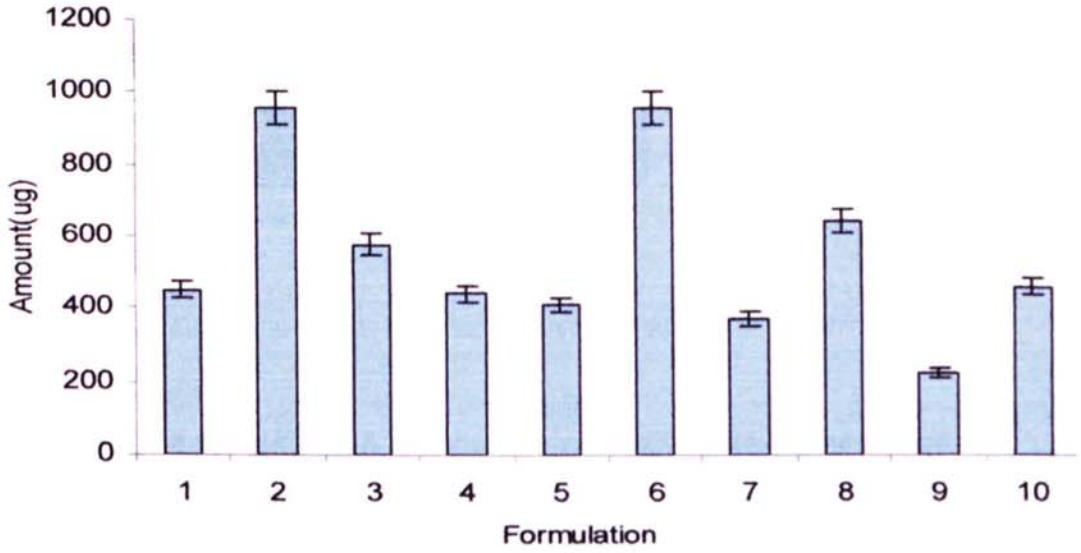


Figure 5.1: Amount of flurbiprofen left in the skin after 24 hours application of formulation 1- 10. Mean ± s.d. N = 3

The amount of flurbiprofen, which saturated the porcine skin, is shown in figure 5.1. One-way anova (see appendix) showed that the amount of drug left in the skin after 24 hours permeation study was significantly different ($p = 0.00$) from one another for each formulation, with the exception of formulations 1, 4, 5 and 7 ($p > 0.00$). The lowest amount left was from formulation 9. This could be due to reduced amount released of the drug released.

The higher amounts were left in the skin from formulations 2 and 6, both of which contains 1 % and 2 % (w/w) respective loading of Tween 20 while 5 % (w/w) menthol was also combined with the latter. These high amounts (close to 10% w/w of the total drug loading) could be linked to increased release from the drug-matrix mixture due to the effect of the added excipients. There was a limited correlation ($r = 0.05$, see appendix) between the amount left in the skin and the permeation results discussed above (see section. 5.3.1).

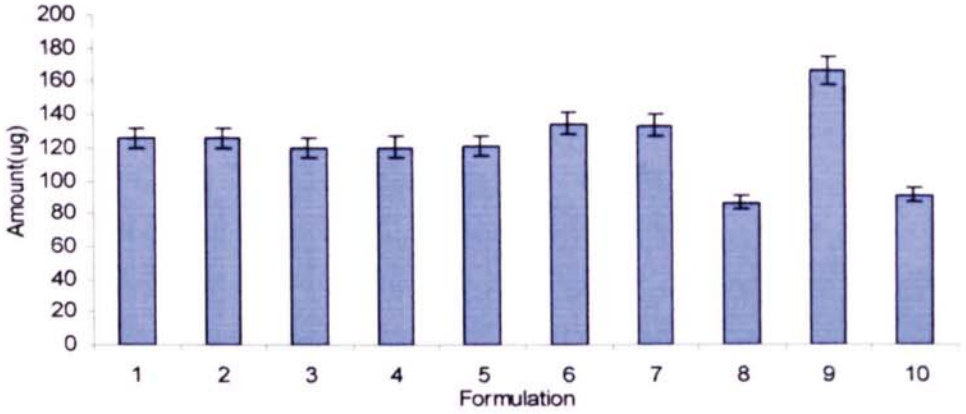


Figure 5.2: Amount of naproxen in the skin after 24 hours application of formulation 1- 10. Mean ± s.d. N = 3.

As seen in figure 5.2 and table 5.1b above, the correlation between the amount of naproxen that permeated the porcine skin and the amount that saturated after 24h hours was low ($r = 0.14$,see appendix for scattered plot). This indicates that the higher the amount of drug retained within the skin, the lesser the quantity, which permeated through this membrane. The amount of drug that saturated the skin was similar ($p > 0.00$) for the formulations investigated; with the exception of formulations 8 and 10 (both of which are comparable). Formulation 9 had higher amounts retained in the skin and this could be as a result of the increased release from the drug-matrix mixture. The reduced amount left in the skin seen for formulation 8 can be associated with the reduced amount, which permeated the skin (section 5.3.5).

Statistical analysis (one-way anova) showed that the amounts of naproxen left after application of formulation 1, 2, 3, 4, 5, 6, and 7 were not significantly different ($p > 0.00$) from one another, just as formulations 8 and 9.

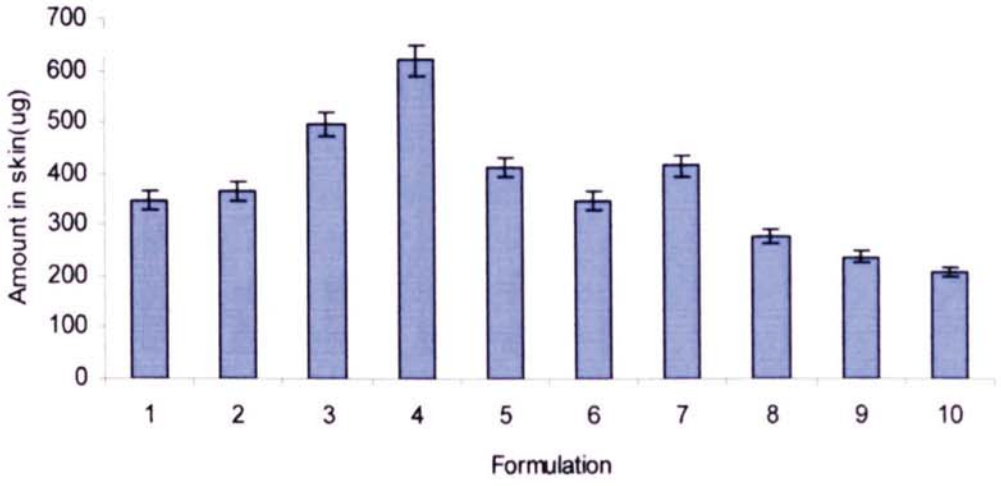


Figure 5.3: Amount of ibuprofen left in the skin after 24 hours application of formulation 1- 10. Mean ± s.d. N = 3.

