A Thesis

entitled

Design, Optimization and Evaluation of a Novel Emulgel of Ibuprofen for Enhanced Skin Delivery using Formulating for Efficacy™ software

by

Aastha Chadha

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Master of Science Degree in Pharmaceutical Sciences

Industrial Pharmacy

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May, 2018

An Abstract of

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Development of novel formulations present unique challenges in selecting the optimum formulation type for a given active ingredient. The topical route has long been used for delivering drugs directly to the affected target site through the skin. Current approaches in design and optimization of topical formulations necessitate extensive decisions in choosing the right components for the formulation to achieve high safety, clinical efficacy, and patient compliance. It is a resource-intensive process, which involves a certain degree of hit-and-trial on the part of the formulator. Solubility of the active ingredient in the vehicle and the skin is an important parameter in selecting and optimizing vehicle components for topical formulation development. The Formulating for Efficacy™ (FFE) software presents a new approach to design topical formulations aimed at selecting the components, which work synergistically to drive the active ingredient past the skin barrier while achieving sufficient solubility in the vehicle. The science behind the FFE software involves the use of Hansen Solubility Parameters (HSPs) of the active ingredient, the vehicle components and the skin to understand the interactions, which determine the solubility of active ingredients in the vehicle and skin. This research project was aimed at

developing an emulgel of ibuprofen using FFE for the design and optimization of the oil phase of the emulgel. Conventionally, ibuprofen is available in the form of gels in most European countries. However, emulgels have been found to be better suited for delivering hydrophobic molecules with an added advantage of sustained release, which is necessary in the management of chronic musculoskeletal conditions. In this study, three emulgel formulations of ibuprofen (5% w/w) were developed and characterized. These emulgels differed in composition of the oil phase with regards to the use of penetration enhancers. Furthermore, the emulgels were compared with a commercially available ibuprofen gel, *AlgoFlex Dolo*™ (AGFD) for viscosity, pH of the formulation, spreadability, *in vitro* drug release and *in vitro* drug permeation. All three emulgels were found to have a lower viscosity and greater spreadability than the commercial product, which may translate to better patient acceptability and compliance. The pH of the emulgels and the marketed formulation was found to be close to that of the human skin. All three emulgels exhibited higher drug release and permeation than AGFD when tested using Franz diffusion cells. Additionally, it was found that the emulgels showed a sustained delivery over a longer period of time, while AGFD exhibited a relatively low lag time and lower permeability across the Strat-M® membrane in the *in vitro* permeation study carried out using Franz diffusion cells. In summary, the results of this research establish a new and useful approach in designing the oil phase of a formulation where the active ingredient needs to be dissolved in the oil phase. Additionally, the use of emulgels for topical delivery of hydrophobic drugs has been found to be better at achieving a higher drug concentration over a longer period of time across the skin barrier as compared to gels.

Dedicated to my parents, Meena and Inder Vir Chadha - The pillars of my life

Acknowledgements

I would like to acknowledge the faculty at University of Toledo for sharing knowledge, providing feedback and motivation throughout my stint as a graduate student. I would like to thank Dr. Gabriella Baki, Dr. Caren L. Steinmiller, Dr. Sai Hanuman Sagar Boddu, Dr. Jerry Nesamony, Dr. Amit Tiwari and Dr. Dragan Isailovic for imparting knowledge and providing subject-expertise, which made this work of research possible. I would also like to thank the College of Graduate Studies for the continued financial support throughout my graduate studies.

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List of Abbreviations

AA.....Arachidonic Acid
ACN.....Acetonitrile
AGFD.....AlgoFlex Dolo™
ANOVA.....Analysis of Variance

BCS.....Biopharmaceutical Classification System

COX.....Cyclooxygenase
COX-1.....Cyclooxygenase 1
COX-2.....Cyclooxygenase 2

DCMS.....Decylmethylsulfoxide
DGME.....Diethylene Glycol Monoethyl Ether
DMAC.....Dimethylacetamide
DMF.....Dimethylformamide
DMI.....Dimethyl Isosorbide
DMSO.....Dimethylsulfoxide

EP.....European Pharmacopoeia

FAs.....Fatty Acids
FDA.....Food and Drug Administration
FFE.....Formulating for Efficacy™

GI.....Gastrointestinal

HPLC.....High-Performance Liquid Chromatography
HPMC.....Hydroxypropyl Methylcellulose
HSPs.....Hansen Solubility Parameters

IPM.....Isopropyl Myristate

JP.....Japanese Pharmacopoeia

KPS 60.....Kolliphor™ Polysorbate 60

LFN.....LexFeel Natural™

LOD .....Limit of Detection
LOQ .....Limit of Quantitation
LPS.....Lipopolysaccharide
LTs .....Leukotrienes

MHRA.....Medicines and Healthcare products Regulatory Agency
MWCO.....Molecular Weight Cut-Off

NMF .....Natural Moisturizing Factor
NSAIDs.....Non-Steroidal Anti-Inflammatory Drugs

OSAL .....Octyl Salicylate
OTC.....Over-the-Counter
O/W.....Oil-in-Water

PCA.....Pyrrolidone Carboxylic Acid
PGs.....Prostaglandins
PGD_2 .....Prostaglandin D_2
PGE_2.....Prostaglandin E_2
$PGF_{2\alpha}$ .....Prostaglandin $F_{2\alpha}$
PGG_2 .....Prostaglandin G_2
PGH_2 .....Prostaglandin H_2
PGI_2.....Prostaglandin I_2
PVP .....Polyvinyl Pyrrolidone

RSD.....Relative Standard Deviation

SC.....Stratum Corneum
SG .....Stratum Granulosum
SMILES .....Simplified Molecular Input Line Entry System

TC .....Target Concentration
TDDS .....Transdermal Drug Delivery Systems
TEWL .....Transepidermal Water Loss
Tx .....Thromboxane
TxA_2.....Thromboxane A_2

USP .....United States Pharmacopeia
UV.....Ultraviolet

W/O.....Water-in-oil
w/w.....Weight by weight or weight percent

List of Symbols

% .....Percent
°C .....Degree Celsius
® .....Registered
™.....Trademark

α_v .....Thermodynamic activity of drug in vehicle
δ.....Solubility parameter
δD.....Dispersion parameter
δH.....Hydrogen-bonding parameter
δP .....Polar parameter
η .....Viscosity
γ_s.....Activity coefficient of the drug in the skin
γ_v .....Activity coefficient of the drug in the vehicle
µL.....Microliter
µm .....Micrometer

A.....Cross-sectional area
C_{donor}.....Concentration of drug molecules in the donor chamber
cm.....Centimeter
$C_{receptor}$ .....Concentration of drug molecules in the receptor chamber
C_v.....Concentration of the drug substance in the vehicle
$C_{v(solubilized)}$ .....Concentration of the "solubilized" drug substance in the vehicle
D.....Diffusional coefficient
Da.....Dalton
g.....Gram(s)
hr .....Hour(s)
J .....Flux
J_{ss}.....Steady-state flux
K_m.....Partition coefficient
K_p .....Permeability coefficient
L .....Length of diffusion pathway
Log P.....Logarithm of partition coefficient
m .....Mass
mg .....Milligram
min .....Minute(s)
mL.....Milliliter
mm .....Millimeter
MPa.....Megapascal
n.....Number of replicates
nm .....Nanometer

N_0 .....Avogadro's number
Pa.....Pascal
P_{app} .....Apparent permeability coefficient
pH.....Negative logarithm of hydrogen ion concentration
pK_α .....Negative logarithm of ionization constant
pps.....Points per second
R_a.....HSP distance
rpm.....Rotations per minute
s.....Seconds
t .....Time
T .....Absolute temperature
T_{lag}.....Lag time
x.....Times (magnification)

Chapter 1

Introduction

1.1 Skin as a barrier

Skin, the largest organ in the human body, serves as a physical barrier between the body and the surrounding environment. It also poses as a first line of defense against pathogens, prevents loss of water and impedes the entry of chemicals by functioning as a barrier [1]. In addition to serving as a sensory organ, it helps regulate body temperature, provide immunity, protect against ultraviolet rays and synthesize vitamin D. The two main structural layers of the skin are the epidermis and dermis. The epidermis consists of five strata: corneum, lucidum, granulosum, spinosum and basale [2]. The dermis consists of layers of collagen fibers, elastic fibers, blood and lymph vessels, soft connective tissue and nerve endings [3].

The barrier function of the skin is primarily provided by the stratum corneum (SC) of the epidermis. Keratinocytes, melanocytes, and Langerhans cells are the three types of cells found in the epidermis. Amongst these, the keratinocytes, which originate in the basal layer, are the predominant cells in the epidermis. These cells migrate to the stratum granulosum (SG) and are transformed into corneocytes, which essentially play the role of "bricks" in the brick and mortar model of the SC. The SG, which is only few cells thick,

plays an essential function in the formation of cells, which serve as a barrier [1]. The transformation of keratinocytes to corneocytes occurs in the SG layer by a process called cornification. Cornification is a programmed cell death, which results in enucleation of the keratinocytes followed by disappearance of cytoplasm and release of lipids into the intercellular space [4]. The keratin intermediate filaments organize to form microfibrils and transform into a complex scaffold serving as a tight mechanical barrier. The water repellant barrier is formed by the lipids present in the intercellular space, which stack against each other, and provide the "mortar" in the brick and mortar model of the SC [1, 4].

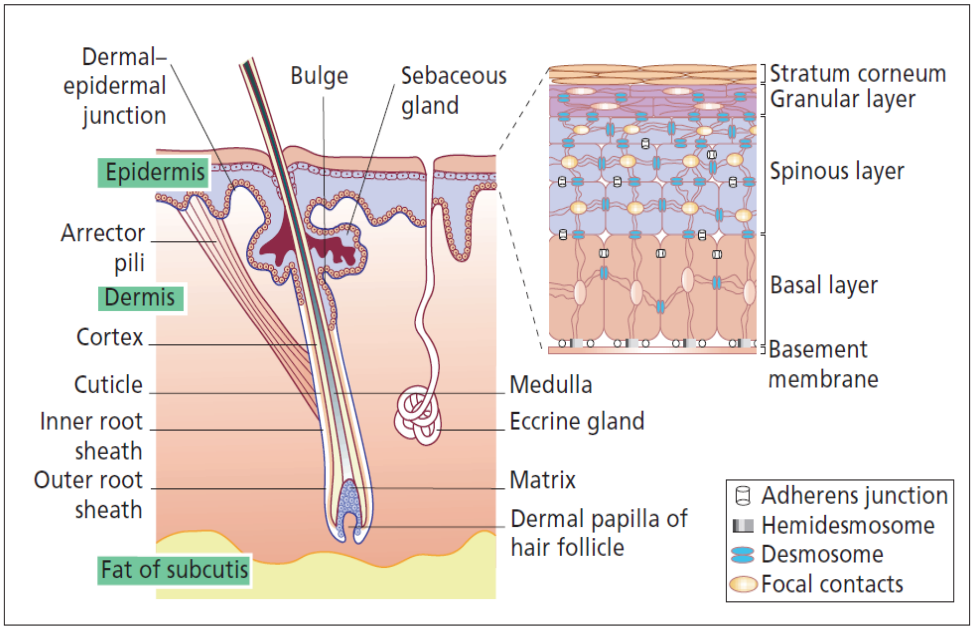


Fig. 1-1 The skin and its appendages [5]

The "brick and mortar" mosaic of corneocytes embedded in lipid-rich extracellular environment composed of ceramides, free fatty acids and cholesterol is the basis of the barrier function of the skin. This barrier functions not only to restrict the transepidermal

water loss (TEWL), but also impedes the entry of certain chemicals or penetrants [6]. A full understanding of the penetration barrier function of the SC is imperative to drug delivery via the topical route.

1.2 The bricks and mortar model of SC barrier

The bricks are composed of corneocytes along with the keratin intermediate filaments, which form the structural proteins of skin, hairs and nails. The acidic type I keratin, which is negatively charged, interacts with neutral to basic type II keratin possessing positively charged amino acids. The two types of keratin are responsible for forming a *coiled-coil* structure, which helps maintain the integrity of the keratinocytes in the basal cell layer. During cornification, the *coiled-coils* aggregate to form microfibrils, which lie parallel to the surface of skin. These microfibril structures strengthen the corneocytes and limit the SC swelling by stratification [1].

The two types of granules formed at the SG are called keratohyalin granules and lamellar bodies. The keratohyalin granules consist of structural proteins, filaggrin and loricrin, which help in the formation of the "bricks". The filaggrin helps in the formation of the keratin coiled-coils and gets digested by proteolytic enzymes during the process of cornification. This digestion results in the release of amino acid components of the natural moisturizing factor (NMF), which helps maintain the hydration of the SC. NMF is composed of lactates, pyrollidone carboxylic acid (PCA) and amino acids from the filaggrin digestion [1, 5, 7].

The phospholipid bilayer of the cell membrane of keratinocytes gets transformed into a resistant cell envelope of corneocytes during the cornification process. The cross-

linking of proteins, involucrin and loricrin, by the enzyme transglutaminase forms the basis of the cell envelope [8]. Keratin filaments, which are also cross-linked to the envelope, and lipids (ceramides), bound to involucrin protein, now form the resistant cell envelope which is impermeable to water [9].

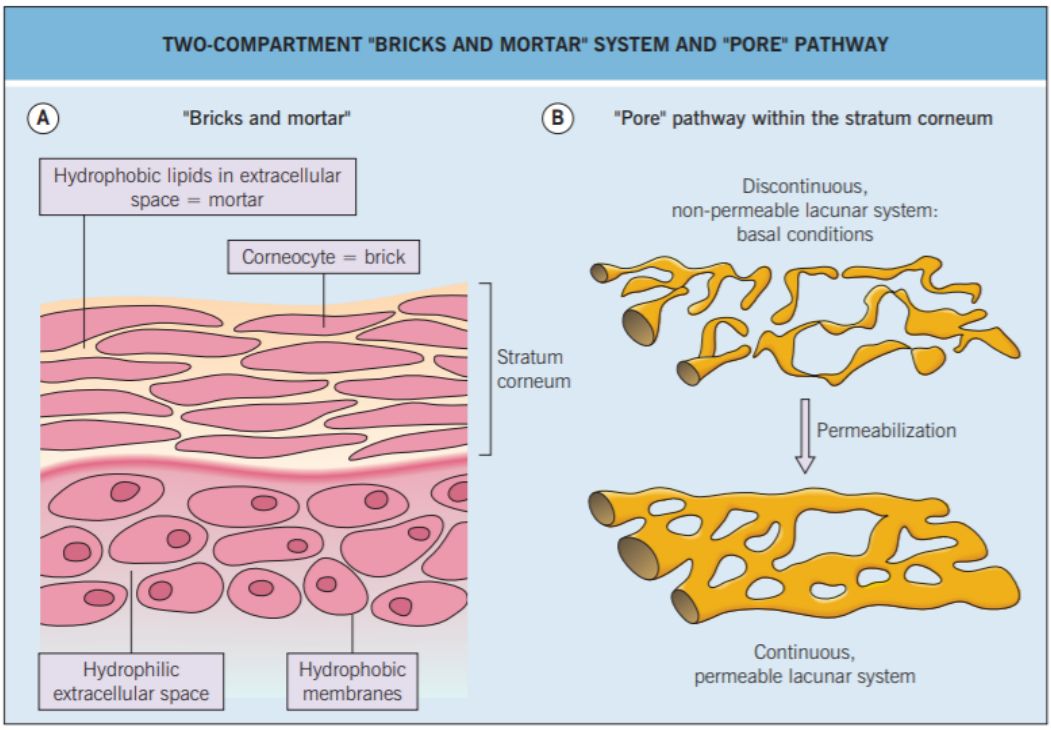


Fig. 1-2 A. “Brick and mortar” model and B. “pore” pathway. Permeabilization under conditions of occlusion, prolonged hydration, sonophoresis leads to rearrangement of the lacunae to form relatively permeable domains [10]

Desmosomes, the primary cellular junctions of the epidermis, are composed of glycoproteins. During the transformation of keratinocytes to corneocytes, these cellular junctions are modified by the addition of *corneodesmosine* protein [11]. The desmosomes of the SC are thus called corneodesmosomes. During desquamation (i.e., cell-shedding),

corneodesmosomes are digested by the proteolytic enzymes by the time the cells reach the skin surface [12] The degradation of the corneodesmosomes results in the formation of discontinuous lacunar domains, which form the “pore” pathway. These pathways also pose as a permeability barrier to certain topically applied drugs[10].

The intercellular lipids in the SC, which provide water resistance to the SC, are a critically important part of the overall barrier layer of skin [13]. These lipids are composed of long-chain fatty acids, glucosyl ceramides, cholesterol and cholesterol esters. The glucosyl ceramides form ceramides during the cornification process [14]. Ceramides are sphingolipids linked to long-chain fatty acids and serve an important function in the organization of the lipids in the SC [13].

The pH of the skin surface is approximately 4-5.5 and it is primarily due to the fatty acids present in the acid-mantle of the skin [15]. In addition to the fatty acids, other processes, like surface deposits of eccrine gland and sebaceous gland products and local generation of protons within the lower SC layers also play a role in the acidification of skin surface [10]. The pH of the skin not only plays a role in protection against bacterial infections, [16] but is also critical in maintaining the permeability barrier [10].

The dynamic “brick and mortar” serving as a barrier shows remarkable properties to restrict the entry of most topically applied drugs, except those that are lipid-soluble and of low-molecular weight [10]. The extracellular lipid-rich matrix serves as a “reservoir” for lipid-soluble drugs, such as topical corticosteroids, which accumulate and are released slowly into the systemic circulation through the dermis [17].

1.3 Routes of drug penetration into the skin

Drugs applied onto the skin can enter through different routes of penetration. Drugs enter either via SC (transepidermal route) or the appendages (transappendageal route). The transappendageal route, also called the shunt route, as it circumvents the SC cells, consists of a drug transport via the eccrine glands and pilosebaceous unit (i.e., hair follicles with their associated sebaceous glands). As appendages cover less than 0.1% of total surface area of skin, it is hypothesized that the transappendageal route is not the predominant route of penetration [18, 19]. However, transient diffusion upon application of a drug occurs mainly through the appendageal route rather than the transepidermal route [20]. The eccrine sweat glands produce hydrophilic by-products, while the follicles produce hydrophobic by-products due to the presence of sebum. These secretions also affect the water content of the SC, thereby altering the percutaneous absorption [21]. Additionally, sweat ducts and hair follicles are surrounded by a capillary network, which facilitates the transport of molecules directly into the systemic circulation [18]. The hydrophobic drugs tend to accumulate in the sebaceous units and this “pool” serves to release the drug into the systemic circulation [19]. Similarly, hair follicles also act as long-term reservoirs for drugs or drug carriers, which range within the size limits of the follicle aperture [22, 23].

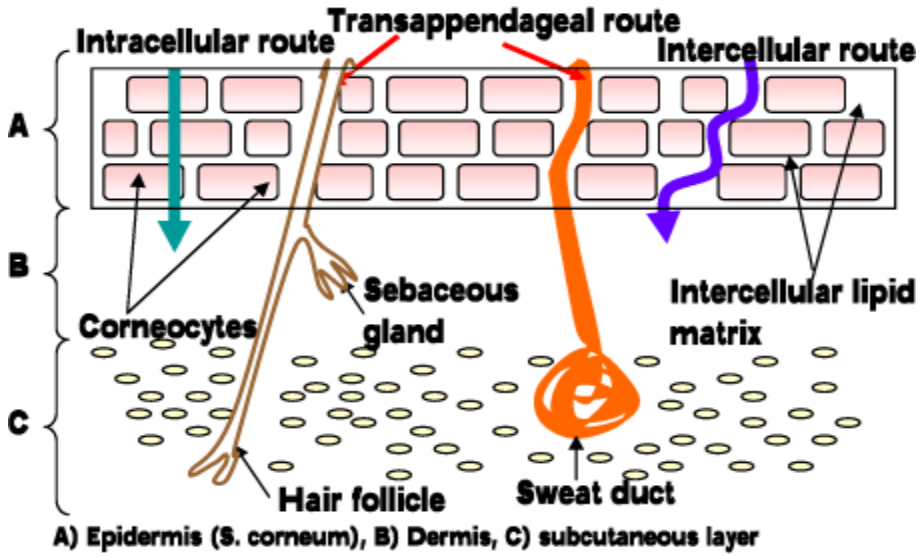


Fig. 1-3 Pathways of drug penetration through the skin [24]

The transepidermal route through the SC consists of two pathways: intercellular and intracellular. The intercellular pathway consists of lipids, which are rich in ceramides, free sterols, free fatty acids, along with low quantities of glycolipids, sterol esters, triglycerides, cholesterol sulfate and hydrocarbons. Since the glycolipids, cholesterol sulfate and sphingolipids are amphipathic in nature, the lipid layer of the SC exists as a bilayer, which reinforces the barrier properties of the SC [25, 26]. The lipid bilayer provides a transport pathway for most hydrophobic drugs. The intracellular pathway, consisting of corneocytes bound by lipoidal envelope, is utilized by hydrophilic drugs [18, 26]. However, it is imperative for hydrophilic molecules to cross the intercellular lipid matrix to enter the corneocytes. The bilayer structure, which is believed to be impervious to hydrophilic substances, possesses an orthorhombic packing at room temperature and the packing is transient at even slightly higher temperatures. This lipid reorganization affects

the transport properties of the skin for hydrophilic substances [26]. This fluidity in the lipid structure also forms the basis for the action of penetration enhancers [27].

1.4 Factors affecting skin permeability

As normal, healthy skin poses a significant barrier to permeation of drug molecules, it is important to understand, modulate and overcome this barrier for a successful delivery of molecules through the topical route. The target molecules may either be aimed for local or systemic effects through the topical route. While a local effect is achieved by topical application of suspended and/or dissolved drugs in ointments or creams, systemic delivery is typically achieved through transdermal drug delivery systems (TDDS) [28]. In both cases, the drug formulation or delivery system is applied to the skin with the aim of diffusion of the drug from the vehicle to the SC and subsequent layers of the skin [10]

1.4.1 Passive diffusion of drug substances

The kinetics of movement of drug molecules forms a theoretical basis for understanding the percutaneous absorption of drugs through the topical route [29]. The term “flux” is typically used to measure the mass transport of molecules across a solution or barrier. Flux is defined as the mass or number of molecules passing through a given cross-sectional area in a given period of time. It can be mathematically expressed as:

$$J = \frac{m}{A\,t} \qquad \text{Eq. 1.1}$$

Where *J* is the flux of a molecule with mass *m* moving across *A* cross-sectional area in time *t* [30].

Passive diffusion is the driving force for the transport of molecules in solution across a concentration gradient [31]. The velocity of diffusion is governed by the diffusional coefficient (*D*), which is dependent on the size of the solute molecules and viscosity of the solvent as described by Stokes-Einstein equation:

$$D = \frac{R\,T}{6\,\pi\,\eta\,N_0\,r_a} \qquad \text{Eq. 1.2}$$

where *R* is the gas constant, *T* is the absolute temperature, η is the viscosity of the solvent, N_0 is the Avogadro's number and r_a is the radius of the spherical solute molecule [30].

Fick's law of diffusion governs the passive movement of drug molecules across a concentration gradient. Accordingly, the rate of absorption, i.e., flux (*J*) of any substance across a barrier is directly proportional to its concentration difference across that barrier. [32].

For biopharmaceutical studies of drug permeation across barrier membranes, a typical setup consists of a donor compartment with a defined initial concentration of the compound and a defined volume, a membrane with a defined cross-sectional area and thickness, and a receptor compartment with a defined initial concentration and defined receptor fluid volume. Continuous stirring in the receptor compartment ensures that there is no concentration gradient within the two compartments and the only concentration gradient is across the barrier membrane. In such cases, flux across the barrier only transports negligible amount of solvent and the concentration gradient across the barrier is maintained constant. The flux is then of zero order, i.e. constant, since flux occurs as a function of concentration gradient. In such case, Fick's law can be simply expressed as

$$J = K_p(C_{donor} - C_{receptor}) \qquad \text{Eq. 1.3}$$

where K_p is the permeability coefficient, C_{donor} and $C_{receptor}$ are the concentration of the drug molecules in the donor and receptor chamber, respectively, and J is the flux across the two chambers. Assuming that the concentration gradient is linear and constant, i.e., time-independent, and $C_{receptor}$ is negligible as compared to C_{donor}, Fick's law can be expressed as [30]:

$$J = K_p\, C_{donor} \Leftrightarrow K_p = \frac{J}{C_{donor}} \qquad \text{Eq. 1.4}$$

C_{donor} is essentially equal to the concentration of the drug substance in the vehicle, C_v. The equation can then be rewritten as

$$J = K_p C_v \qquad \text{Eq. 1.5}$$

The permeability coefficient K_p, is then the ratio of the flux and concentration of the drug substance in the vehicle, C_v [33]. K_p is dependent on factors related to the drug and the barrier, such as the partition coefficient K_m, diffusion coefficient D and length of the diffusion pathway L. Taking into account these factors, Fick's law can be expressed as[10]:

$$J = \left(\frac{D\, K_m}{L}\right) C_v \qquad \text{Eq. 1.6}$$

From eq. 1.6, it is evident that flux can be increased by increasing the diffusion coefficient D, partition coefficient K_m, and/or concentration of the drug in the vehicle C_v.

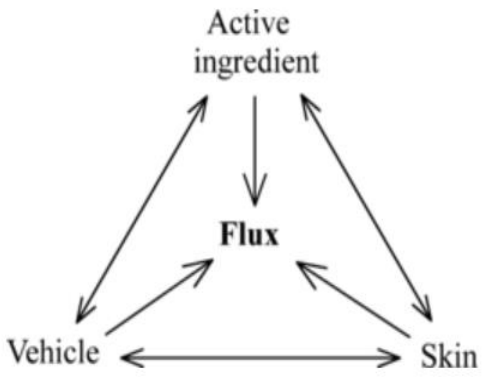


Fig. 1-4 Flux and possible interactions between the vehicle, drug (active) and skin [34]

All these factors are dependent on the vehicle or formulation and are also influenced by interactions between the drug and the formulation, the formulation and the skin, and the drug and the skin. Hence the choice of the vehicle/formulation becomes vital in driving the drug substance into the skin [35].

The percutaneous absorption of a drug is a multi-step process where the drug moves sequentially from the top layer of the skin, i.e., epidermis to the lower layers, i.e., dermis and subcutaneous fat layer. For the absorption of most substances, the barrier layer of SC acts as a rate limiting membrane and therefore the percutaneous absorption can be understood by analyzing the kinetics of drug movement through the SC. For a finite amount of drug applied topically, i.e., thin layer of drug with a definite amount of drug in contact with skin, the flux never attains a constant value, and this is called a non-steady state phenomenon. The process of percutaneous absorption can be divided into three phases:

i) *Lag phase:* it is the period with no apparent absorption of the drug into the membrane. Although there is drug diffusion, i.e., movement of the drug

from the vehicle to the SC, the flux is zero at this point because no drug has passed through the entire length of the SC.

ii) *Rising phase:* as the drug remains in contact with the skin for an increased period of time, drug absorption rises as the molecules make their way into the barrier layer, and the concentration of drug increases in the SC. This establishes a concentration gradient between the SC and viable epidermal layers. The rate of movement of the drug from the SC to the lower layers of epidermis increases and flux of the drug can be observed.

iii) *Falling phase:* with further increase in time, the initial concentration gradient reverses and the concentration of the drug in the topically applied film decreases. This results in a falling portion of the curve towards the end of the absorption process [29].

The total amount of drug absorbed can then be calculated by integrating the area under the curve from the three phases. The concept of steady-state kinetics is observed when the amount of drug applied to the skin is large enough to be considered an infinite dose. In steady-state, the flux attains a constant value with increasing time. It is most commonly observed in *in vitro* studies of percutaneous absorption of drugs and is rarely achievable under clinical conditions [29].

From equation 1.6, a linear relationship between the concentration of the drug and the flux can be deduced. However, the concentration of drug is linearly related to the flux only at low values. As the drug concentration rises, the solubility of drug in the vehicle poses as a limiting factor. As only the solubilized drug diffuses through the vehicle into the

skin, it is more apt to relate concentration of the solubilized drug with flux in equation 1.6. As a result, Fick's law can be modified as follows:

$$J = \frac{D\,K_m}{L}\,C_{v\,(solubilized)} \qquad \text{Eq. 1.7}$$

where $C_{v\,(solubilized)}$ is the concentration of "solubilized" drug in the vehicle. Therefore, the total concentration of drug in the vehicle, which is often listed on the drug formulation, becomes misleading as it sheds no light on the soluble fraction of drug. As a result, the same concentration of drug in different vehicles with varying solubility profiles can lead to different rates of absorption. This can in turn lead to different potencies with the same concentration of the drug. The selection of the vehicle with optimum solubility for the drug thus becomes imperative to achieving therapeutic efficacy through the topical route [29].

1.4.2 Role of Vehicle

As discussed above, the choice of the vehicle is an important consideration to achieve maximum therapeutic efficacy of a drug. The physio-chemical properties of the vehicle impact the amount of the drug, which first diffuses through the vehicle to the skin surface and then permeates through the SC layers to its site of action. The effect of the vehicle can be studied through its effect on drug release, its interaction with the skin and solubility of the drug in the vehicle, respectively.

i) *Release of the drug from the vehicle:* the physio-chemical properties of the combination of a drug and a vehicle play an important role in the release of the drug. Most importantly, drug solubility and rate of release of the solubilized drug in the presence of other components of the vehicle, such as

penetration modifiers, determine the flux of the drug from the vehicle. Other properties of the vehicle such as viscosity, do not pose as rate-limiting factors and indirectly impact the release of the drug from the vehicle [18]. From the discussion on Fick's law, it is evident that the concentration of the solubilized drug in the vehicle is related to the flux. However, this is true only in indefinitely dilute solutions of the drug where molecular interactions of drug-vehicle and drug-drug are negligible. In concentrated solutions, the thermodynamic activity of drug molecules comes into play. The thermodynamic activity describes the escaping tendency of the drug from the vehicle into the skin. The relation between thermodynamic activity and concentration of the drug in vehicle can be described as follows:

$$a_v = \gamma_v C_{v(solubilized)} \qquad \text{Eq. 1.8}$$

Where a_v is the thermodynamic activity, γ_v is the activity coefficient and $C_{v(solubilized)}$ is the concentration of the solubilized drug in the vehicle. Similarly, the partition coefficient in a concentrated solution is described by the activity coefficients of the drug in the vehicle and the skin ($K_m = \gamma_v/\gamma_s$). The equation describing flux (J) in a concentrated solution can then be rewritten as:

$$J = D\frac{a_v}{L\,\gamma_s} \qquad \text{Eq. 1.9}$$

The thermodynamic activity of drug molecules is related to their solubility in the vehicle. Generally, thermodynamic activity (escaping tendency) of a drug is higher in a vehicle with lower drug solubility. By knowing the

solubility of the drug in the vehicle, the thermodynamic activity can be predicted from the ratio of concentration of the solubilized drug and solubility of the drug in the vehicle. Flux can thus be optimized by increasing the thermodynamic activity of the drug molecules [35].

ii) *Effect of the vehicle on the skin:* Different components of the vehicle interact with the skin to varying degrees. Some occlude the skin to prevent water loss, while some extract lipids from the SC to reduce the barrier properties [36]. Various penetration modifiers used in vehicles modulate skin permeability by disordering or "fluidizing" the lipids in the SC and forming micro-cavities in the lamellar SC structure. Other components may penetrate and mix into the extracellular lipids of the SC [37, 38]. Some chemicals like depilatory agents have also been found to disrupt the cellular integrity of the corneocytes thereby altering the flux of active agents [39]. A novel class of peptide-based penetration enhancers also interact with the skin and are internalized by either transfollicular pathways, ionic interactions with amino acids, or by membrane disruption [40]. Thus, components of the vehicle play a vital role in increasing the flux across the barrier membrane.

iii) *Supersaturation:* the thermodynamic activity a_v is unity at the saturation concentration of the drug in the vehicle. At concentrations beyond saturation, the thermodynamic activity increases with an increase in the concentration of drug in the vehicle. Thus, the flux is increased with higher degree of supersaturation [41-43]. However, supersaturated vehicles are

thermodynamically unstable due to the high thermodynamic activity. As a result, the drugs tend to recrystallize in the vehicle, which leads to a drop in the flux. Using anti-nucleating agents, such as polyvinyl pyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC) has been found to impede recrystallization and improve the stability of the supersaturated vehicles [44].

1.4.3 Physio-chemical properties of a drug

The diffusion coefficient and partition coefficient of the drug, which depend on the molecular weight, size, structure and degree of ionization of the molecules, are important factors in determining the skin permeability of a drug [45]. The concentration gradient is the driving force for percutaneous absorption. For drug molecules to reach the deeper layers of skin, the concentration of the soluble drug should be high in the outermost layer while low in the subsequent deeper layers [10, 18]. Thus, partition coefficient (K_m) of the drug, i.e., its tendency to leave the vehicle and enter the skin, plays an important role in determining the drug concentration in the SC. Partition coefficient (K_m) is expressed as a ratio of equilibrium solubility of the drug in the surface of the SC (barrier) and the vehicle [29, 46]. An increase in the partition coefficient brought on by changing the characteristics or composition of the vehicle such that the drug has higher solubility in the SC than in the vehicle, increases the flux. For a drug to traverse the subsequent layers of skin, the viable epidermis must act as a sink. The deeper layers of skin are relatively more permeable to molecules with good aqueous solubility, in comparison to the lipoidal SC barrier. Thus, it is difficult to achieve optimum permeation except when drug, carrier or components of the

vehicle have suitable solubilities in both lipid and aqueous environments. Highly lipophilic compounds tend to form a reservoir in the SC with very low penetration to the deeper epidermal layers [18, 29].

The partition coefficient is also influenced by charge and degree of ionization of the penetrating molecule. As per pH partition theory, non-ionized species can better penetrate through the epidermis [47]. Thus, pH of the vehicle plays an important role in determining the extent of percutaneous absorption of a penetrant. The pH of the vehicle and ionization constant, $pK\alpha$, determine the relative concentration of ionized and non-ionized species. The ionized species penetrate through the intracellular route, whereas the non-ionized species penetrate predominantly through the intercellular and transappendageal route. [18, 48]. For uncharged molecules with a high dipole moment, such as amphiphilic molecules, the polar part has a better affinity towards the hydrophilic epidermal layers and the non-polar part prefers the SC [49].

The diffusion coefficient, D, depends on the molecular weight and size of the penetrating molecule. Molecules with similar polarity have different rates of permeation based on their molecular weight. Lower molecular weight species tend to diffuse faster due to a decreased diffusivity in a liquid medium with increasing molecular volume [18, 29, 50].

The Rule of 5, proposed by Lipinski et al., can be used as a thumb rule for selecting drugs for non-oral delivery. It states that non-oral delivery of drugs can only be achieved if the drug has molecular weight below 500 Da, less than five H-bond donors, less than ten H-bond acceptors and a log P value below 5 [51].