From figure 5.3, the amount of ibuprofen left in the skin is not statistically different ($p > 0.00$) for formulations 1, 2, 5, 6 and 7 while formulations 8, 9 and 10 are also similar ($p > 0.00$). There was no correlation between this result and the permeation data stated in table 5.1.a above ($r = -0.00$). The highest amount left in the skin was for formulation 4. This could be attributed to increased amount released from the matrix via the action of both Tween 20 and Carbopol® as explained earlier (section 5.3.6). The reduced amount left in the skin for formulations 9 and 10 was due to decreased amount released for permeation (see table 4.1). Not only did 2 % w/w Tween 20 (formulation 4) increase the release of ibuprofen (table 4.1), this increase led to a significant amount left in the skin after permeation.

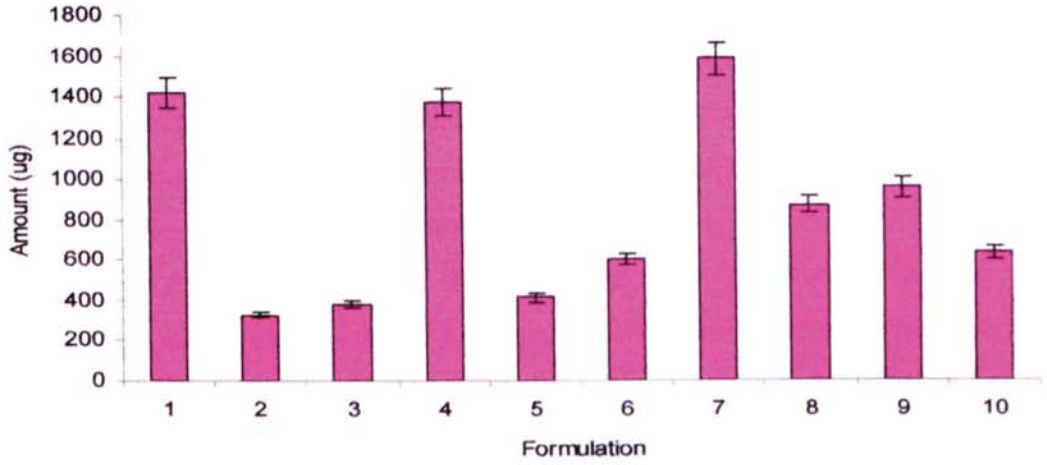


Figure 5.4: Amount of diclofenac sodium left in the skin after 24 hours application of formulation 1- 10. Mean ± s.d. N = 3.

Table 5.4 above shows that there was no difference between the amount of drug left in the skin for formulations 2, 3 and 5 ($p > 0.00$). Formulations 6 and 10 are also not different statistically while formulations 1, 4 and 7 are equally similar ($p > 0.00$) in the amount of diclofenac left in the skin after permeation study. The result reported is similar to the permeation results shown in table 5.1.b above except for formulation 5, which is quite reduced in this case. This could be associated to reduced amount of drug released due the effect of increased concentration of Carbopol® 971 in this formulation from 1 % w/w (formulation 4) to 2 % w/w (formulation 5). This data correlated well with the release results shown in table 4.1. The reduced amount released would mean reduced quantity to diffuse to the skin surface from the matrix during the permeation process.

In conclusion, from this study, the association between the amounts of drug that saturated the skin with the amount which permeated through the skin could be linked with the quantity of drug released from the drug-matrix mixture prior to the permeation as well as the presence of other factors that can influence permeation in the formulations.

5.5. Comparative permeation study of diclofenac products

For the purpose of product development, further permeation studies were carried out using the diclofenac formulation 7 (table 4.1) as a model. This formulation was then compared with the market leader, Voltarol Emulgel.

Voltarol Emulgel (Novartis) contains 1.16% diclofenac diethylammonium, which is equivalent to 1% diclofenac sodium. The other excipients present in this formulation are; diethylamine, carbomer, macrogol cetostearyl ether, cocyl caprylocaprate, isopropyl alcohol, liquid paraffin heavy, perfume creme 45, propylene glycol and distilled water (http://emc.medicines.org.uk/).

Formulation 7 contains 10% sodium diclofenac, 1% Tween, 1% Carbopol® 971 and 5% menthol.

Table 5.3 shows a summary of the calculated flux, Kp and amount of diclofenac left in the skin after 24 hours permeation studies.

5.5.1. Permeation

Formulation	Flux, J ($ug/cm^2/h$) ± s.d	Permeability coefficient, Kp(cm/h) x 10^{-3}	Mass in tissue ± s.d (µg)
Voltarol Emulgel	3.36 ± 0.12	1.68	43.21 ± 4.83
Formulation 7	33.037 ± 6.39	123.18 ± 2.64	1582.58 ± 35.59

Table 5.3: Flux, Kp and amount of drug left in the skin after 24 hours permeation study of Voltarol emulgel. Mean ± s.d. N = 3.

From table 5.3, formulation 7, chosen as the lead from previous studies (table 5.2) gave a higher flux, J and permeability coefficient value in comparison to Voltarol emulgel. The flux of diclofenac sodium from the lead formulation was about 10 times more while Kp was about 120 times greater. Although the lead formulation contained 10 % w/w of the active while 1 % w/w was present in the commercial formulation.

The ability of formulation 7 to enhance permeation of diclofenac sodium could be due to ability of Tween 20 to act as a surfactant to solubilise the skin as reported by Rigg and Barry (1990) and Williams and Barry (2004). In addition to this, effects of Carbopol® 971 in promoting the release of drugs were mentioned earlier in this thesis.

Interestingly, the amount of drug left in the skin seems to correspond well with the permeation data. For saturation studies, the amount left in the skin was approximately 9 times more for formulation 7 than Voltarol emugel.

5.5.2. Stability testing

A stability study was carried out on the lead formulation of diclofenac at 25° C (room temperature) and at 4°C (refrigeration). Although the drug was stable at 4°C (refrigeration) with protection from air, it degraded rapidly at 25°C (room temperature), particularly when unprotected from air and light. The method employed can be found in section 2.13.

Figure 5.5 shows the data obtained for stability data of the lead formulation of diclofenac. The results shows that 15 % of the drug was degraded after storage in the refrigerator for a year while almost 50 % was lost on storage at room temperature for the same duration. There was no significant change in the drug content after seven days of storage under the two conditions considered. The degradation of the drug might have being caused by the peroxidation of the wax lipids on exposure to air. However, storage of this formulation at extreme conditions may result in faster degradation of the drug.

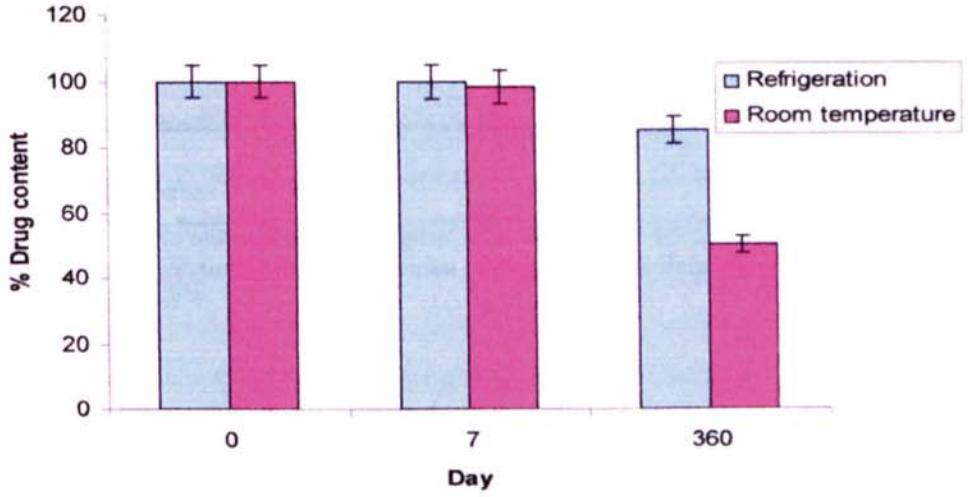


Figure 5.5. Stability data of diclofenac lead formulation (7). Mean ± s.d. N = 3.

5.6. Comparative permeation study of ibuprofen products

For the purpose of completeness, the results from the permeation studies of the lead formulations of ibuprofen were compared with the permeation data of available commercial products of the drug.

Another permeation study was carried under similar condition with 100mg of Ibugel and Ibugel Forte gels (each of which contains 5 % and 10 % w/w of the active (ibuprofen) respectively). Each also contain inactive ingredients IMS, carbomers, diethylamine and purified water (http://emc.medicines.org.uk/). These products were made by Dermal™ and bought from the local pharmacy.

Table 5.4 shows the flux and permeability coefficient of ibuprofen when the formulations prepared in the laboratory were compared with the two available commercial products mentioned above.

Sample	Formulation	Flux, J (ug/cm^2/hr)	Permeability coefficient, Kp(cm/h) x 10^{-3}	Mass in tissue (µg)
A	Ibugel	36.64 ± 2.01	7.32	135.48 ± 3.63
B	Ibugel Forte	76.54 ± 2.25	7.65	169.08 ± 0.77

Table 5.4: Comparative result of the Flux (µg/cm^2) and Kp (cm/hr x 10^{-3}) of Ibuprofen that permeated through full dorsal skin after 24 hours application of Ibugel (5%w/w ibuprofen) and Ibugel Forte (10%w/w ibuprofen). Mean ± s.d. N = 3.

From table 5.1.a and 5.4, the flux of ibuprofen from the commercial products was observed to be lower when compared with all the formulations investigated in section 5.3 with the exception of formulations 6 and 7. This was expected as both formulations produced a reduced release rate of the drug (section 4.3.1). Meanwhile, as expected the flux for Ibugel (5% w/w ibuprofen) was lower when compared with Ibugel forte (10% w/w ibuprofen). This difference could be attributed to the thermodynamic difference caused by difference in the concentration of active in both formulations. Although, 5% w/w ibuprofen was loaded into each of the formulations considered (except Ibugel Forte), the high flux derived from formulations 1- 10 (with the exception of formulations 6 and 7) can be attributed to the presence of enhancers and possibly enhancing effect of the wax matrix. Furthermore, the effect of achieving high solubility in relation to a higher thermodynamic activity for the drug via interactions between the active ingredient, the excipients and the wax base might have positive effect in promoting ibuprofen flux when compared with the commercial products.

However, amongst the formulations, Kp value calculated for Ibugel Forte was comparable and even better in some cases for some of the formulations investigated. Overall, the lead formulation (2) was more effective in delivering ibuprofen across the full dorsal porcine skin than all other products investigated.

5.7. Conclusions

In this chapter, various results have been reported that demonstrated the effects of hydrophilic polymer Carbopol® 971, Tween 20, a non-ionic surfactant and by menthol on

the permeation of each of the investigated drugs from the wax-based transdermal tablets. The increased flux observed for formulation 1 of diclofenac and ibuprofen when compared to those of flurbiprofen and naproxen might be due to a possible interaction (as described in section 3.4.3) between these former drugs and the wax that led to their partitioning into the skin.

On the one hand, a reduced flux was calculated for all formulations containing Tween 20 as excipient for each of the drug investigated. This could be due to retention of more drugs in the skin (formulation 2, figure 5.1, 5.2 and formulation 3, figure 5.3) since there was increased drug released from the matrix (section 4.4).

On the other hand, Carbopol® did not enhance permeation of drug. It could be concluded interference of Carbopol® led to a lower drug released from the wax-drug matrix (table 4.1) and produced reduced permeation as evidenced by low drug amount that saturated the skin (figure 5.1, 5.3 and 5.4).