1.4.4 Regional Variation and Skin Condition

All body sites have a different thickness of the epidermal layers, distribution of hair follicles and sebaceous glands, hydration status, temperature and barrier integrity [18, 52]. Thicker skin is a greater barrier to the permeability of molecules with the same physicochemical properties [53, 54]. Areas with a higher number of appendages show increased transfollicular transport of molecules, although this route is not predominant for percutaneous absorption [55, 56].

The extent of hydration of the SC affects the thickness of the barrier layer. The thickened barrier layer, due to increased hydration, expands the reservoir volume available to drugs and affects the subsequent penetration into the viable epidermis [57]. Also, water molecules interact with the polar head groups of the lipid bilayer causing the formation of hydration shells around the polar heads. This results in an increased fluidity and permeability for the substances that permeate through the lipoidal layer. Additionally, the extended hydrophilic domain also improves the penetration of polar molecules [18, 58, 59]. Occlusion of the skin causes increased hydration by preventing TEWL [18, 60]. Increasing the time of hydration causes structural changes in skin, which is characterized by swelling and softening of the keratin filaments. This opens up a pore pathway to facilitate the diffusion of molecules [57]. The effect of hydration on the diffusion of lipophilic molecules can be partly explained by the decreased affinity of the drug to the SC in a hydrated condition vs. non-hydrated condition. This decreased affinity drives the diffusion towards the deeper layers of the epidermis [18, 61].

Temperature of the skin influences the degree of vascularity and lipid structure of the epidermal layers. Increased blood circulation in the viable epidermis layer causes slight increase in uptake of drug molecules into the blood, but since this step is not the rate limiting step in percutaneous absorption, it is hardly measurable [62].

The integrity of the SC barrier is compromised in pathological conditions such as atopic dermatitis [63], ichthyosis [64], psoriasis [65], and severe xerosis [66]. It has been noted that percutaneous absorption is higher in skin with a less intact barrier [67]. Thus, any conditions affecting the barrier functions of the SC may influence the diffusion of a drug from the vehicle to the SC [68].

1.5 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are the most commonly prescribed drugs to treat pain, edema and inflammation arising from conditions like arthritis, musculoskeletal injuries, joint disorders and myalgia [69]. NSAIDs have been proven to be clinically efficacious for pain and inflammation management in several disorders, such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, dysmenorrhea, dental pain and headache [70]. These agents act by inhibiting the cyclooxygenase (COX) enzyme, which is responsible for catalyzing the biosynthesis of prostaglandins (PGs) and thromboxane (Tx). Arachidonic acid (AA), an unsaturated fatty acid present in cell membranes, serves as a precursor for synthesis for PGs and leukotrienes (LTs). The free AA is acted upon by COX enzymes resulting in synthesis of prostaglandin G_2 (PGG_2). The next step involves a reduction of PGG_2 to PGH_2 by a peroxidase reaction. Subsequently, cell-specific isomerases and synthases catalyze the conversion of PGH_2 to biologically active prostaglandins: PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2,

and TxA_2. These PGs are responsible for maintenance of homeostatic functions and modulation of inflammatory response in the body [71-73].

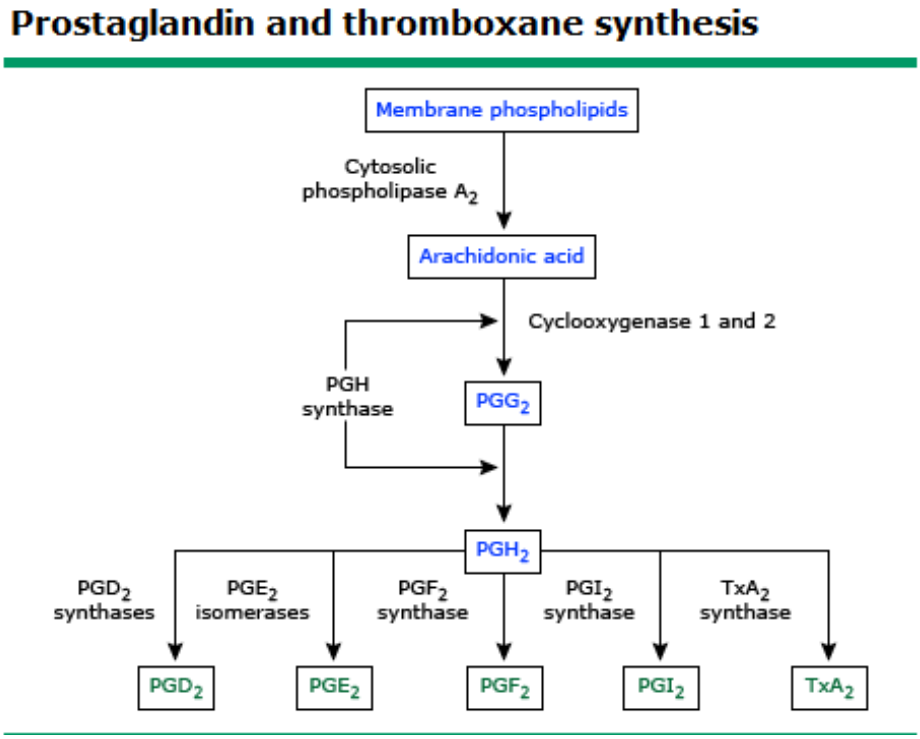


Fig.1-5 Schematic representation of the prostaglandin synthesis pathway [74]

The two isoforms of COX, namely COX-1 and COX-2, have been found to have structural and functional differences [69]. The constitutive isoform COX-1 is expressed in most tissues and mediates physiological functions like cytoprotection of gastric mucosa, regulation of renal blood flow and platelet aggregation. The inducible COX-2 has been associated with modulation of inflammatory activities and tumorigenesis. Recent findings suggest that COX-1 is induced during lipopolysaccharide (LPS)-mediated inflammatory response and cellular differentiation, while COX-2 also supports homeostatic functions, such as kidney development, ovulation and embryo implantation [71, 75, 76]. Research in

mice models suggest that both isoforms may contribute to an inflammatory response and COX-2 plays an anti-inflammatory role in the later phase of the inflammatory cascade [77-79].

NSAIDs have been classified according to the relative affinity of the agent towards COX-1 and COX-2 (Table 1.1).

Table 1.1 Classification of NSAIDs based on COX inhibition [69]

COX inhibition activity	**Examples**
Non-selective irreversible inhibitors of both COX-1 and COX-2 (<5-fold COX-2 selectivity)	Aspirin, triflusal [80]
Non-selective competitive inhibitors of both COX-1 and COX-2 (<5-fold COX-2 selectivity)	Ibuprofen, diclofenac, piroxicam, naproxen
COX-2 selective competitive inhibitors of both COX-1 and COX-2 (5 to 50-fold COX-2 selectivity)	Celecoxib, meloxicam, nimesulide, etodolac
Strong COX-2 competitive inhibitors with very low affinity for COX-1 (> 50-fold COX-2 selectivity)	Refecoxib (withdrawn in 2004), NS-398
Weak competitive inhibitors of both COX-1 and COX-2	Sodium salicylate, namubutone

1.6 Topical delivery of NSAIDs

NSAIDs are available in oral, topical, transdermal, ophthalmic, rectal and parenteral dosage forms. Oral NSAIDs have been associated with dose-related adverse events, such as increased risk of upper gastrointestinal (GI) complications, cardiovascular toxicity, renal toxicity, hepatic toxicity and hemorrhagic stroke [81-83]. Recently, the

United States (US) Food and Drug Administration (FDA) has issued new guidelines with respect to label warnings placed on over-the-counter (OTC) and prescription NSAIDs. These new guidelines emphasize the risk of heart attack and stroke in patients with or without heart disease or risk factors for heart disease [84]. Typically, oral NSAIDs cause GI damage by disrupting the epithelial layer of the mucosa, which is attributed to COX inhibition causing reduction in PG synthesis in the gastric mucosa [85, 86]. GI complications include ulceration, bleeding, diarrhea, nausea, obstruction, and/or perforation [85, 87].

To overcome the toxicity of NSAIDs, several strategies have been adopted. The use of simple analgesics and/or rubefacients rather than NSAIDs has been shown to be equally efficacious in the management of osteoarthritis [88-90]. As the toxicity of oral NSAIDs is primarily dose-related, rational use of the lowest effective doses of the least toxic NSAID for the shortest duration of time has been linked to a reduction in adverse effects [91, 92]. Topical NSAID therapy has also been widely explored as an alternative to oral therapy. Globally, various NSAIDs such as diclofenac, eltenac, ketoprofen, ibuprofen, piroxicam and felbinac are available as topical preparations in the form of ointments, gels, patches and/or topical solutions [83]. In the US, diclofenac sodium is the only NSAID that has been approved for topical use [93]. Currently, diclofenac sodium is available as gel and topical solution at different strengths ranging from 1% to 3% w/w [94]. The American Academy of Orthopedic Surgeons recognizes topical NSAIDs as the first-line pharmacologic therapy for patients with increased risk of GI risk for management of osteoarthritis [83].

Topical formulations of NSAIDs are designed to achieve a high local concentration of the active ingredient at the affected site while bypassing the systemic circulation and

first-pass metabolism [95, 96]. The local enhanced concentration of the active ingredient is achieved by direct diffusion into the skin and subsequent penetration into the deeper tissues [95, 97]. Several pharmacokinetic studies have reported that the direct penetration of drugs into the skin layers is responsible for the high local concentration of NSAIDs [98, 99]. The cutaneous blood supply not only helps maintain sink conditions for percutaneous absorption by absorbing and diluting drugs passing through the epidermis, but it also impedes drug penetration by removing the drug through the systemic circulation [100]. The evidence for the role of cutaneous blood flow has been found by using vasoconstrictors (phenylephrine) concomitantly with other topical drugs. The penetration of topical agents was found to be higher with the use of vasoconstrictors [101].

The distinct advantages of topical delivery of NSAIDs include a lower toxicity and better tolerability due to low systemic concentration, avoidance of enterohepatic recirculation, direct administration onto target site, ease of administration and higher patient compliance, more cost-effectiveness due to a lower incidence of adverse events, avoidance of drug-drug interaction, and faster onset of action due to the elimination of dosage titration [96, 102, 103]. The therapeutic efficacy of topically applied NSAIDs has been established by comparing the extent of absorption of the drug via topical and oral routes. Muller et al. found a 12-fold higher concentration of topically applied diclofenac in the interstitial fluid of the skeletal muscles than the plasma [104]. Similarly, another group found a similar concentration of ibuprofen in the muscle tissue when applied topically and administered orally in two groups of healthy volunteers [105, 106]. Evidence from several clinical studies comparing the two routes of administration, oral and topical, suggests that NSAIDs administered topically possess a comparable efficacy as oral NSAIDs [107-111].

The most commonly reported adverse effects of topically administered NSAIDs are dermatological in nature [87, 96]. These include rash and/or pruritus at the site of application, dry skin, erythema, irritation, and paresthesia. A very low incidence of GI adverse effects, including bleeding, constipation, diarrhea and nausea, has been reported [87]. In summary, topical NSAIDs have been reported to have fewer adverse effects and fewer treatment withdrawals due to less adverse effects [112].

1.7 Emulgels

Emulgels are two phase systems with an emulsion entrapped into the matrix of an outer gel phase [113, 114]. Gels have been extensively used as topical drug delivery systems for hydrophilic drugs. Although gels possess certain advantages, such as greater dissolution of the drug, easy migration of the drug through the matrix, faster onset of action than in the case of creams or ointments, and better aesthetic appeal as compared oily formulations, they are not suitable vehicles for hydrophobic molecules unless some solubility enhancer and/or an agent to modify the intermolecular interactions is used [115, 116]. Emulsions, both oil-in-water (O/W) and water-in-oil (W/O) type, have long been used as topical drug delivery systems for different molecules. However, the inherent thermodynamic instability and limited drug loading capacity are two main challenges with emulsions as drug-delivery systems [117].

Several approaches have been used to overcome these limitations. Microemulsions, which are inherently thermodynamically stable, utilize a high proportion of surfactant and co-surfactant to form optically clear emulsions. Such high content of surfactants may not always be desirable due to their irritation and SC disrupting potential even though these

show remarkable penetration enhancement properties [118]. By adjusting the specific gravity of the dispersed phase, emulsions are rendered more stable towards creaming (i.e., rising or sedimentation of droplets, depending on the difference in specific gravities between the phases, under the influence of gravity). Gums, clays and synthetic polymers have traditionally been used in the continuous phase of emulsions to impart better rheological characteristics and stability [117]. The use of a gelling agent in the outer phase not only imparts thixotropy but also facilitates a controlled release of the lipophilic active ingredient in the oil-phase of the emulsion system [119, 120]. Additionally, emulgels are more stable, do not require intense sonification and possess advantages of both emulsions and gels [120, 121].

Several emulgel formulations are available in the market globally. The most noted product is Voltaren Emulgel®, which contains diclofenac diethylamine in 1.16% w/w. The formulation has been claimed to have a unique skin penetrating property as a virtue of the combination of a cream and a gel. It is also available in a higher strength at 2.32% w/w. These formulations are widely used in Canada, Australia and United Kingdom (commercialized as Voltarol Emulgel P®) for relief of mild arthritic pain [122-124]. However, Voltaren Emulgel® is not yet approved in the US. These products have been shown to be clinically effective at relieving muscle pains and aches. Voltarol Emulgel P® has been found to be as effective as taking 400 mg oral ibuprofen for the arthritis of the fingers [124].

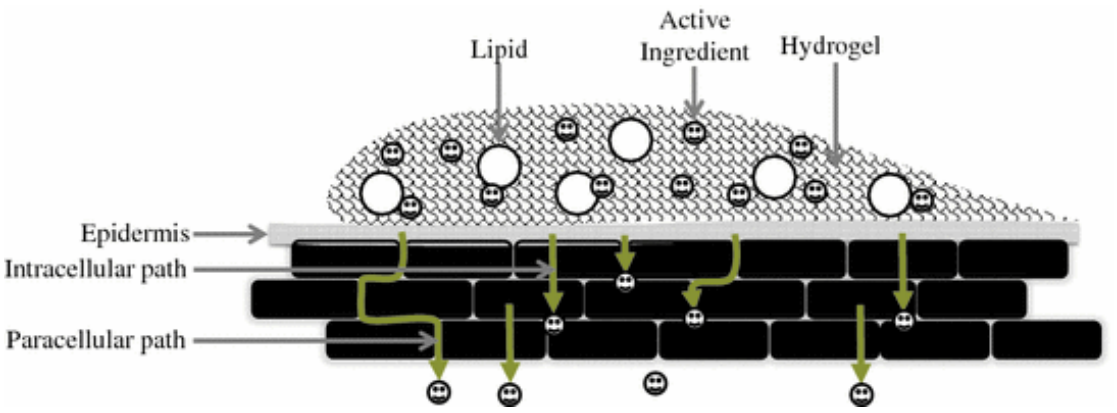


Fig. 1-6 Schematic representation of an emulgel system [120]

Prior studies have shown that permeation of ibuprofen from gels is more rapid compared to creams, while creams deliver a higher amount of drug over time [125]. It was also demonstrated that emulgels provide better solubility and skin-permeability for poorly water-soluble drugs, e.g., ibuprofen, than gels alone [126].

1.8 Penetration modifiers

Conventionally, the terms ‘chemical penetration enhancers/ sorption promoters/ accelerants’ have been used to refer to agents that, when present as constituents of a topical delivery vehicle, partition into the skin and interact with components of the SC to cause an increase in skin permeability towards the active ingredient in a temporary and reversible manner [127, 128]. However, there is a broader class of agents that act as either enhancers or retardants for a specific active species depending upon the vehicle used for topical delivery. These agents are collectively termed as ‘penetration modifiers’ [129-131].

These agents have two distinct mechanisms of action as penetration modifiers. Firstly, the chemical agent enters the skin, increases the solubility of the active ingredient

in the skin and causes an increase in partition coefficient (*Km* or *Log P*) of the active ingredient between the skin and vehicle. Secondly, these agents can diffuse into the SC and cause disruption of the lipid matrix by "fluidizing" or extracting the lipids and affect the diffusion coefficient of the active ingredient in the skin [127, 132].

Certain properties are desirable for these agents to be effective penetration enhancers. They should be non-toxic, non-irritant, chemically stable and hypoallergenic at the permissible range used in the vehicle. They should have a predictable, reproducible and reversible activity with less variation when applied to the skin as a constituent of the vehicle. They should not have any inherent pharmacological activity and should be compatible with other excipients of the formulation. They should ideally work in a unidirectional manner by enhancing the penetration of the permeant into the skin and simultaneously, not causing any loss of endogenous components of the skin [27, 130].

Different classes of penetration enhancers have been identified based on their chemical structures (Table 1-2). These agents can have more than one type of mechanisms of action on the SC (Figure 1-7).