Meanwhile, formulations containing menthol gave a reduced permeation for ibuprofen and naproxen. This was due to reduced release of both drugs (as explained in section 4.4). The decreased permeation of flurbiprofen and diclofenac formulations containing menthol could be attributed to increased retention of these drugs in the skin (formulation 6, figure 5.1 and formulation 7, figure 5.4).

However, the highest flux was only attained for each of the drugs investigated by varying the amount of excipients in the preferred formulations (table 5.2). The increased permeation observed was due to either increased drug released or the direct effect of the excipients on the skin to promote drug flux.

Ultimately, the lead formulations (for diclofenac and ibuprofen) were shown to be as effective as some of the available products on the market. Also, result obtained indicated that the formulations are better stored in refrigeration.

More importantly, it was shown that wax-based tablet could be an effective approach for the delivery of transdermal candidates.

Chapter Six

Concluding remarks and further work

6.1. Concluding remarks

The aim of the present investigations was to develop delivery systems that will contain appropriate amount of the actives (some selected non-steroidal anti-inflammatory drugs; ibuprofen, diclofenac, flurbiprofen and naproxen) and effectively deliver them through the skin. A novel delivery technology was employed.

The single most important property in the design of topical/transdermal drug delivery is the choice of vehicle of formulation that is to be used. This vehicle not only imparts cosmetic desirability but also can control the rate and extent of drug delivery to the skin, in essence the flux of the drug.

Generally the rate of delivery of drug is related to the solubility of the drug within the vehicle therefore determination of the solubility within the vehicle is crucial for effective design of novel drug delivery systems. Solubility of drugs in matrices is a vital step in product development. It not only helps in to avoid wastage of the actives once saturation is attained but it could serve as a determinant factor for comparing efficiency of the product with others.
In this thesis, attempt was made to determine the solubility of ibuprofen, naproxen, and flurbiprofen and in some cases, diclofenac sodium, in Witepsol H-15, using a range of techniques.

To enhance the permeation of these drugs, their solubility was first optimized in the wax and furthermore, chemical excipients that double as penetration enhancers were added to modify the barrier properties of the skin.

It is well known fact is that permeation across the skin is driven mainly by the thermodynamic activity of any penetrants. This factor is often taken as the effective saturated concentration of the substance in the delivery vehicle. Measuring this is problematic in semi-solids. In chapter three, microscopy, Higuchi release kinetics, saturation solution flux, Hildebrand solubility and hyper-DSC techniques were employed to investigate solubility in semi-solids. The merits and demerits of each of these techniques as a tool in determining the solubility of the selected drugs in semi-solid formulations such as Witepsol® H15 used in these investigations was also described.

A novel technique, hyper-DSC, was employed to determine the solubility of the selected drugs in the wax. The solubility of ibuprofen, flurbiprofen and naproxen was calculated to be 12.7 %, 22.1 % and 20.1% (w/w). Results from these approaches were comparable. Microscopy and Higuchi release approaches were concluded to simple and established techniques that could be used to validate results of the hyper-DSC studies. However, it was concluded that the choice of technique should be determined by the objective of the investigation and the availability of equipment. The preferred methods were suggested in this thesis.

After the solubility of these drugs have been characterised, in chapter four, the effect of menthol, Carbopol® 971 and Tween 20 on the release kinetics of the drugs was carried out. This study revealed that Tween 20 increased the release rate of all the drugs investigated while Carbopol® 971 only increased the dissolution of diclofenac significantly, naproxen and ibuprofen slightly but reduced the release rate of flurbiprofen. Menthol on the other hand, increased the release of diclofenac sodium and flurbiprofen and reduced the release of ibuprofen and naproxen. This could be as a result of the lower pKa value of the former drugs. It was concluded that proper optimisation of the release rates of a drug from the wax matrix demanded a good balance of added excipients.

The results also provided an insight into the possible interactions between the excipients, the wax and the drugs. Hence, release was increased or decreased depending on the

solubility of the drugs within the wax matrix as well as the conduciveness of the wax base environment. It was concluded that achieving a good balance between increasing the solubility of the drugs within the lipophilic fatty base as well as promoting their release entails having a thorough understanding of the relationship between the drugs, the added excipients and the wax matrix. Therefore, the added excipients should not only improve the release of the drugs in the matrix but also (if possible), they must enhance the wetting of the drugs for an enhanced release from the wax matrix.

The optimisation procedures concerned the potential use of increased thermodynamic activity of the drugs in the matrix to serve as driving force for the enhancement of penetration. The investigations showed that 5% (w/w) menthol resulted in increase in K_H (release rate) value of diclofenac, naproxen and flurbiprofen formulations (provided no other excipients were present in the latter cases) but K_H value was decreased for all ibuprofen formulations.
At 1% (w/w) loading, Carbopol® 971 enhanced the release rate of each of the drugs considered in the presence of Tween 20 at 1 % (w/w). However, at 2 % (w/w) loading, Carbopol® 971 a significant increase in K_H value was only observed for naproxen compared to other formulations.

In chapter five, various results were reported that demonstrated the effects of hydrophilic polymer Carbopol® 971, Tween 20, a non-ionic surfactant and by menthol on the permeation of each of the investigated drugs from the wax-based transdermal tablets. It was shown that permeation might be optimised in the presence or mixture of any or combination of these excipients. The results obtained further showed that there was a good correlation between the amount of drugs released with the amount of drug that penetrated the skin and the amount of drugs that was retained in the skin after permeation experiments. It was concluded that at the chosen concentrations, the preferred formulations that gave optimum release for the drugs in the presence of Tween 20, menthol and Carbopol® 971 are ibuprofen (wax + 1 % w/w Tween 20), flurbiprofen (wax + 1 % w/w Tween 20), naproxen (wax + 1% w/w Tween 20 + 1 % Carbopol®) and diclofenac (wax + 1 % w/w Tween 20 + 1 % w/w Carbopol® + 5% w/w menthol).

More importantly, it could be concluded that wax-based tablet proved to be an effective approach for the delivery of transdermal candidates. In addition, the stability results showed a very stable lead formulation over a period of one year.

The release rate, solubility and flux value for each drug was discovered to be dependent on their intrinsic physicochemical properties. Furthermore, attempt was made to use mathematical models to predict solubility. The effect of the characteristics of each drug on the parameters investigated was also discussed. It could be concluded that various factors inter-play affected these variables from the experiments conducted.

There was evidence of possible interactions between the drugs and the wax as shown by lowered melting point of drug –wax mixture, reduced or enhanced release rate and decreased or increased flux. It was concluded that in the presence or absence of the excipients, some of the selected drugs (most noticeable for ibuprofen) interacted with the wax to form eutectic mixture. Further studies will be carried out to verify this conclusion.

In addition, throughout these investigations, unit dosed tablets were prepared using simple conventional cold tableting techniques. These tablets are formulated to contain exact amount of actives and they offer potential means as topical vehicle.

The technology described in this thesis is a semi-solid, easy to melt-on the skin, unit-dose formulation manufactured by use of acceptable excipients for delivery of suitable for topical or transdermal use. The technology is water-free, stable and solid at room temperature but melts on contact with the skin. It also offers the advantage of one application at a time with ease of removal in case of adverse effects.
The matrix is commonly used as a suppository base while the excipients (menthol, Carbopol® 971 and Tween 20) are either generally recognised as safe compounds or already used in pharmaceutical formulations in the past, thus eliminating the issue of irritancy or 'allerginicity' commonly seen with some transdermal dosage forms.

The tablet is more accurate and cost effective because known amount of drugs can incorporated into each.
The manufacturing processes (cold tableting and granulation) described there in are cheap, environmentally friendly as it neither involve use of solvents nor high energy and it can be made using available tableting instrumentation.

6.2. Further work

Despite the success of these investigations, further studies need to be carried out to optimize some of the formulations via use of appropriate wax base, additional excipients to be added and furthermore, the final fate of the base is a vital study that should be conducted. For example, investigations will be carried out to see effect of lower and higher concentration of menthol and possibly other members of the terpenes series especially those with alcoholic functional groups such as nerolidol and farnesol, on the release and permeation of these drugs.

In addition, attempt will be made to select other drugs especially those that are more hydrophilic in nature. Correlation will be drawn between the results obtained from this study with the future studies. Also, excipients such as anionic and cationic surfactants should be incorporated into these formulations in varied concentrations. The use of other bases (both in the Witepsol® H15 class or other lipophilic bases) is another important study that can be carried out in the future.

The effect of other hydrophilic polymers such as the common hydroxyl methylcellulose and methylcellulose on the solubility, release and permeation of these drugs would be an imperative investigation for the future.

One vital study would be to investigate how the use of another membrane in the permeation approaches to determine solubility as reported in chapter four. There is a

possibility that similar values in comparison with the results of other techniques will be obtained.

USP-II basket method was used throughout this thesis for the dissolution experiments. It will be interesting to know how the result from this method will correlate with the use of other methods of release studies that are specifically designed for transdermal dosage forms.

During formulation preparation, attempt was made to characterise the size of the granules used for making the tablets. Reduction of particle size is a common approach for enhancing drug release. Effort should be made in future studies to investigate how well reducing the granular sizes will affect the release of these drugs and ultimately, their permeation.

Future research will be carried out on how to exploit hyper-DSC as an analytical tool to investigate solubility of a range of drugs in wax matrices especially for semi-solid formulations. Emphasis should be made on how to validate the technique. Effort will be made to investigate the effect of varying scan rates on the normalised heat of fusion used for solubility calculations.

Attempt will also be made in the future to explore the use of complex mathematical models to ascertain in details how the physicochemical properties of the drugs can influence their solubility in a semi-solid matrix. It would be interesting to be able to develop a model for prediction of solubility of drugs in any chosen combination of drug and wax matrix prior to experimental investigations.

Only five techniques were used in this thesis. Other techniques such as x-ray powder diffraction and DSC will be employed and results will be compared with the methodology used in this thesis. The pH of the wax will be investigated using appropriate instrumentation. Effort will be made to manipulate this factor, if possible to optimise the efficacy of the lead formulations.

Veterinary application of the present technology especially as spot on or rub -on product for application to pet animals is an interest that will be keenly pursued in the future. While, investigations into the *in vivo* pharmacokinetics of investigated drugs from the lead formulations for prediction of effectiveness and efficacy in paediatric populations as a rub-on product due to the easiness of melting of the wax will be a top priority of future studies.

Ultimately, it will be interesting to pursue new therapeutic areas for small and large molecules employing this technology and also incorporating other applications such as active delivery systems (micro-needles or disposable applicator devices), if possible.

The storage of this technology into acceptable and patient friendly packs is another top priority that will be considered in the near future.

To conclude, this study has presented a possible solution for topical delivery of selected non-steroidal anti-inflammatory candidates and also to solve the problems of wastage of active ingredients and safety issue commonly associated with the administration of transdermal formulations with the development of novel unit dosed tablets using established but cost effective methods and pharmaceutically acceptable excipients. These formulations have the potential to be used as future solution for effective delivery of drugs through the skin.

Appendix

pH / buffer type	Component A / (ml)	Component B /(ml)	Final volume(ml) / 18°C
Sorensen's glycine I	7.5g glycine + 5.88g NaCl in 1 litre off double distilled water	0.1 Molar HCl	100
1.8	45.70	54.30	100
2.6	70.20	29.80	100
3.4	90.30	9.70	100
Sorensen's citrate I	21g citric acid dissolve in 200ml 1molar NaOH, made up to 1 litre of double distilled water	0.1 Molar HCl	
4.0	55.80	44.20	100
Sorensen's phospate	9.0g KH_2PO_4 in 1 litre of double distilled water	11.88g $Na_2HPO_4.2H_2O$ dissolved in 1 litre of double distilled water	
5.2	98.00	2.00	100
6.0	87.70	12.30	100
7.4	19.60	80.40	100
8.0	5.50	94.50	100

Table A1: Sorensens' buffer systems (Diem, 1962).