Table 1.2 Classification of chemical permeation enhancers [127]

No.	Class	Mechanism(s) of action	Examples
1.	Terpenes	For hydrophilic drugs: increasing drug diffusivity into the SC by disrupting lipid matrix of the SC For lipophilic drugs: increasing both drug diffusivity and partitioning into the SC	D-limonene, 1,8-cineole, L-menthol, anethol, nerolidol, eugenol, carvone, thymol
2.	Azone® and analogs	Fluidizing the lipid barrier, interacting with ceramides to increase fluidity in the lamellae that facilitate drug penetration	Azone® (laurocapram), alkyl azones
3	Fatty acids (FAs)	Disrupting lipid packing to increase bilayer fluidity [133]	Unsaturated FAs: Oleic acid, linolenic acid Saturated FAs: undecanoic acid, lauric acid, myristic acid, pelargonic acid [133]

No.	Class	Mechanism(s) of action	Examples
4	Alcohols	Extracting lipids and proteins, fluidizing lipids, increasing drug partitioning and solubility in the SC, and changing the thermodynamic activity of drug	Ethanol, isopropyl alcohol, 1-butanol, 1-propanol, 1-octanol, decanol
5.	Glycols	Permeating rapidly into the skin and altering the thermodynamic activity of the drug, modifying the drug solubility by interacting with lipids	Propylene glycol
6.	Surfactants	Anionic: interacting with lipids and keratin in the SC Cationic: interacting with SC proteins through polar and hydrophobic interactions Nonionic: increasing fluidity of lipid bilayer, solubilizing and extracting lipids, interacting with keratin filaments in the corneocytes, and altering the thermodynamic activity of the drug	Anionic: Sodium lauryl sulfate Cationic: benzalkonium chloride, cetylpyridinium chloride (not used as penetration enhancers due to irritant and toxic nature) Nonionic: polysorbates

No.	Class	Mechanism(s) of action	Examples
		Amphoteric surfactants: fluidizing lipids	Amphoteric: dodecyl betaine, hexadecyl betaine, hexadecylsulfobetaine
7.	Sulphoxides	Extracting lipids, interacting with keratin, displacing bound water within keratin, interacting with lipid alkyl chain	Dimethylsulfoxide (DMSO), dimethylformamide (DMF), dimethylacetamide (DMAC), decylmethyl sulfoxide (DCMS)
8.	Esters	Integration within lipid matrix and increasing fluidity of lipids	Isopropyl myristate, octyl salicylate (OSAL), sorbitan monoleate, glyceryl monooleate, glyceryl monolaurate
9.	Ethers	Increasing solubility of the penetrant in the SC [134]	Diethylene glycol monoethyl ether (transcutol®), dimethyl isosorbide (DMI), diethylene glycol monoethyl ether (DGME) [135]

No.	Class	Mechanism(s) of action	Examples
10.	Water	Increasing hydration of skin and increasing solubility of hydrophilic drug in the SC, increasing partitioning of the drug, forming aqueous pore pathway to enhance drug permeation	Water

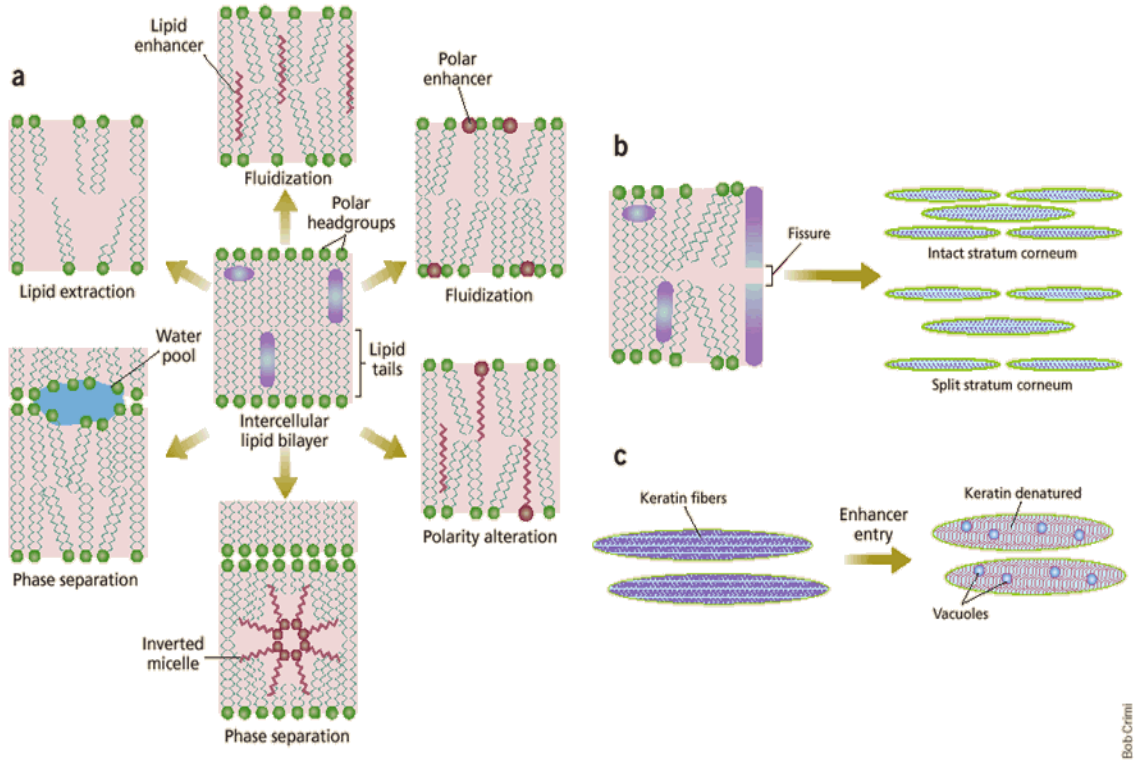


Fig. 1-7 Different mechanisms of action of penetration modifiers: a. action at the intercellular lipid matrix, b. action at the cellular junctions and protein structures, c. action within corneocytes [136]

1.9 Ibuprofen

Ibuprofen is a commonly used NSAID available as oral tablets, capsules (liqui-gels), suspensions and intravenous injectable solution in the US [94]. Globally, it is also available as gel for topical use [137]. It is one of the safest NSAID, which is generally well-tolerated [138].

1.9.1 Physiochemical properties

Ibuprofen (2-[4-(2-methylpropyl) phenyl] propanoic acid) is a propanoic acid derivate with anti-inflammatory, analgesic and antipyretic effects. It is an aromatic compound with a benzene ring. It has a three-carbon carboxylic acid functional group with phenyl group attached to the second carbon. In the para-position to the propanoic group is the isobutyl group [139]. It consists of a racemic mixture of R(-)-ibuprofen and S(+)-ibuprofen, with S(+)-ibuprofen showing COX inhibitory activity [140].

Molecular formula:$C_{13}H_{18}O_2$

Molecular weight: 206.285 g/mol

Melting point: 75-77° C

Solubility: 21 mg/L (at 25° C) in water, very soluble in alcohol and readily soluble in most organic solvents; low aqueous solubility at pH 1.2 and 4.5, high aqueous solubility at pH 6.8

Partition coefficient (Log P): 3.97

Dissociation constant (pKα): 4.91 [139]

Biopharmaceutics Classification System (BCS) class: IIa (low aqueous solubility and high intestinal membrane permeability)

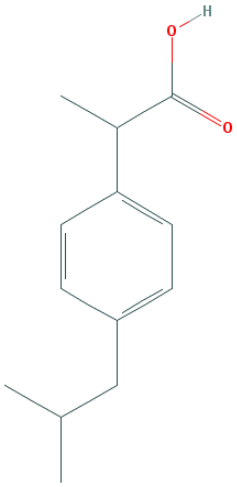


Fig. 1-8 Molecular structure of ibuprofen [139]

1.9.2 Pharmacology

Ibuprofen is a non-selective COX-1 and COX-2 inhibitor, which causes decreased production of prostaglandin via the COX pathway. It also causes decreased formation of TxA_2, thereby inhibiting platelet aggregation [139]. Antipyretic effects have been attributed to the action on the hypothalamus, resulting in increased peripheral blood flow, vasodilation and subsequent heat dissipation [141].

1.9.3 Dosage forms

Ibuprofen is available as oral tablets, capsules, suspensions and topical gels in varying strengths. Table 1-3 is a summary of the various dosage forms of ibuprofen with their strength and marketing status.

Table 1.3 Various dosage forms of ibuprofen and its salts

Active ingredient	**Strength**	**Dosage form/route**	**Marketing status**
Ibuprofen	50 mg, 100 mg	Tablet, chewable; oral	OTC
Ibuprofen	200 mg	Tablet; Oral	OTC
Ibuprofen	400 mg, 600 mg, 800 mg	Tablet; Oral	Prescription
Ibuprofen	40 mg/mL	Suspensions/drops; oral	OTC
Ibuprofen	100 mg/ 5 mL	Suspension; oral	Prescription
Ibuprofen	Eq. 200 mg free acid and potassium salt	Capsule; oral	Prescription
Ibuprofen lysine	Eq. 10 mg base/mL	Injectable; intravenous	Prescription
Ibuprofen	5.0 % w/w	Spray solution; topical	Prescription
Ibuprofen	5.0 % w/w, 10 % w/w	Gel; topical	OTC and prescription, respectively

1.10 Regulatory status of ibuprofen for topical delivery

While ibuprofen is approved for topical delivery in most European countries, it is not approved as a topical dosage form in the US. In 2009, the FDA issued warning letters to several manufacturers of topical ibuprofen formulations on safety grounds of these formulations. Several topical ibuprofen gels with strengths varying from 5-15% w/w have been withdrawn from the market post these warning letters. Although the FDA proposed to include orally administered ibuprofen to the applicable OTC monograph in 2009, it did not add topical ibuprofen to any OTC monographs [142].

The Medicines and Healthcare products Regulatory Agency (MHRA) has approved several topical ibuprofen formulations for rheumatic pain, muscle aches and pain, strains, sprains, sports injuries and non-serious arthritic pain. While formulations with 5% w/w strength are available as OTC, those with 10% w/w strength are typically labeled as *Max Strength* and are available as "prescription only" or "pharmacy only" [143].

Chapter 2

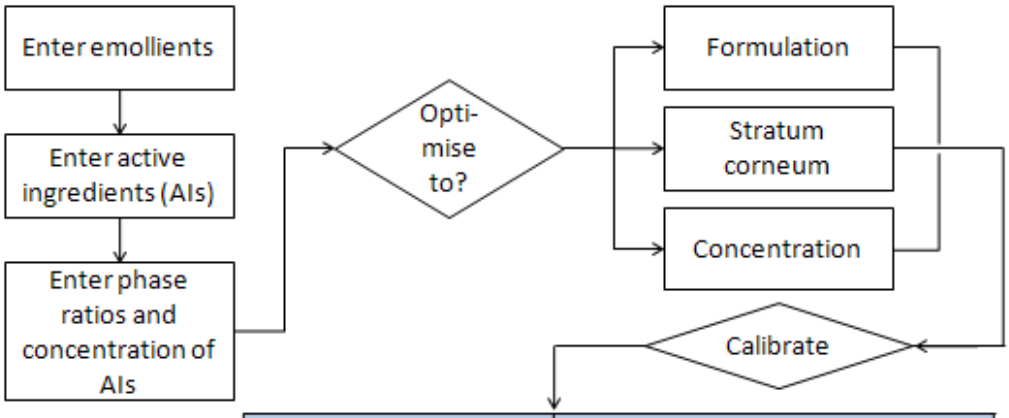
Formulating for Efficacy™ Software

2.1 Capabilities of the software

Formulating for Efficacy™ (FFE) is a software designed to aid the formulator in making the right choice of excipient(s) for an active ingredient(s) in a formulation. The aim of the formulator is to select such formulation components at the right concentrations, which optimize skin delivery of the active ingredient(s). The software aids in selecting the right blend of formulation excipients that not only dissolve the active ingredient(s) but also drive these into the skin layers [144].

FFE can be used in different modes to optimize a list of selected ingredients, or add new ingredients to an existing formula, or create an entirely new formula for the selected active ingredient(s), which can be a single species or a blend of different actives in definite ratios. New ingredients and new actives can be added to the list of default ingredients by entering their canonical SMILES (i.e., Simplified Molecular Input Line Entry System) [144]. Canonical SMILES is a unique line notation for representing molecules with their isotopic and chiral specifications. For most active ingredients and excipients, SMILES notation can be readily found in online database, such as PubChem. SMILES can also be generated using online tools like PubChem Skecher or ChemSpider by simply drawing the

accurate chemical structure of the moiety [145]. When creating a formula, certain information, such as % oil phase in the formulation, % active in the oil phase, % active required overall, % solvent required, % active as supplied and target concentration needs to be entered. The formulation can be optimized 1) towards the active ingredient to dissolve the maximum amount of active ingredient in the formulation; or 2) towards the SC to ensure that a large proportion of active ingredient penetrates the SC and subsequent layers of skin; or 3) towards the target concentration (TC) to make sure that the selected target concentration on the 'actives' page is close to the maximum solubility limit and the flux of the drug is high enough to leave the formulation and enter the skin. [144].



New ingredients	Formulation
None *(add to existing formulation)*	Optimize ratios of existing ingredients
One *(add to existing formulation)*	Identify best new emollient and its required concentration
Two /Three *(create new formulations)*	Identify best new emollients and their required concentrations

Fig. 2-1 Schematic outline of the FFE software [144]

Precisely, FFE can be used to find the best extra ingredient in the oil phase for a current formulation, find the best two ingredients, find the best three ingredients, or to optimize the selected ingredient list. These four different options can be used in different situations. If there is an existing formulation and the formulator does not want to change or add any new ingredients but only modulate the ratio towards achieving best possible penetration of the active ingredient, then the software can be used to 'optimize the selected ingredient list' to identify the most optimal ratio of the chosen ingredients that help drive the active into the skin [144].

Fig. 2-2 Choices for selecting and optimizing ingredients of a formulation in FFE [144]

If a new ingredient is to be added to the existing formulation, FFE can optimize the formulation as per formulator's choice and add one best ingredient to the list. Similarly, two new ingredients can also be added to an existing list of selected ingredients. The option to 'find three best ingredients' can be used to identify the three best ingredients in optimal ratios for a drug to achieve the best possible outcome. Although, FFE does not give a final

formula, it helps optimize the phase in which the active ingredient is placed by identifying new ingredients or adjusting the ratio of existing ingredients. These ingredients can be emollients or penetration modifiers, which work by increasing the solubility of the active ingredient in the formulation. Other components of the formulation, such as surfactants, stabilizers, preservatives etc. still need to be identified, but they are not part of the calculations in FFE. The major task of identifying the best solvents, which balance the two aspects of topical delivery i.e., dissolving the active ingredient in the formulation while driving the solubilized active ingredient into the skin, is made easier and is less time consuming with FFE [144].

2.2 How it works?

As discussed in section 1.4, optimum solubility of the drug in the vehicle and in the skin is important for achieving a high flux J of the drug into the skin. The partition coefficient K_m and the diffusion coefficient D of the active ingredient directly influence the flux J [18, 35]. The partition coefficient K_m can be increased by increasing the solubility of the active ingredient in the SC while keeping the concentration in the formulation the same or by reducing the concentration in the formulation to a level where the active ingredient is soluble in the formulation while keeping the concentration in the SC same [146]. Hansen Solubility Parameters (HSPs) are used for quantitatively predicting the various factors, which affect the solubility behavior of two or more solvents. HSPs are composed of δD (the "dispersion" parameter), δP (the "polar" parameter) and δH (the "hydrogen-bonding" parameter) as represented by equation 2.1. The total of these

parameters represents the solubility parameter δ. The units of the solubility parameters are $MPa^{1/2}$ [147, 148].

$$\delta^2 = \delta D^2 + \delta P^2 + \delta H^2 \qquad \text{Eq. 2.1}$$

By comparing the HSPs of one substance (or a solvent) with another substance, the "likeness" of the two substances can be predicted. Because like dissolves like, the closer the HSPs of two substances, the better the affinity of the two substances. The HSP distance between two molecules, represented by R_a, is the measure of how alike they are. Conventionally, the smaller the R_a, the more likely that two substances are compatible in terms of solubility. The HSP distance R_a is calculated using the following formula [148]:

$${R_a}^2 = 4(\delta D_1 - \delta D_2)^2 + (\delta P_1 - \delta P_2)^2 + (\delta H_1 - \delta H_2)^2 \qquad \text{Eq. 2.2}$$

A HSP sphere can be constructed by using the three parameters as coordinates in three dimensions [148]. FFE calculates these HSPs of the active ingredient, the formulation components and the SC. By convention, the solubility of an active ingredient is higher in a formulation component if the HSPs of two chemicals are closer together, i.e., R_a is smaller. As a result of these speedy calculations, it is possible to predict best ingredients to be used in a definite ratio to achieve a desired outcome of either optimizing the formulation towards the active ingredient, or to increase the partitioning of the active ingredient into the skin, or to reach maximum solubility of the active ingredient in the formulation [146].

Chapter 3

Aim of the Research

The aim of the research was to develop and characterize emulgel formulations for ibuprofen using different penetration enhancers identified and optimized by FFE and literature. As mentioned in section 1.10, ibuprofen is not yet approved for topical delivery by the FDA, while it is available as a gel approved by the MHRA. This research explored the prospect of using ibuprofen in an emulgel-based formulation, which is suitable for topical delivery of a hydrophobic drug, while simultaneously investigating the effect of penetration enhancers dimethyl isosorbide (DMI) and isopropyl myristate (IPM). The prepared emulgel formulations have also been compared to a marketed gel product *AlgoFlex Dolo*™ (AGFD) containing 5% w/w ibuprofen marketed by Sanofi-Aventis in Hungary.

Using FFE presents an innovative new approach towards designing and optimizing the oil phase of the formulations. The results from this research with regards to designing the oil phase of the formulation can serve as a basis for using FFE for designing and optimizing the oil phase of other formulations where the oil phase contains the active ingredient(s). Not only does it prove to be a time-saving and efficient approach, but it also saves resources and delivers useful information on topical formulation development.

Chapter 4

Materials and Methods

4.1 Materials

4.1.1 Active Ingredient

Ibuprofen 25 US Quality (PDR-No. 30076166/Lot no.: SB1W0030) was received from BASF (Ludwigshafen, Germany). It is a white crystalline powder with a characteristic odor, mean particle size of about 25 µm and melting range of 75-78°C. This sample meets the current United States Pharmacopeia (USP), European Pharmacopoeia (EP) and Japanese Pharmacopoeia (JP) monographs.