Summary of statistical result for release rate data of ibuprofen (table 3.2)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	4	36.39	0.5	0.25
2	4	89.32	0.37	0.185
3	4	164.5	1.64	0.82
4	4	228.7	1.61	0.805
5	4	296.3	0.38	0.19
6	4	459	0.01	0.005
7	4	559.7	0.25	0.125
8	4	590	0.56	0.28
9	4	629.3	0.44	0.22
10	4	633.2	1.41	0.705

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	1.946e+06	9	2.162e+05
Within Groups	25.11	30	0.8371
Total	1.946e+06	39	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{2.162e+05}{0.8371} = 258310.00 \quad P = 0.000$$

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	36.39	0.5	0.25
2	4	89.32	0.37	0.185

Difference -52.93 0.311

95% confidence interval for difference: -53.69 to -52.17

t = -170.189 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	459	0.01	0.005
2	4	559.7	0.25	0.125

Difference -100.7 0.1251

95% confidence interval for difference: -101 to -100.4

t = -804.716 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	629.3	0.44	0.22
2	4	633.2	1.41	0.705

Difference -3.85 0.7385

95% confidence interval for difference: -5.657 to -2.043

t = -5.213 with 6 degrees of freedom; P = 0.002

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	164.5	1.64	0.82
2	4	590	0.56	0.28

Difference -425.5 0.8665

95% confidence interval for difference: -427.6 to -423.4

t = -491.063 with 6 degrees of freedom; P = 0.000

Summary of statistical result for release rate data of flurbiprofen (table 3.2)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	4	34.04	0.29	0.145
2	4	91.81	0.2	0.1
3	4	178.7	0.4	0.2
4	4	216.3	4.86	2.43
5	4	267.8	1.01	0.505
6	4	381.7	3.96	1.98
7	4	390.8	4.21	2.105
8	4	398.2	1.84	0.92
9	4	422.9	0.24	0.12
10	4	433.7	0.72	0.36

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	7.639e+05	9	8.488e+04
Within Groups	186.9	30	6.229

Total 7.641e+05 39

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{8.488e+04}{6.229} = 13626.64 \quad P = 0.000$$

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	34.04	0.29	0.145
2	4	91.81	0.2	0.1

Difference -57.77 0.1761

95% confidence interval for difference: -58.2 to -57.34

t = -327.979 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	216.3	4.86	2.43
2	4	267.8	1.01	0.505

Difference -51.54 2.482

95% confidence interval for difference: -57.61 to -45.47

t = -20.766 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	422.9	0.24	0.12
2	4	433.7	0.72	0.36

Difference -10.8 0.3795

95% confidence interval for difference: -11.73 to -9.871

t = -28.461 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	178.7	0.4	0.2
2	4	398.2	1.84	0.92

Difference -219.5 0.9415

95% confidence interval for difference: -221.8 to -217.2

t = -233.142 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	398.2	1.84	0.92
2	4	422.9	0.24	0.12

Difference -24.7 0.9278

95% confidence interval for difference: -26.97 to -22.43

t = -26.622 with 6 degrees of freedom; P = 0.000

Summary of statistical result for release rate data of naproxen (table 3.2)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	4	47.27	0.3	0.15
2	4	61.69	0.41	0.205
3	4	96.71	0.36	0.18
4	4	127.5	3.02	1.51
5	4	158.5	1.46	0.73
6	4	213.1	3.7	1.85
7	4	280.2	2.3	1.15
8	4	303.3	0.68	0.34
9	4	311.9	0.37	0.185
10	4	321.1	0.76	0.38
11	4	335	0.45	0.225

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	4.899e+05	10	4.899e+04

Within Groups	96	33	2.909
Total	4.9e+05	43	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{4.899e+04}{2.909} = 16841.21 \quad P = 0.000$$

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	47.27	0.3	0.15
2	4	61.69	0.41	0.205

Difference -14.42 0.254

95% confidence interval for difference: -15.04 to -13.8

t = -56.768 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	280.2	2.3	1.15
2	4	303.3	0.68	0.34

Difference -23.14 1.199

95% confidence interval for difference: -26.07 to -20.21

t = -19.296 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	127.5	0.37	0.185
2	4	158.5	1.46	0.73

Difference -31 0.7531

95% confidence interval for difference: -32.84 to -29.16

t = -41.164 with 6 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	4	321.1	0.76	0.38
2	4	335	0.45	0.225

Difference -13.88 0.4416

95% confidence interval for difference: -14.96 to -12.8

t = -31.430 with 6 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	4	96.71	0.36	0.18
2	4	311.9	0.37	0.185

Difference -215.2 0.2581

95% confidence interval for difference: -215.8 to -214.5

t = -833.649 with 6 degrees of freedom; P = 0.000

Numerical data for enthalpy of fusion and variability of melting points of ibuprofen from hyper-DSC studies (see section 3.4.3)

Ibuprofen

	ΔHf(J/g)	Onset(°C)
	218.76	75.42
	220.586	73.48
	221.42	74.47
Ave	220.255333	74.45667
stdev	1.36047982	0.970069

Solubility determination(Ibuprofen)

5%	10%	20%	25%	30%	35%	40%	50%
0	0	0	0	16.15	19.26	22.85	31.35
0	0	0	0	15.8	19.35	21.46	35.37
0	0	0	0	15.27	17.24	25.43	31.52
0	0	0	0	14.94	22.9	18.27	34.16
0	0	0	0	12.98	23.55	30.51	32.94

Ave(J/g)	0	0	0	0	15.028	20.46	23.704	33.068
stdev	0	0	0	0	1.236475	2.671245028	4.59874766	1.721618

Numerical data for enthalpy of fusion of flurbiprofen from hyper-DSC studies (see section 3.4.3)

	Solubility determination (Heat of fusion J/g)									
Conc(%w/w)	0.50%	1%	2%	10%	20%	25%	30%	35%	40%	45%
	0	0	0	0	0	4.55	14.81	16.08	24.49	27.86
	0	0	0	0	0	4.17	15.73	16.63	24.59	28.86
	0	0	0	0	0	4.31	13.16	16.7	25.08	28.24
	0	0	0	0	0	4.16	13.11	16.54	24.9	30.19
	0	0	0	0	0	4.22	13.16	16.63	25.16	28.26
Ave(J/g)	0	0	0	0	0	4.28	13.99	16.52	24.84	28.68
Stdev	0	0	0	0	0	0.16	1.21	0.25	0.295	0.916

Numerical data for enthalpy of fusion of naproxen from hyper-DSC studies (see section 3.4.3)

	Naproxen					
Conc(%w/w)	▲H (J/g)			Ave	stdev	
1	0	0	0	0	0	0
2	0	0	0	0	0	0
5	0	0	0	0	0	0
10	0	0	0	0	0	0
20	0	0	0	0	0	0
30	13.75	17.25	17.19	16.42	16.153	1.65
35	21.51	24.35	24.18	21.56	22.9	1.58
40	29.4	29.96	30.12	30.42	29.975	0.43
45	45.68	44.42	44.74	43.28	44.53	0.99

Summary of statistical result for release rate data of diclofenac (table 4.1)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	3	31.70	2.20	1.27
2	3	41.30	0.40	0.2309
3	3	65.20	2.30	1.328
4	3	86.10	1.70	0.9815
5	3	53.80	0.60	0.3464
6	3	382.60	4.10	2.367
7	3	468.30	7.50	4.33
8	3	641.80	3.84	2.217
9	3	649.00	0.76	0.4388
10	3	326.70	4.61	2.662

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	1.705e+06	9	1.895e+05
Within Groups	246.4	20	12.32
Total	1.706e+06	29	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{1.895e+05}{12.32} = 15382.50 \quad P = 0.000$$

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	31.7	2.2	1.27
2	3	41.3	0.4	0.2309

Difference -9.6 1.291

95% confidence interval for difference: -13.18 to -6.016

t = -7.436 with 4 degrees of freedom; P = 0.002

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	641.8	3.84	2.217
2	3	649	0.76	0.4388

Difference -7.2 2.26

95% confidence interval for difference: -13.47 to -0.9251

t = -3.186 with 4 degrees of freedom; P = 0.033

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	86.1	1.7	0.9815
2	3	53.8	0.6	0.3464

Difference 32.3 1.041

95% confidence interval for difference: 29.41 to 35.19

t = 31.033 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	41.3	0.4	0.2309
2	3	65.2	2.3	1.328

Difference -23.9 1.348

95% confidence interval for difference: -27.64 to -20.16

t = -17.732 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	649	0.76	0.4388
2	3	326.7	4.61	2.662

Difference 322.3 2.698

95% confidence interval for difference: 314.8 to 329.8

t = 119.484 with 4 degrees of freedom; P = 0.000

Summary of statistical result for release rate data of ibuprofen (table 4.1)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	3	57.19	0.42	0.2425
2	3	5.64	0.51	0.2944
3	3	66.52	0.03	0.01732
4	3	69.2	0.26	0.1501
5	3	67.81	0.53	0.306
6	3	59.34	0.83	0.4792
7	3	59.1	0.29	0.1674
8	3	53.75	0.46	0.2656
9	3	58.54	0.01	0.005774
10	3	52.19	0.18	0.1039

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	8992	9	999.1
Within Groups	3.606	20	0.1803
Total	8995	29	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{999.1}{0.1803} = 5541.20 \quad P = 0.000$$

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	57.19	0.42	0.2425
2	3	58.64	0.51	0.2944

Difference -1.45 0.3814

95% confidence interval for difference: -2.509 to -0.3909

t = -3.801 with 4 degrees of freedom; P = 0.019

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	66.52	0.34	0.1963
2	3	69.2	0.26	0.1501

Difference -2.68 0.2471

95% confidence interval for difference: -3.366 to -1.994

t = -10.845 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	67.81	0.53	0.306
2	3	59.34	0.83	0.4792
Difference		8.47		0.5686

95% confidence interval for difference: 6.891 to 10.05

t = 14.897 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	69.2	0.26	0.1501
2	3	67.81	0.53	0.306
Difference		1.39		0.3408

95% confidence interval for difference: 0.4437 to 2.336
t = 4.078 with 4 degrees of freedom; P = 0.015

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	59.34	0.83	0.4792
2	3	59.1	0.29	0.1674
Difference		0.24		0.5076

95% confidence interval for difference: -1.169 to 1.649

t = 0.473 with 4 degrees of freedom; P = 0.661

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	53.75	0.46	0.2656
2	3	58.54	0.01	0.005774
Difference		-4.79		0.2656

95% confidence interval for difference: -5.528 to -4.052

t = -18.032 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	58.54	0.01	0.005774
2	3	52.19	0.18	0.1039

Difference 6.35 0.1041

95% confidence interval for difference: 6.061 to 6.639

t = 61.009 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	58.54	0.01	0.005774
2	3	57.19	0.42	0.2425

Difference 1.35 0.2426

95% confidence interval for difference: 0.6766 to 2.023
t = 5.566 with 4 degrees of freedom; P = 0.005

Summary of statistical result for release rate data of flurbiprofen (table 4.1)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	307.4	2.4	1.386
3	3	350.3	2.91	1.68
4	3	345.3	2.38	1.374
5	3	330.2	3.19	1.842
6	3	288.9	2.68	1.547
7	3	268	2.32	1.339
8	3	315.6	3.61	2.084
9	3	245.1	1.22	0.7044
10	3	247.7	1.48	0.8545

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	4.449e+04	9	4944
Within Groups	121.4	20	6.069

Total 4.461e+04 29

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{4944}{6.069} = 814.56 \quad P = 0.000$$

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	307.4	2.4	1.386
Difference		-56.32		1.539

95% confidence interval for difference: -60.59 to -52.05

t = -36.595 with 4 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	350.3	2.91	1.68
2	3	345.3	2.38	1.374
Difference		4.95		2.17

95% confidence interval for difference: -1.076 to 10.98

t = 2.281 with 4 degrees of freedom; P = 0.085

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	330.2	3.19	1.842
2	3	288.9	2.68	1.547
Difference		41.37		2.405

95% confidence interval for difference: 34.69 to 48.05

t = 17.198 with 4 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	245.1	1.22	0.7044
2	3	247.7	1.48	0.8545

Difference -2.68 1.107

95% confidence interval for difference: -5.755 to 0.3946

t = -2.420 with 4 degrees of freedom; P = 0.073

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	268	2.32	1.339
2	3	315.6	3.61	2.084

Difference -47.62 2.478

95% confidence interval for difference: -54.5 to -40.74

t = -19.221 with 4 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	247.7	1.48	0.8545