4.1.2 Inactive ingredients

Carbomer 940 and triethanolamine (Lot no. J9822H3060B) were procured from Making Cosmetics (Snoqualmie, WA). Gransolve® DMI (USP/NF: dimethyl isosorbide, Lot no.: 152320933) was procured from Grant Industries, Inc (Elmwood Park, NJ). Kollicream® IPM (USP/NF: Isopropyl Myristate, Lot no.: 0010084422) and Kolliphor® PS 60 (KPS 60) (USP/NF: polyethylene(20)sorbitan monostearate, Lot No.: 0010149655) were procured from BASF Pharma Solutions (Tarrytown, NY). LexFeel® Natural (LFN) (INCI: heptyl undecylenate, Lot No.: DL4189) was procured from Inolex Inc.

(Philadelphia, PA). Deionized water supplied at the University of Toledo Health Science Campus was used.

4.1.3 Analytical Reagents

Sodium hydroxide, potassium phosphate monobasic, acetonitrile (ACN), triethylamine and orthophosphoric acid were procured from Fischer Scientific (Hampton, NH). All reagents used were of analytical grade.

4.2 Methods

4.2.1 Preparation of formulations

The three emulgel formulations F1, F2 and F3 were comprised of a carbomer 940 gel base and an oil phase with different compositions as mentioned in table 4-1. Each formulation contained 5% w/w ibuprofen as the active ingredient. The two phases were prepared separately as per the procedures detailed below.

Table 4.1 Composition of F1, F2 and F3 emulgels

Ingredient	Amount (% w/w)		
	F1	**F2**	**F3**
0.5% w/w Carbomer 940 gel	78.0	78.0	78.0
Ibuprofen	5.0	5.0	5.0
LFN	14.0	5.0	-
DMI	-	9.0	9.0
IPM	-	-	5.0
KPS 60	3.0	3.0	3.0

4.2.1.1 Preparation of 0.5% w/w carbomer 940 gel

A 0.5% w/w Carbomer 940 gel was prepared by dispersing a pre-weighed amount of carbomer 940 into deionized water using an overhead mixer (IKA RW20 Digital, Wilmington, NC) at 750 rpm for about 30 minutes. The pH of the gel was brought to 6.0 using triethanolamine.

4.2.1.2 Preparation of the emulgels

An accurately weighed amount of ibuprofen was added to LFN, a mixture of DMI and LFN and a mixture of DMI and IPM to prepare the oil phase of F1, F2 and F3, respectively. KPS 60 was added to a pre-weighed amount of carbomer gel base and mixed using overhead mixer at 750 rpm for about 5 minutes. The oil phase was added to the

carbomer gel base containing KPS 60 and mixed using overhead mixer at 750 rpm for about 15 minutes until the two phases were completely homogenous.

4.2.2 High-Performance Liquid Chromatography (HPLC)

An HPLC method was developed and validated for the analysis of ibuprofen in the formulations. HPLC (Waters Alliance e2695 separations module, Milford, MA) equipped with Waters 2489 UV/Visible detector and Accucore XL C18 column with a 4 µm particle size and 150 x 4.6 mm length was used for the analysis with a mobile phase containing a buffer (pH 2.5) and acetonitrile in 40:60 ratio pumped isocratically at 1 mL/min flow rate. The buffer consisted of HPLC grade water: triethylamine: orthophosphoric acid (1000 mL:1 mL:0.5 mL). The column was maintained at ambient room temperature and injection volume was kept at 10 µL throughout. The absorbance of ibuprofen was measured at a λ_{max} of 222 nm. A stock solution of ibuprofen at a strength of 1 mg/mL was prepared in acetonitrile and calibration standards ranging from 0.195 µg/mL to 100 µg/mL were prepared from the stock solution. Calibration standards were run (n=6) and the average peak area was obtained. A calibration curve was made by plotting the average peak area against the concentration of ibuprofen (µg/mL). Method validation parameters including Limit of Detection (LOD), Limit of Quantitation (LOQ), accuracy, inter-day precision and system suitability were determined.

4.2.3 Determination of viscosity

Viscosity of the three formulations F1, F2 and F3 and AGFD was determined using a Discovery HR 3 hybrid rheometer with a peltier plate with 60 mm radius at a shear rate

ranging from 0-1000 s^{-1} at both 20°C and 32°C in triplicate. Average viscosity values (Pa.s) were reported at both the temperatures.

4.2.4 Determination of pH

The pH of the three formulations F1, F2 and F3 and AGFD was determined using a Mettler Toledo Seven Compact pH meter (Billerica, MA). The pH meter was calibrated with standard buffer solutions of pH 4, 7 and 10 before each run. The electrode was dipped directly into the formulations and the gel product and readings were recorded in triplicate for each sample. Average pH values were reported.

4.2.5 Determination of drug content

The drug content of the formulations F1, F2 and F3 was determined by dissolving accurately weighed quantities of each formulation in ACN. The resultant solution was filtered using EMD Millipore membrane filter and serially diluted with ACN to obtain suitable dilution. Drug content was analyzed using the HPLC method developed for ibuprofen and determined quantitatively from the calibration curve.

4.2.6 Determination of spreadability

Spreadability of formulation F1, F2, F3 and AGFD was determined using TA.XT*Plus* texture analyzer (Texture Technologies Corp., Hamilton, MA) with a TTC spreadability fixture comprising of male and female Perspex 90-degree cones at 25°C. The instrument was calibrated using 5 kg load cell before each run. For probe calibration, the male cone was lowered into empty female cone (sample holder) so that the two were

practically touching. The starting point was then set at 25.0 mm above the female cone. Probe calibration was done before each run. To determine spreadability, each sample was placed into the female cone and pressed down using metal spatula to eliminate air pockets. The test mode was set to 'measure force in compression' and 'return to start' option was used. The starting distance of male and female cone was then set to 23 mm to avoid overloading/underloading. The test speed and post-test speed were set to 2.0 mm/s and 10.0 mm/s, respectively. Data acquisition rate was set to 200 pps. Exponent stable micro systems software (version 6.1.10.0) was used to generate spreadability curves.

4.2.7 Differential Scanning Calorimetry (DSC)

DSC analysis was performed for ibuprofen, blank formulations (blank F1, blank F2 and blank F3), drug-loaded formulations (F1, F2 and F3) and AGFD using DSC 822e Mettler Toledo equipment (Columbus, OH) equipped with a TS0800GCI gas flow system attached to a nitrogen gas cylinder and TSO801RO sample robot. All samples weighing in the range of 5-8 mg were weighed using a Mettler Toledo MT5 microbalance and sealed in a 100 µL aluminum crucible using a Mettler Toledo sealing press for crucibles. DSC studies were performed at a 10°C/min heating rate over a wide temperature range (0-200°C) with nitrogen gas purged at a rate of 10 mL/min. Star^{e} SW 10.0 software was used to generate thermograms.

4.2.8 Optical imaging analysis

Optical imaging analysis was done for F1, F2 and F3 formulations using AmScope MD35 microscope (Irvine, CA) under 10x and 40x magnification. The formulations were

suitably diluted with water before placing on glass slide to observe under 40x magnification. AmScope 3.4 software was used to capture images under the 10x and 40x magnification.

4.2.9 *In vitro* release and *in vitro* permeation studies

Jacketed Franz diffusion cells (PermeGear, Hellertown, PA) with a 15.0 mm orifice diameter and 12.0 mL volume were used to study *in vitro* release and *in vitro* permeation of F1, F2, F3 and AGFD. The cells were placed in a V9-CB stirrer (PermeGear, Hellertown, PA) connected with a water bath assembly set at 32±0.2°C. For the *in vitro* release, Spectra/Por 2 dialysis membrane of 12-14 kDa molecular weight cut-off (MWCO) and 25.0 mm diameter was used. For the *in vitro* permeation, Strat-M® membrane with 25.0 mm diameter was used. For each cell, water from the water bath was circulated through the outer jacket to maintain constant temperature and a magnetic stirrer bar was placed in the receptor chamber. Phosphate buffer (pH 7.4) was used as the receptor medium. An accurately pre-weighed amount of formulations F1, F2, F3 and AGFD were applied onto the membrane through the donor chamber of the cell. The receptor chamber of each cell was occluded with parafilm to prevent evaporation. For each formulation, the *in vitro* release and *in vitro* permeation studies were carried out over the course of 24 hrs and 12 hrs, respectively, with n=4. Samples of 0.3 mL were taken at the start of the study (i.e., at 0 mins) and subsequently at regular time intervals (i.e., 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 hrs). The sample volume was replaced by an equal volume of fresh phosphate buffer each time. Any air bubbles generated beneath the surface of the membrane were carefully removed by tilting the Franz cell to facilitate bubble escape

through the side arm. Flux (J) was calculated from slope of the line obtained by plotting the cumulative amount of ibuprofen permeated (µg/cm^2) vs. time (hr). Apparent permeability coefficient (P_{app}) was calculated by dividing flux (J) with the initial concentration of ibuprofen in the donor chamber (C_{donor}). Average values of flux (J_{avg}) and apparent permeability coefficient ($P_{app.\ avg}$) were reported. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test for multiple comparisons in SPSS software (version 24). Results were considered to be statistically significant at p-value < 0.05.

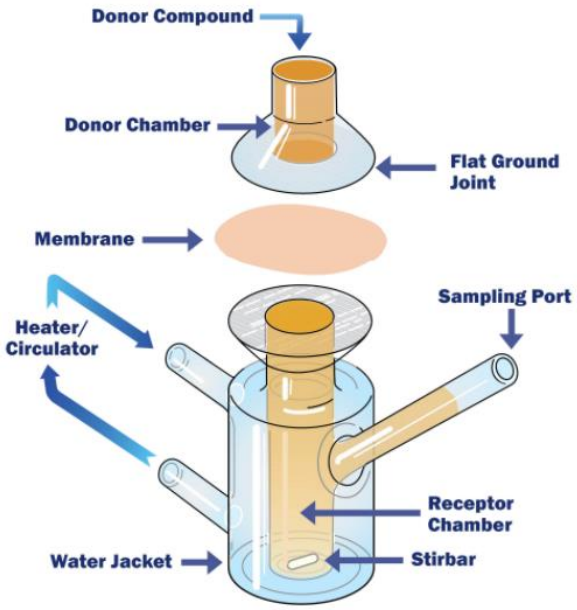


Fig. 4-1 Oblique view of a jacketed Franz cell [149]

Chapter 5

Results and Discussion

5.1 HPLC method development and method validation parameters

An HPLC method was successfully developed and validated for the analysis of ibuprofen in formulation F1, F2 and F3. From the optimized parameters, the retention time of ibuprofen was found to be 3.1 mins as shown in the chromatogram (Fig. 5-1). From the calibration curve (Fig. 5-2), a straight line with the following line equation was obtained with an R^2 value of 0.9999:

$$y = 22176x - 577.29 \qquad \text{Eq. 5.1}$$

The summary of the method validation parameters is presented in table 5-1.

Table 5.1 Values of the method validation parameters

Parameter	Value
LOD	0.264 µg/mL
LOQ	0.801 µg/mL
Linearity	R^2 = 0.9999; y-intercept = 577.29; slope = 22176
Accuracy	99.61-101.95 %
Precision	Relative Standard Deviation (RSD) = 0.74

From the method validation parameters, the method was found to be linear over a wide range of ibuprofen concentrations. The accuracy of the method, as determined by the percent recovery of ibuprofen, was found to be well within the suggested limits of 95-105% [150]. The precision of the method, determined from repeatability, was found to be well within the limits. The RSD of the measurements was found to be less than 2, which indicates a suitable precision [150].

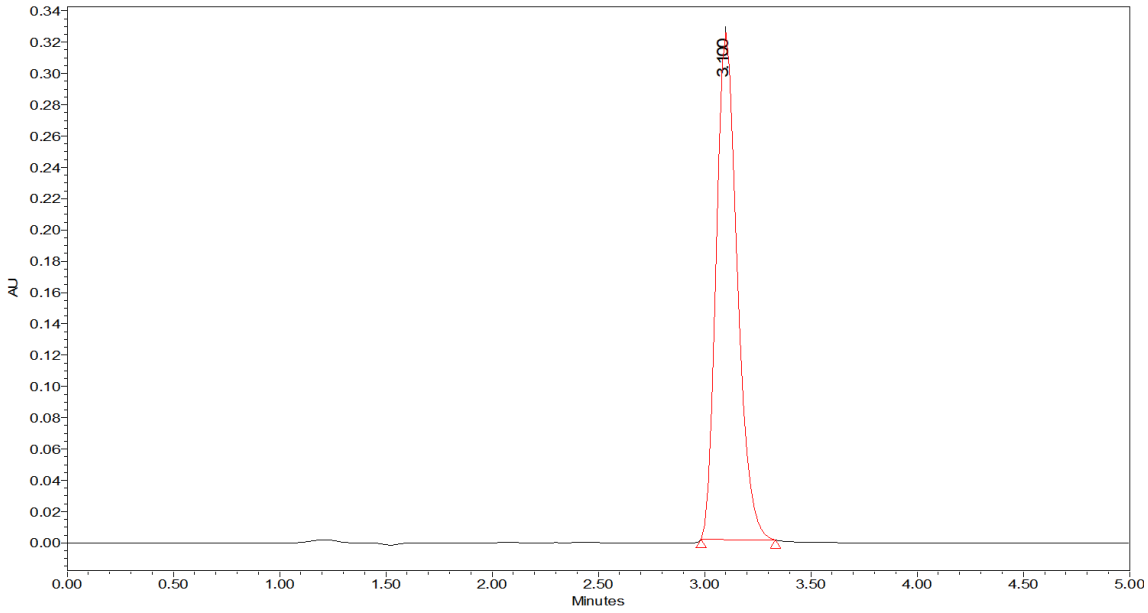


Fig. 5-1 Representative HPLC chromatogram of ibuprofen

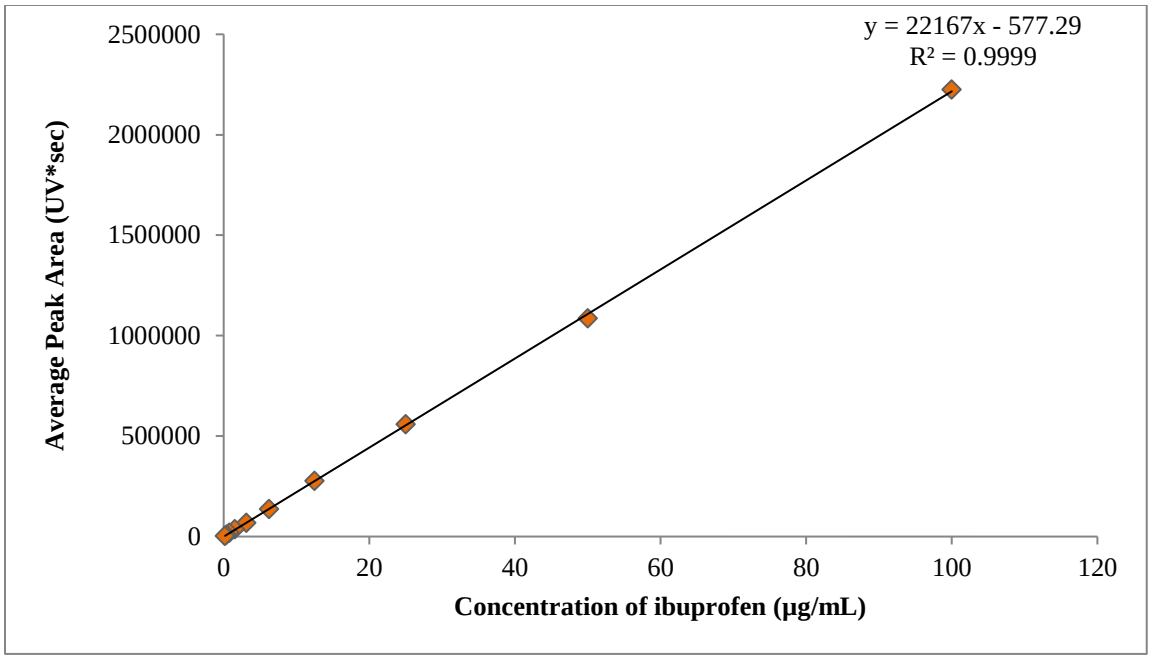


Fig. 5-2 Calibration curve of ibuprofen in ACN

5.2 Viscosity

All three formulations F1, F2, and F3, and the marketed gel product AGFD exhibited non-Newtonian pseudoplastic behavior. Pseudoplastic fluids are also called shear-thinning fluids as these fluids exhibit a reduction in viscosity with an increase in shear rate [151, 152]. This flow behavior is evident from the flow curves represented in Fig. 5-3 and Fig. 5-4. The change in viscosity with increasing shear rate is presented in Fig. 5-5 and Fig. 5-6. It can be observed that all the formulations and AGFD tended to behave like Newtonian fluids at low shear rates [151, 153].

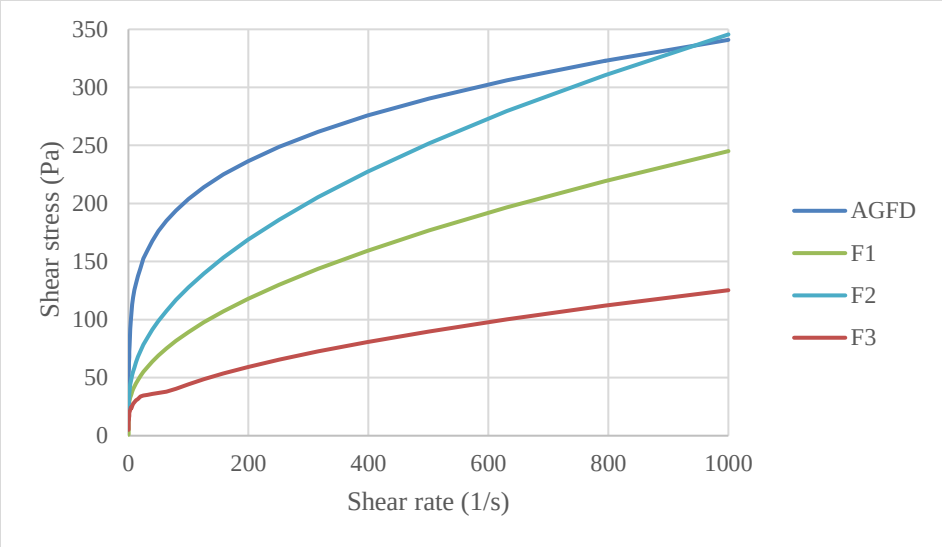


Fig. 5-3 Flow curves of F1, F2, F3 and AGFD at 20°C

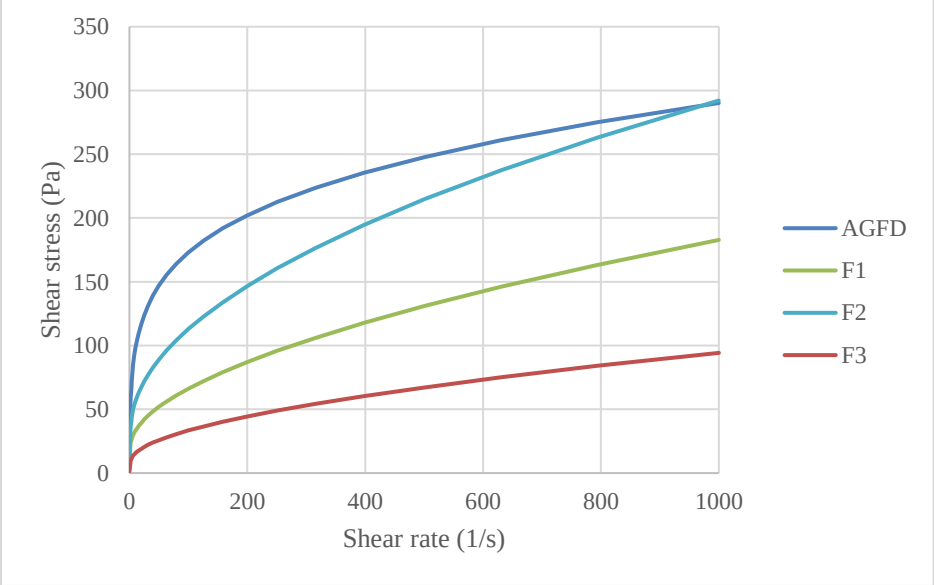


Fig. 5-4 Flow curves of F1, F2, F3 and AGFD at 32°C

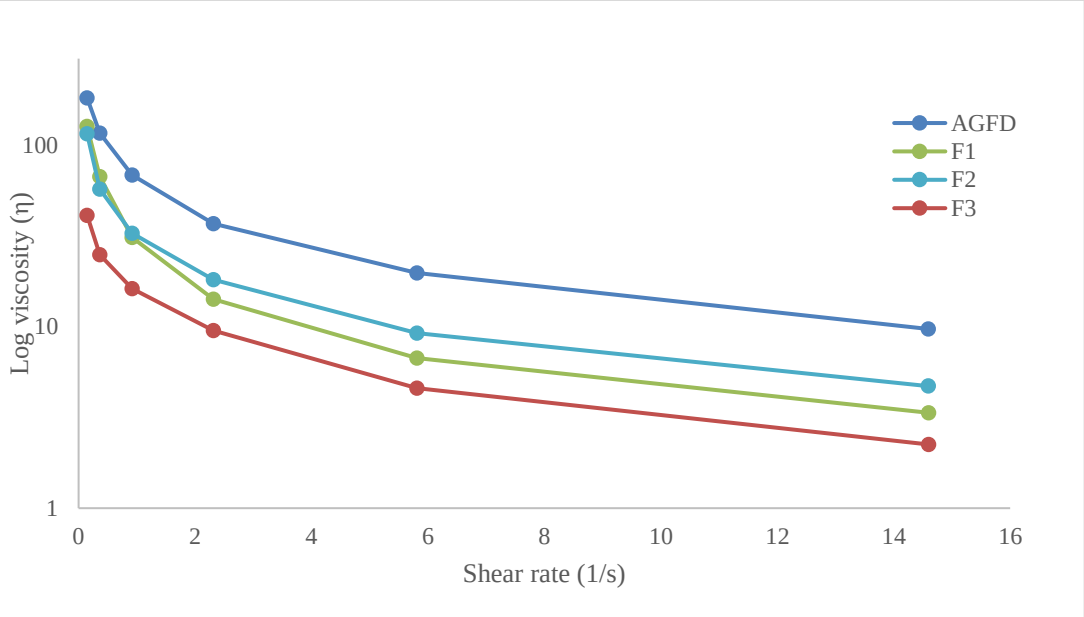


Fig. 5-5 Log viscosity (η) vs. shear rate at 20°C

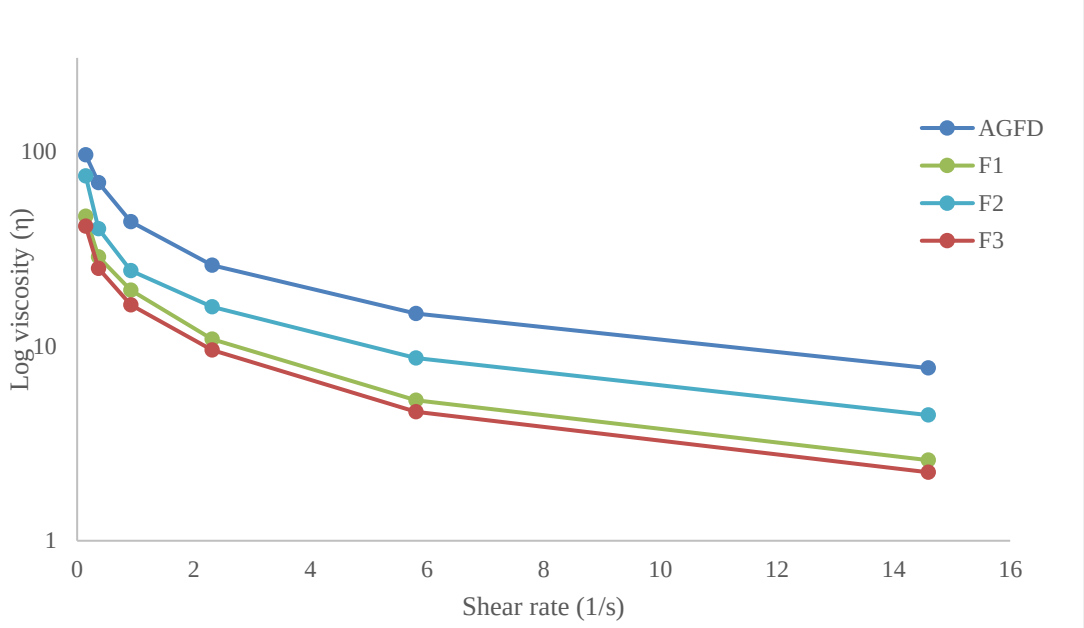


Fig. 5-6 Log viscosity (η) vs. shear rate at 32°C

The average viscosity of the emulgel formulations was found to be in the range of 38-141 Pa.s at 10 rpm at 20°C while AGFD displayed a higher average viscosity of 175 Pa.s at 10 rpm at 20°C, which emulates the temperature for storage. Similarly, the emulgel formulations displayed significantly lower average viscosity values ranging from 67-11 Pa.s at 10 rpm at 32°C, while AGFD had an average viscosity value of 93 Pa.s at 10 rpm at 32°C, which is the temperature of human skin. The average viscosity values at 10 rpm at both temperatures are reported in table 5-2. A comparative profile of average viscosity of F1, F2, F3 and AGFD is presented in Fig. 5-3. It is evident that AGFD had a significantly higher average viscosity than F2 and F3. Although lower, the average viscosity of F1 is comparable to AGFD. The higher viscosity of AGFD can be attributed to a higher amount of gelling agent in the monophasic structure. In contrast, emulgels are biphasic semi-solid formulations with an oil phase, which tends to influence the viscosity. The inter-particle interactions between the oil droplets and the continuous gel phase affects the viscosity of the biphasic system. Primarily, the viscosities of the oil phase components are critical in determining the final viscosity of a formulation [154-156]. When comparing the emulgels, F1 had the highest viscosity and F3 had the lowest viscosity at 20°C. At 32°C, F2 had the highest viscosity and F3 has the lowest viscosity. These differences may be attributed to the inherent difference in the composition of the oil phase of the emulgels [157, 158].

It can also be observed that the viscosity of all the formulations decreased with an increase in temperature from 20°C to 32°C. This can be explained by the fact that

intermolecular forces of cohesion decreased with increasing temperature and this caused a decrease in the resistance to flow [159].

Table 5.2 Average viscosity of F1, F2, F3 and AGFD at 20°C and 32°C at 10 rpm

Formulation	**Average viscosity and SD (Pa.s) at 10 rpm**	
	20°C	**32°C**
F1	140.63 ± 0.06	42.57 ± 0.09
F2	100.89 ± 0.09	66.89 ± 0.07
F3	37.88 ± 0.02	10.93 ± 0.03
AGFD	174.95 ± 0.07	92.96 ± 0.05

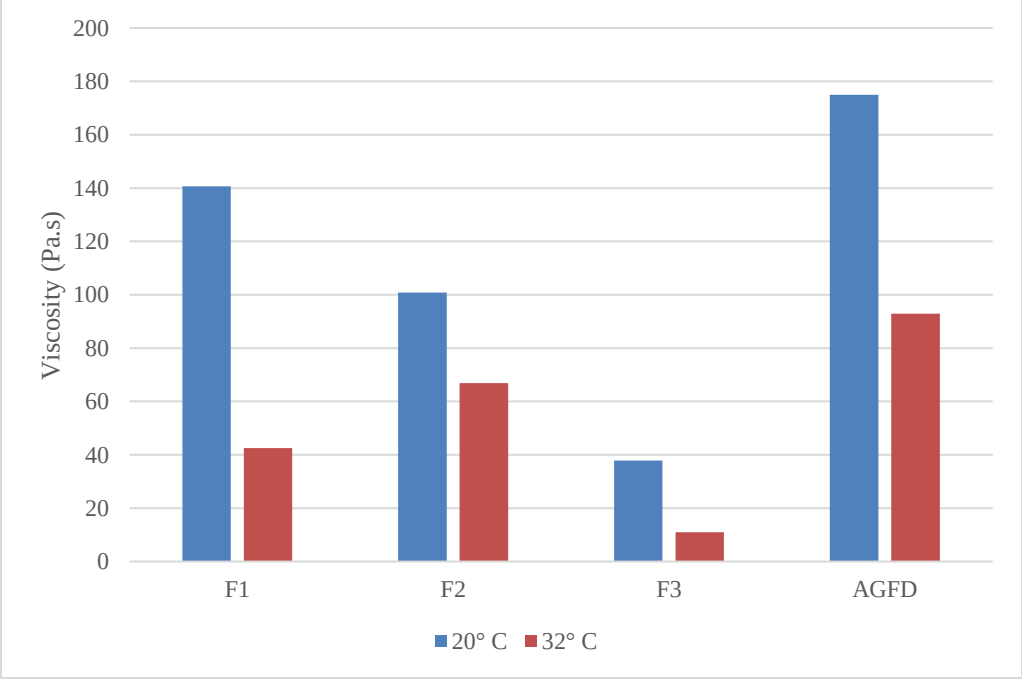


Fig. 5-7 Average viscosity of F1, F2, F3 and AGFD at 20°C and 32°C

5.3 pH

The average pH of the formulations was found to be in range of 5.48 to 5.65, while the average pH of AGFD was found to be 6.22. The values are reported in table 5-3. The pH of the skin ranges from 4 to 6 [160], depending on the gender, age and body part. The pH range of the emulgel formulations was considered optimal for topical delivery as there is a minimal potency for causing irritation to the skin [160, 161]. However, the pH of the formulation also plays an important role in determining the ratio of ionized to unionized species of a drug [162, 163]. The pK_α of ibuprofen is 4.91 [139] and at a pH range of 5.48 to 5.65, the percent of unionized ibuprofen ranges from 21.2 to 15.4%, respectively, as calculated using Henderson-Hasselbalch equation for weak acids:

$$pH = pk_\alpha + log\frac{(A^-)}{(HA)} \qquad \text{Eq. 5.2}$$

where (A^-) and (HA) represent the concentration of ionized and unionized species [164]. As per the pH partition theory, only the unionized form of a drug is able to pass through the lipoidal barrier of the SC. However, it has been reported that the transport of ionized species also contributes to the permeation of drug molecules [165-167]. The optimal pH value of the formulation has been shown to be one unit below the pK_α of a weakly acidic drug provided that such a pH value is within the acceptable range for topical delivery of drugs [165]. Not only does the pH determine the percent of ionized and unionized species, but it also impacts the solubility of the drug. Ibuprofen has been shown to have a pH-dependent solubility and the flux has been shown to be highest at pH 7 where the drug exists in predominantly ionized state. Conversely, the permeability coefficient of ibuprofen decreases with an increase in pH. This paradoxical behavior can be partly explained by the

fact that at a higher pH, the increased solubility compensates for the decreased permeability of the ionized species [168]. It is important to note that the pH of the receptor medium in the Franz cells was 7.4, which is representative of the physiological pH of the blood. In the light of the above facts, this pH may have contributed to the permeation of ibuprofen across the membrane in the permeation study as the receptor fluid's pH has been found to influence the permeability coefficient of drugs under asymmetric testing conditions, i.e., different pH of the receptor and donor components [169].

Table 5.3 Range of pH of F1, F2, F3 and AGFD (values displayed as average ± SD)

Formulation	**pH range**
F1	5.65 ± 0.02
F2	5.48 ± 0.01
F3	5.63 ± 0.01
AGFD	6.22 ± 0.01

5.4 Drug content

The average drug content of all three formulations was found to be within the optimal range of 90-110% with a RSD value of 1.72%. The results are presented in table 5-4. As per the USP 40-NF 35, the assay values for content uniformity of topical semi-solid dosage form should range from 90-110% of the stated amount of drug with a RSD of not more than 6% [170]. The values for F1, F2 and F3 are indicative of an acceptable content uniformity.

Table 5.4 Average drug content values (± RSD)

Formulation	Average drug content (%)
F1	97.59 ± 1.82
F2	97.75 ± 1.47
F3	96.50 ± 1.85

5.5 Spreadability

Table 5-5 summarizes the mean maximum positive force and mean positive area obtained from the texture analysis of F1, F2, F3 and AGFD. The texture analysis curves are presented in Fig. 5-8. Spreadability, as a textural property, refers to the ease of spreading a product from the point of application to the adjacent areas [171]. It is often one of the most important attributes of topical dosage forms that affect patient acceptability, the ease of application and clinical efficacy [172-174]. Firmness, expressed as the maximum force required to obtain a given deformation, is highly correlated to spreadability [175]. From the texture analysis curve in Fig. 5-8, it can be observed that AGFD has the maximum value for force and positive area under the curve, followed by F2>F1>F3. Consequently, the emulgels are easier to spread and rub in to the affected area as compared to AGFD.

Table. 5.5 Texture analysis parameters of F1, F2, F3 and AGFD

Formulation	Maximum positive force (g)	Positive area under the curve (g.s)
F1	79.61	59.83
F2	109.58	90.14
F3	60.97	44.14
AGFD	214.99	189.81

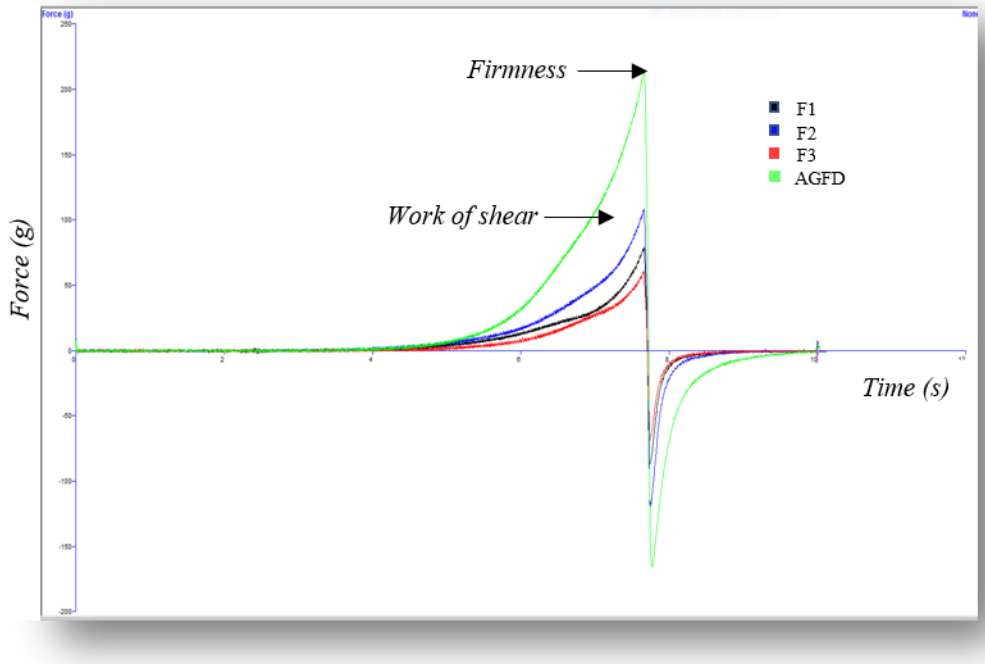


Fig. 5-8 Texture analysis curves of F1, F2, F3 and AGFD

5.6 DSC

The DSC thermograms of pure ibuprofen, the blank formulations of F1, F2 and F3, drug-loaded formulations of F1, F2 and F3, and AGFD are presented in Fig. 5-9 to Fig. 5-13. The DSC thermogram of ibuprofen showed a sharp endothermic peak at 80.48°C, which corresponds to the melting point of ibuprofen [139]. The DSC thermograms of the blank and drug-loaded F1, F2, F3, and AGFD showed a characteristic broad melting endotherm. These are indicative of the semi-crystalline nature of the formulation in both the emulgels and the gel [176]. In the light of the results from the optical imaging analysis, it can be concluded that undissolved ibuprofen is present in crystalline form in the formulation base. The lack of endotherm of drug can be attributed to the melting and solubilization of drug in the formulation base during the heating process. Such behavior has been characterized in the thermograms of physical mixtures of crystalline drugs and polymers [176-178]. It is also reported that some transitions in DSC thermograms are missed as these are smaller than the major transitions or due to a faster scan rate [179].