Difference 3.37 1.086

95% confidence interval for difference: 0.3557 to 6.384

t = 3.104 with 4 degrees of freedom; P = 0.036

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	245.1	1.22	0.7044

Difference 6.05 0.9719

95% confidence interval for difference: 3.351 to 8.749

t = 6.225 with 4 degrees of freedom; P = 0.003

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	288.9	2.68	1.547

Difference -37.75 1.686

95% confidence interval for difference: -42.43 to -33.07

t = -22.390 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	268	2.32	1.339

Difference -16.87 1.498

95% confidence interval for difference: -21.03 to -12.71

t = -11.265 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	245.1	1.22	0.7044

Difference 6.05 0.9719

95% confidence interval for difference: 3.351 to 8.749

t = 6.225 with 4 degrees of freedom; P = 0.003

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	251.1	1.16	0.6697
2	3	247.7	1.48	0.8545

Difference 3.37 1.086

95% confidence interval for difference: 0.3557 to 6.384

t = 3.104 with 4 degrees of freedom; P = 0.036

--- Linear Regression and Correlation ---

n: 10
Slope: -6.011e-06
y Int: 38.64
SE Slope: 0.01145
SE Int: 4.473
SE Est: 4.211
r: -0.0001856
t: -0.0005251
DF: 8
P: 0.9996

Summary of statistical result for release rate data of naproxen (table 4.1)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	3	30.2	0.79	0.4561
2	3	33.45	0.31	0.179
3	3	45.25	1.07	0.6178
4	3	35.43	0.71	0.4099
5	3	37.76	0.62	0.358
6	3	31.75	0.66	0.3811
7	3	33.13	0.74	0.4272
8	3	29.89	0.75	0.433
9	3	34.29	0.55	0.3175
10	3	34.39	0.53	0.306

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	532.1	9	59.12
Within Groups	9.765	20	0.4883
Total	541.9	29	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{59.12}{0.4883} = 121.08 \quad P = 0.000$$

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	30.2	0.79	0.4561
2	3	33.45	0.31	0.179

Difference -3.25 0.49

95% confidence interval for difference: -4.61 to -1.89

t = -6.633 with 4 degrees of freedom; P = 0.003

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	45.25	1.07	0.6178
2	3	35.43	0.71	0.4099

Difference 9.82 0.7414

95% confidence interval for difference: 7.762 to 11.88

t = 13.245 with 4 degrees of freedom; P = 0.000

--- t-test ---

Grp	N	Mean	Std Dev	SEM
1	3	37.76	0.62	0.358
2	3	31.75	0.66	0.3811

Difference 6.01 0.5228

95% confidence interval for difference: 4.558 to 7.462

t = 11.496 with 4 degrees of freedom; P = 0.000

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	33.13	0.74	0.4272
2	3	29.89	0.75	0.433

Difference 3.24 0.6083

95% confidence interval for difference: 1.551 to 4.929

t = 5.326 with 4 degrees of freedom; P = 0.006

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	34.29	0.55	0.3175
2	3	34.39	0.53	0.306

Difference -0.1 0.441

95% confidence interval for difference: -1.324 to 1.124

t = -0.227 with 4 degrees of freedom; P = 0.832

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	30.2	0.79	0.4561
2	3	34.29	0.55	0.3175

Difference -4.09 0.5558

95% confidence interval for difference: -5.633 to -2.547

t = -7.359 with 4 degrees of freedom; P = 0.002

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	30.2	0.79	0.4561
2	3	31.75	0.66	0.3811

Difference -1.55 0.5943

95% confidence interval for difference: -3.2 to 0.1001

t = -2.608 with 4 degrees of freedom; P = 0.060

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	30.2	0.79	0.4561
2	3	33.13	0.74	0.4272

Difference -2.93 0.625

95% confidence interval for difference: -4.665 to -1.195

t = -4.688 with 4 degrees of freedom; P = 0.009

--- t-test ---

Group	N	Mean	Std Dev	SEM
1	3	30.2	0.79	0.4561
2	3	29.89	0.75	0.433
Difference		0.31		0.6289

95% confidence interval for difference: -1.436 to 2.056

t = 0.493 with 4 degrees of freedom; P = 0.648

--- Linear Regression and Correlation ---

n: 10
Slope: 0.02125
y Int: 21.24
SE Slope: 0.05117
SE Int: 6.364
SE Est: 3.431
r: 0.1452
t: 0.4152
DF: 8
P: 0.6889

Summary of statistical result for saturation studies of flurbiprofen (figure 5.1)

--- ANOVA - Analysis of Variance ---

Grp	N	Mean	Std Dev	SEM
1	3	449.4	19.9	11.49
2	3	954.8	12.09	6.98
3	3	575.4	9.61	5.548
4	3	439.8	3.07	1.772
5	3	408.2	4.8	2.771
6	3	956.4	5.18	2.991
7	3	368.4	3.15	1.819
8	3	640.6	9.02	5.208
9	3	223.9	19.29	11.14
10	3	455.7	6.71	3.874

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	1.585e+06	9	1.761e+05
Within Groups	2404	20	120.2
Total	1.588e+06	29	

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{1.761e+05}{120.2} = 1465.01 \quad P = 0.000$$

--- Linear Regression and Correlation ---

n: 10
Slope: 0.0009749
y Int: 35.86
SE Slope: 0.00681
SE Int: 4.042
SE Est: 4.95
r: 0.05055
t: 0.1432
DF: 8
P: 0.8897

Summary of statistical result for saturation studies of ibuprofen (figure 5.3)

--- ANOVA - Analysis of Variance ---

Group	N	Mean	Std Dev	SEM
1	3	347	6.46	3.73
2	3	364.3	9.07	5.237
3	3	496.4	12.78	7.379
4	3	620.3	3.66	2.113
5	3	412.4	19.57	11.3
6	3	347.1	11.11	6.414
7	3	416.7	3.41	1.969
8	3	279.7	4.17	2.408
9	3	237.6	10.45	6.033
10	3	208.8	15.41	8.897

Source of Variation	SS	DF	Variance Est (MS)
Between Groups	4.058e+05	9	4.509e+04
Within Groups	2366	20	118.3

Total 4.082e+05 29

$$F = \frac{s2\_bet}{s2\_wit} = \frac{MSbet}{MSwit} = \frac{4.509e+04}{118.3} = 381.20 \quad P = 0.000$$

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