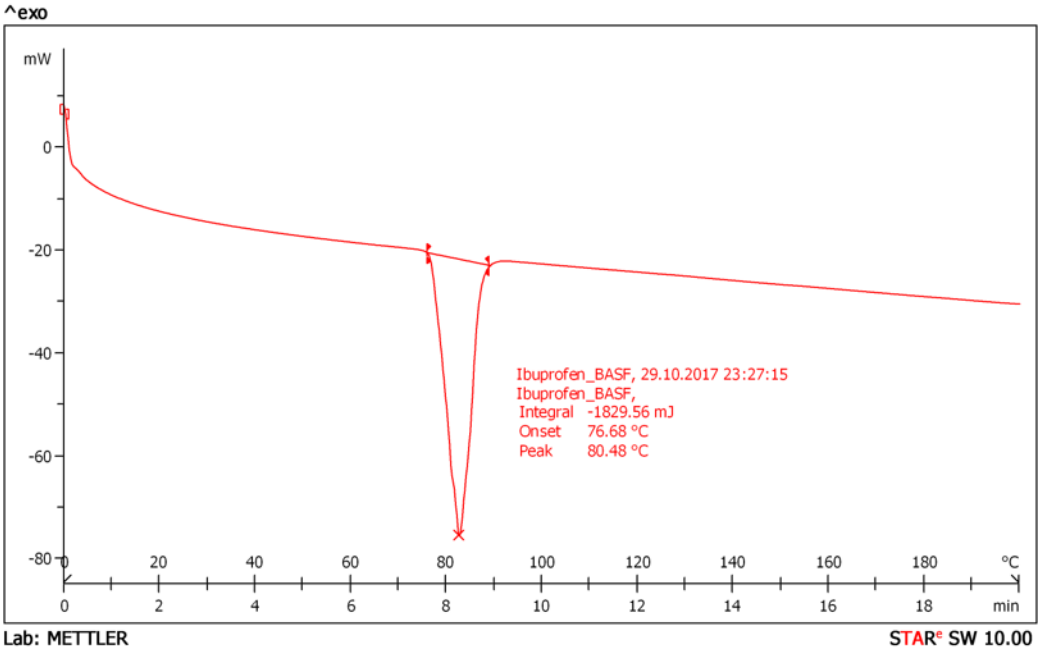


Fig.5-9 DSC thermogram of pure ibuprofen

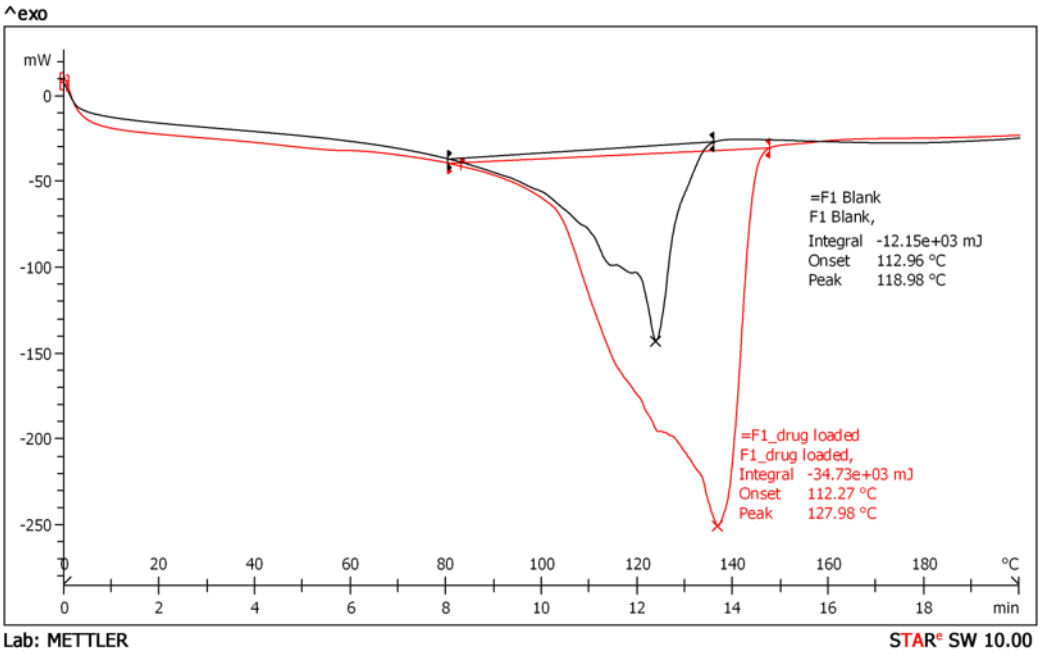


Fig. 5-10 DSC thermograms of blank F1 and drug-loaded F1

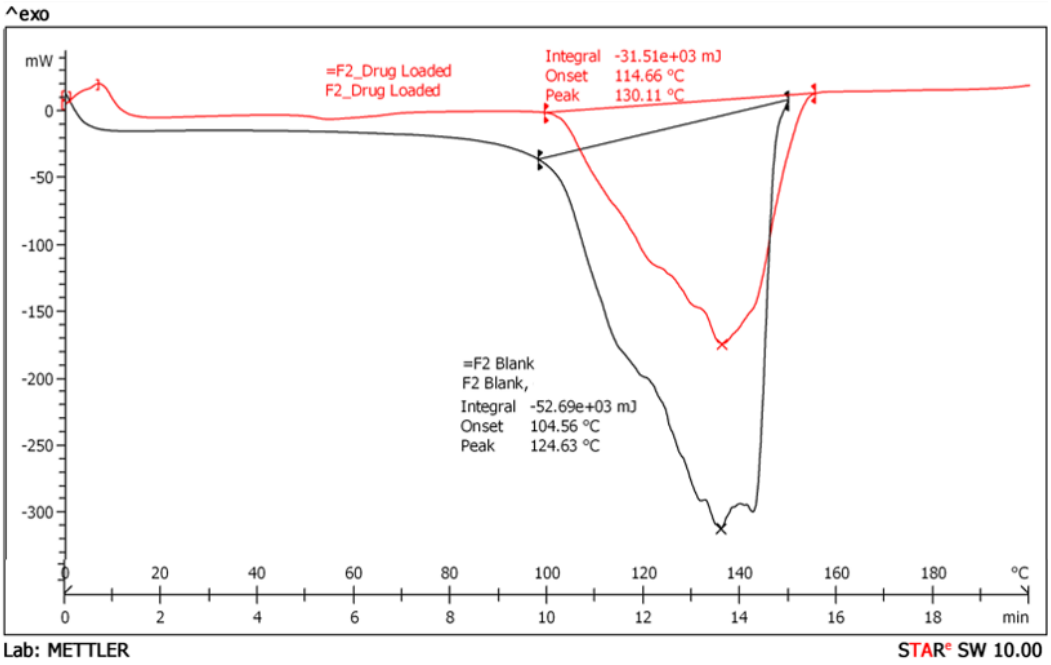


Fig. 5-11 DSC thermograms of blank F2 and drug-loaded F2

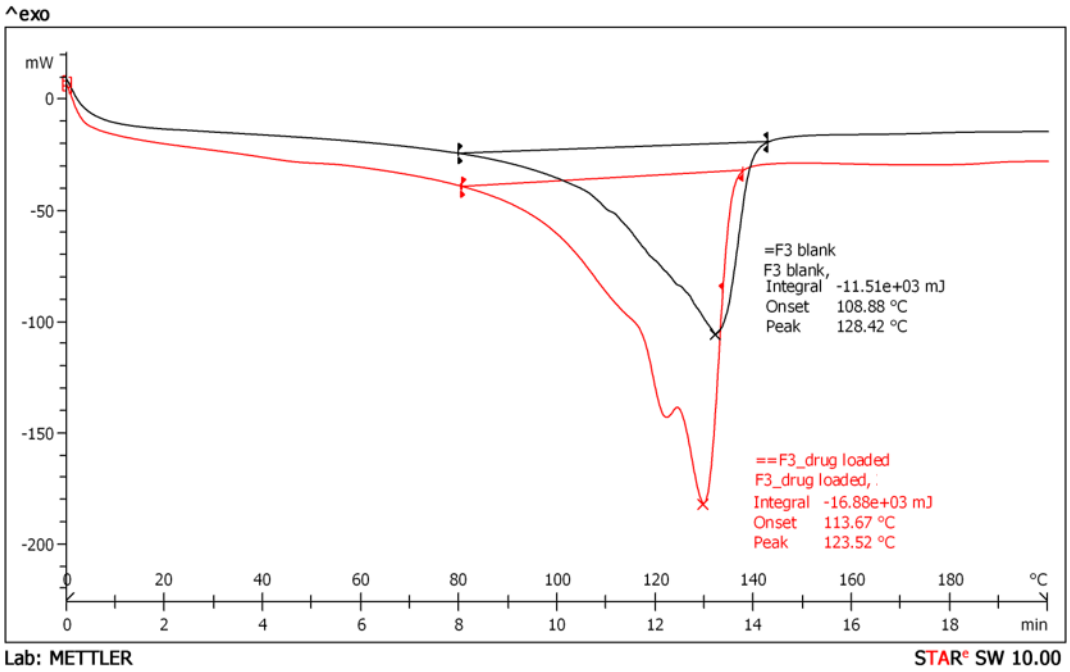


Fig. 5-12 DSC thermograms of blank F3 and drug-loaded F3

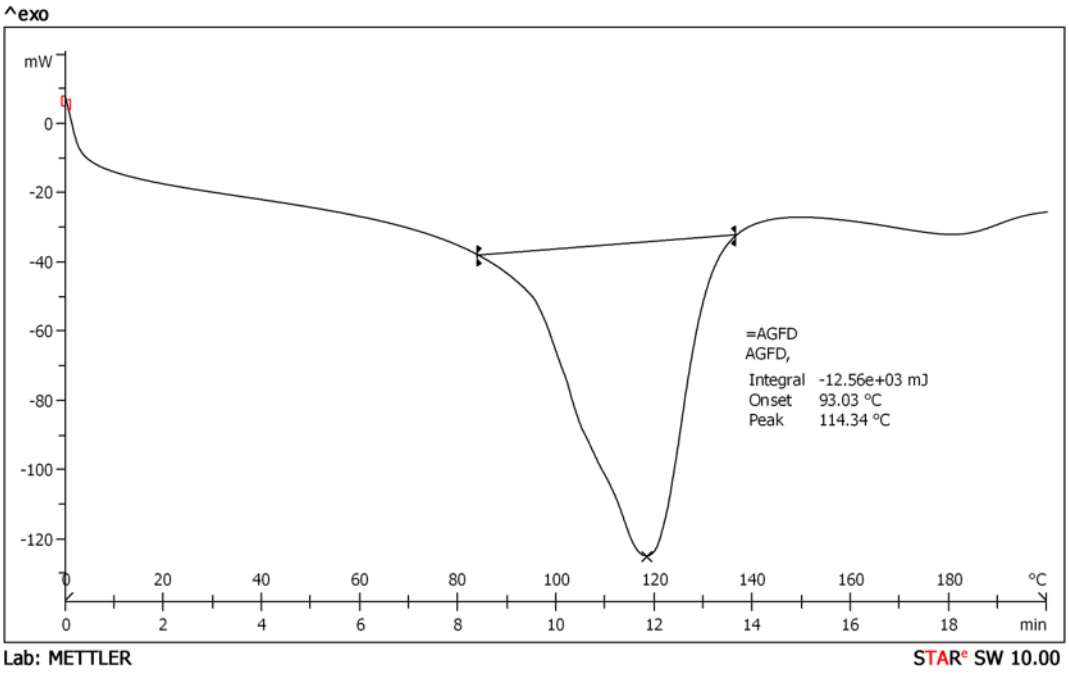


Fig. 5-13 DSC thermogram of AGFD

5.7 Optical imaging analysis

The images of F1, F2 and F3 captured under 10x and 40x magnification are presented in Fig. 5-14. through Fig. 5-19. The presence of insoluble drug crystals is evident in all formulations F1, F2 and F3 under the 10x magnification. This can be attributed to the low solubility of ibuprofen in the continuous phase of the emulgel, which is aqueous in nature [180]. As the drug partitions between the two phases of the emulgel system, it precipitates as an insoluble fraction in the aqueous gel phase [181]. However, the presence of drug in the crystalline form of ibuprofen in the emulgels is not confirmed by DSC thermograms. This may be due to undetectable amount of crystalline drug in DSC samples. It is also reported that some transitions in DSC thermograms are missed as these are smaller than the major transitions or due to a faster scan rate [179]. The presence of insoluble drug crystals may pose an impediment to diffusion of the drug into the skin layers. A drug should be soluble in the vehicle to diffuse readily into the skin [182]. The use of anti-nucleating polymers, such as hydroxypropyl methylcellulose (HPMC) or polyvinylpyrrolidone (PVP) has been associated with the prevention of crystallization in supersaturated systems [44, 183, 184]. Using higher amounts of solubilizing agents, such as DMSO, DMI or propylene glycol may also result in an increased flux due to an enhanced solubility of drug in the vehicle [185, 186].

Under the 40x magnification, the oil droplets of the biphasic emulgels can be clearly observed. The oil phase of the emulgels serves as a reservoir for lipophilic drugs [187]. Due to the complex gel structure, emulgel systems also facilitate a sustained release

of drugs [187-190] making them suitable NSAID-carrying vehicles for the management of chronic musculoskeletal pain.

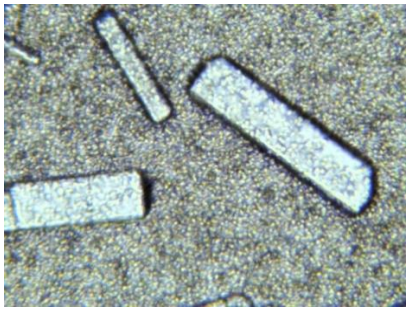


Fig. 5-14 F1 under 10x

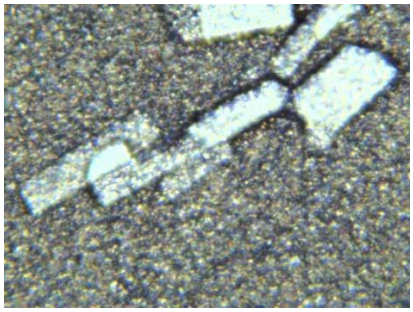


Fig. 5-15 F2 under 10x

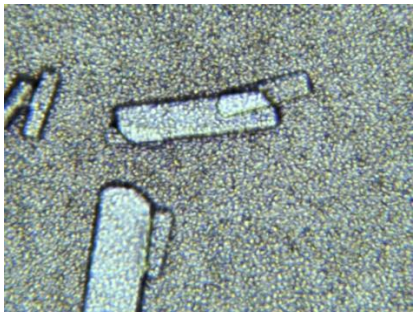


Fig. 5-16 F3 under 10x

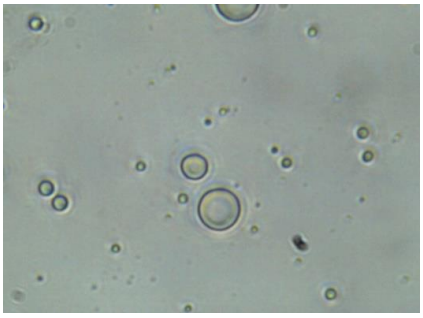


Fig. 5-17 F1 (diluted) under 40x

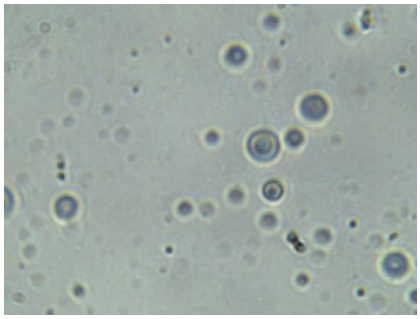


Fig. 5-18 F2 (diluted) under 40x

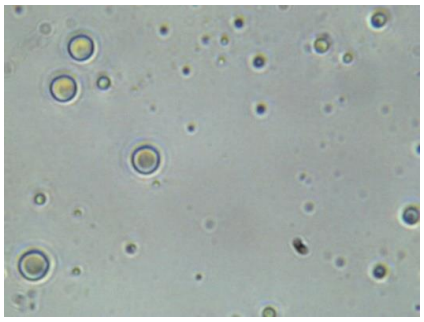


Fig. 5-19 F3 (diluted) under 40x

5.8 *In vitro* release and *in vitro* permeation of ibuprofen

5.8.1 *In vitro* release of ibuprofen

Table 5-6 summarizes the average percent release of ibuprofen from F1, F2, F3 and AGFD at 0.5, 6 and 24 hrs. Fig. 5-20 shows the release profile of ibuprofen from F1, F2, F3 and AGFD. A comparative representation of the percent release of ibuprofen from F1, F2, F3 and AGFD is shown in Fig. 5-21. The release data of F1, F2, F3 and AGFD were fitted to different kinetic models and were found to follow first-order release kinetics with an R^2 value of 0.9911. While no statistically significant difference was found in the percent release of ibuprofen from F1 and F2, F1 and F2 showed highest release followed by F3 and AGFD. All three formulations showed a significantly higher release than AGFD. The fraction of the dissolved drug in the vehicle, the nature of vehicle, the pH of the formulation and the receptor medium, and the log P of the drug are important factors affecting the release of the drug from the vehicle [169, 191-193]. While F1 contained no penetration enhancer, F2 contained DMI, F3 contained both DMI and IPM, and AGFD contained propylene glycol and isopropyl alcohol as mentioned on the product label. In addition to the differences in the composition, the inherent differences in the structure of emulgel and gel should be considered as well to understand the release trend. The aqueous nature of the gel may not allow faster diffusion of lipophilic drugs like ibuprofen whereas the biphasic emulgels with an inner oil phase may offer better diffusion of such molecules. Thus, the effective amount of ibuprofen available for release across the membrane may be higher in the emulgels as compared to AGFD. Furthermore, F1, which did not contain DMI, and F2, which contained DMI, showed a comparable percent release of ibuprofen over 24 hrs. It is

important to note that DMI mainly acts by altering the polarity of the SC [127, 194] and IPM increases the fluidity of the lipids present in the SC [127, 185, 195]. Because the cellulosic membrane used in the release study is not rate-limiting to drug release, the penetration enhancement effect of DMI and IPM is irrelevant to the release of ibuprofen.

Table 5.6 Average release of ibuprofen from F1, F2, F3 and AGFD (±SD)

(* indicates statistically significant figures at $p < 0.05$)

Formulation	**Average release (%)**		
	0.5 hr	**6 hrs**	**24 hrs**
F1	13.6 ± 0.4	86.4 ± 0.6	98.1 ± 0.5
F2	13.0 ± 0.1	77.7 ± 0.5	91.9 ± 1.0
F3	12.5 ± 0.3*	59.7 ± 3.5*	76.1 ± 2.9*
AGFD	7.3 ± 0.7*	32.0 ± 9.8*	35.0 ± 8.1*

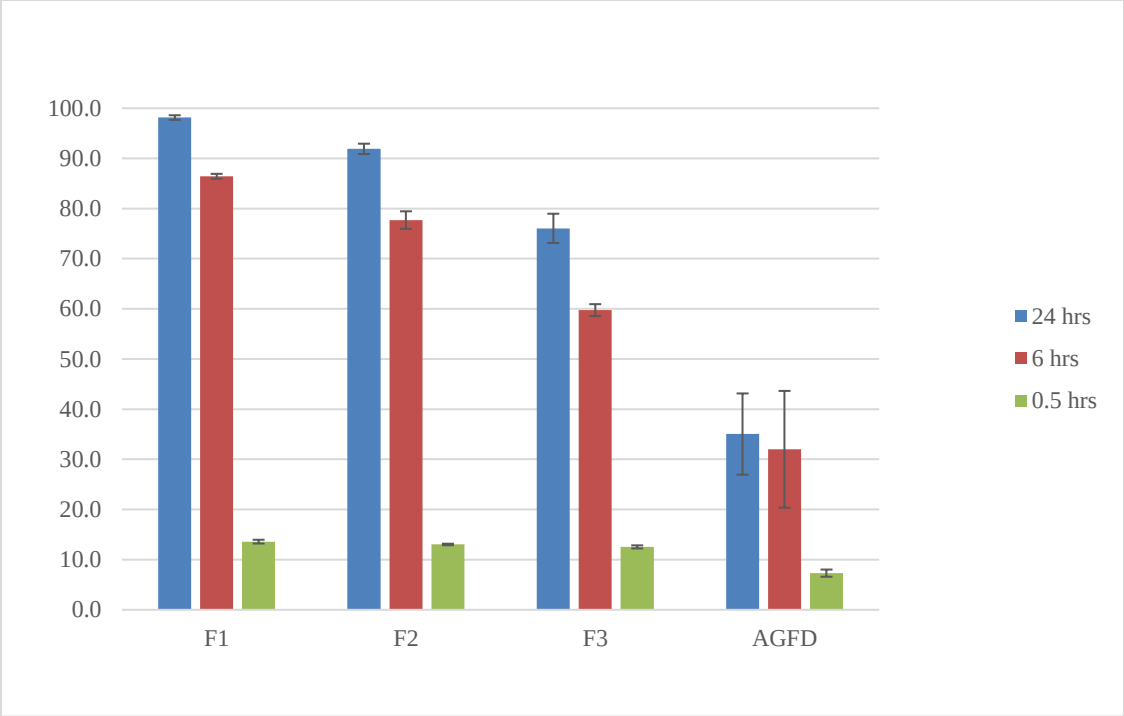


Fig. 5-20 Average release (%) of ibuprofen from F1, F2, F3 and AGFD

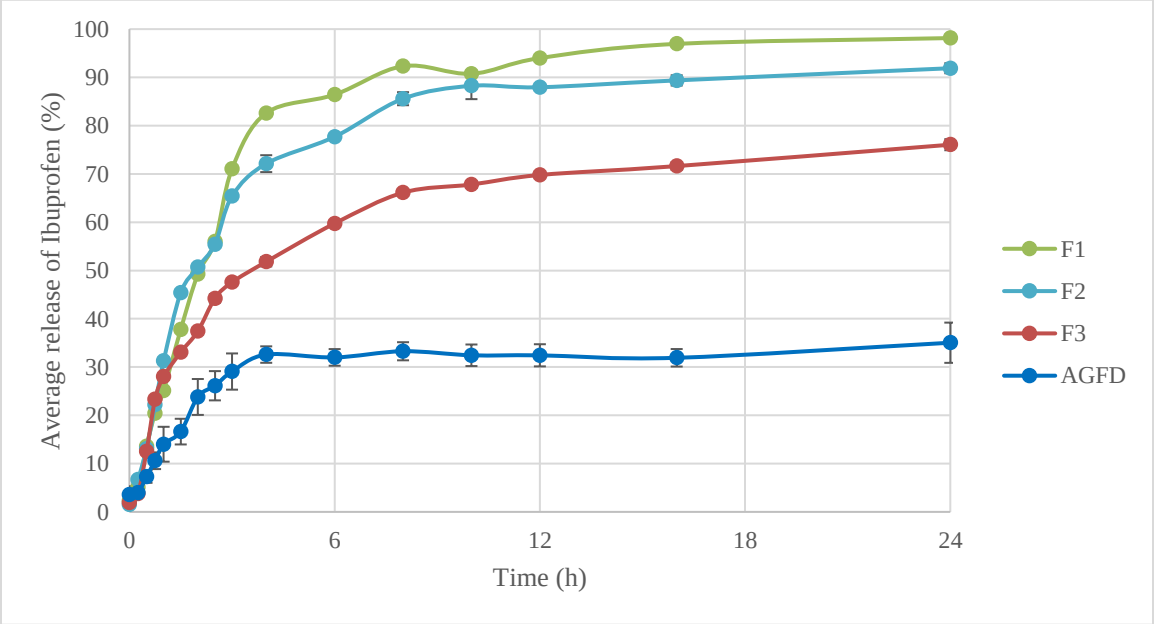


Fig. 5-21 Release profile of ibuprofen from F1, F2, F3 and AGFD

5.8.2 *In vitro* permeation of ibuprofen

Table 5-5 summarizes the average percent permeation of ibuprofen from F1, F2, F3 and AGFD at 2, 6 and 12 hrs. A comparative representation of the average percent permeation is presented in Fig. 5-22. F3 showed the highest permeation over 12 hrs, while no significant difference was found in the permeation of F1 and F2. All three emulgel formulations showed a significantly higher permeation than AGFD. Fig. 5-23 presents the permeation profile over 12 hrs. It was observed that F3 and F2 showed a higher sustained permeation as compared to F1 and AGFD. Also, F3 showed a faster permeation than F1, F2 and AGFD. Table 5-6 summarizes the apparent permeability coefficient (P_{app}), steady-state flux (J_{ss}) and lag time (t_{lag}) of F1, F2, F2 and AGFD. It was observed that F3 exhibited the shortest lag time followed by AGFD<F2<F1. Thus, it can be concluded that the emulgel F3 is better suited for a sustained release of the drug over a longer period as compared to AGFD [196-198]. Interestingly, F3 showed the highest permeation over first the 2 hours followed by F1>AGFD>F2. This indicates that emulgels with suitable penetration enhancers may show a faster permeation as well as a sustained release over a longer period of time. It should be noted that F3 contained both DMI and IPM, while F2 contained only DMI, F1 contained no penetration enhancer and AGFD contained propylene glycol and isopropyl alcohol. Thus, it is imperative to use right penetration enhancers to achieve the desired rate of permeation of the active moiety. As mentioned previously in section 1.8, certain penetration enhancers may function as penetration retardants, or show little to no effect on penetration of an active moiety depending on the vehicle characteristics [129, 131]. In this study, using a combination of penetration enhancers, i.e., DMI and IPM, was found to be more effective at increasing the permeation than using a single penetration

enhancer, i.e., DMI. The different mechanisms of penetration enhancement of these two chemicals should also be considered.

It should also be noted that Strat-M® membrane, which is composed of multiple layers of polyester sulfone, acts as a skin-mimicking artificial membrane. A high degree of positive correlation has been observed between the permeability coefficients obtained through Strat-M® and human/pig ear skin [199, 200], especially for molecules with molecular weight between 155 and 288, and log P value between -0.90 and 3.53 [200]. However, the mechanism of rate-limitation of permeation of an active moiety through Strat-M® remains unknown. Thus, the effect of certain penetration enhancers may not be same when tested on human skin for different active moieties.

Table 5.7 Average permeation of ibuprofen from F1, F2, F3 and AGFD (±SD)

(* indicates statistically significant figures at $p < 0.05$)

Formulation	**Average permeation (%)**		
	2 hrs	**6 hrs**	**12 hrs**
F1	2.7 ± 0.1	17.9 ± 0.9	50.0 ± 1.1
F2	8.6 ± 0.2	28.1 ± 0.5	49.2 ± 0.3
F3	18.1 ± 0.5*	50.8 ± 1.5*	69.8 ± 1.5*
AGFD	8.6 ± 0.9*	16.1 ± 9.8*	26.7 ± 2.8*

Table 5.8 Apparent permeability coefficient (P_{app}), steady-state flux (J_{ss}) and lag time (t_{lag}) of F1, F2, F3 and AGFD

Formulation	**Apparent permeability coefficient (P_{app}) (cm hr^{-1})**	**Steady-state flux (J_{ss}) (µg hr^{-1} cm^{-2})**	**Lag time (t_{lag}) (hr)**
F1	0.007225 ± 0.00004	361.25 ± 2.16	2.75 ± 0.04
F2	0.003127 ± 0.00007	156.35 ± 3.40	1.57 ± 0.16
F3	0.007922 ± 0.00013	396.11 ± 6.33	0.78 ± 0.02
AGFD	0.003213 ± 0.00010	160.66 ± 4.75	0.80 ± 0.04

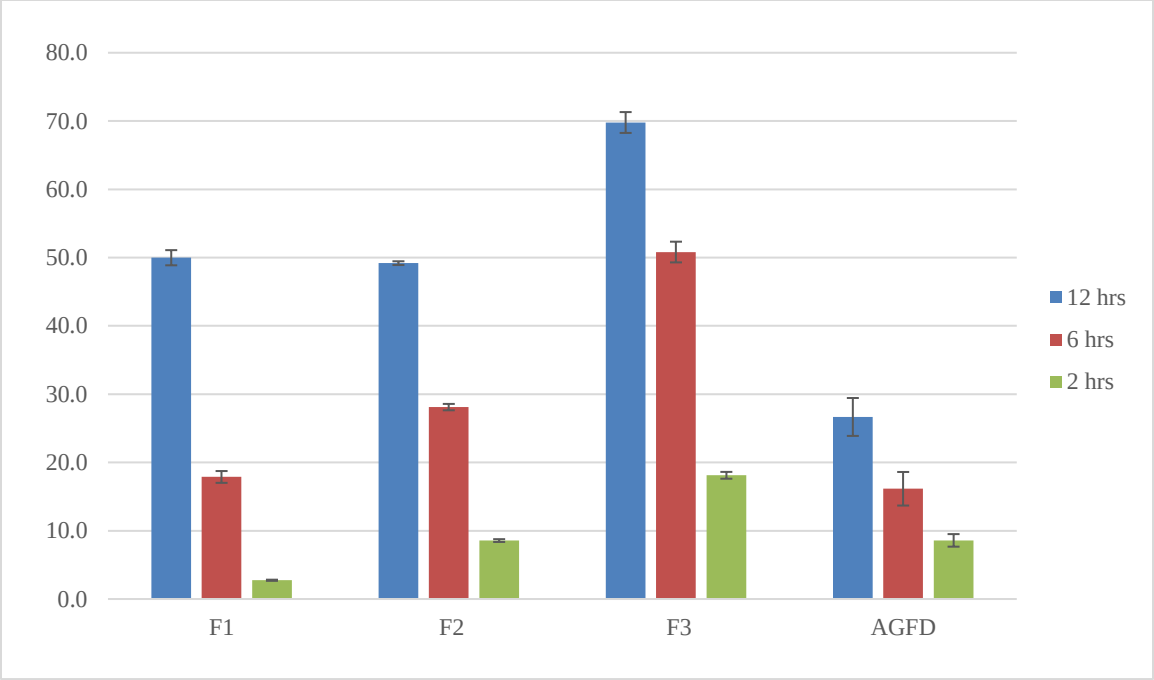


Fig. 5-22 Average permeation (%) of ibuprofen from F1, F2, F3 and AGFD

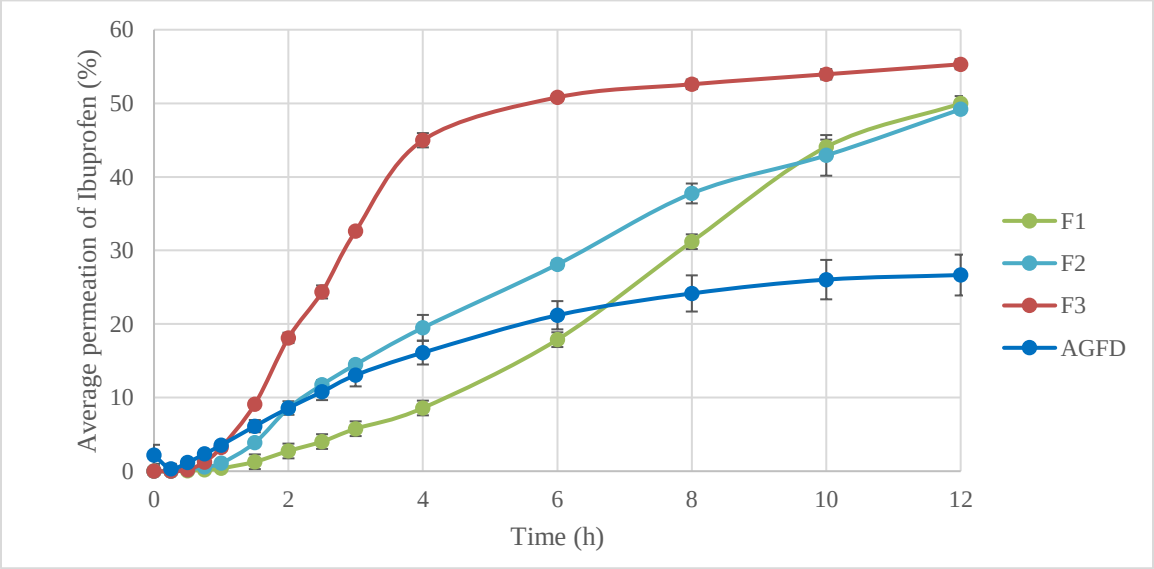


Fig. 5-23 Permeation profile of ibuprofen from F1, F2, F3 and AGFD

Chapter 6

Conclusions

In the present study, three different emulgels of ibuprofen were prepared and evaluated for physiochemical properties, *in vitro* drug release and *in vitro* drug permeation. The oil phase of the emulgel formulations was designed using FFE software. The three emulgels were compared to AGFD in terms of viscosity, pH, spreadability, *in vitro* drug release and *in vitro* drug permeation. All three emulgels were found to be better than AGFD in terms of spreadability, *in vitro* drug release and *in vitro* drug permeation. In terms of viscosity, the lower viscosity of the emulgels can be correlated to their greater spreadability, which facilitates the easier application and improves the patient compliance. The pH of the emulgels was found to be close to that of the human skin, which minimizes the potential for irritation. The role of the formulation pH in determining the fraction of drug that is present in an ionized state was also studied. The release of ibuprofen across a cellulosic membrane was found to be significantly greater for all three emulgels as compared to AGFD. Similarly, the permeation of ibuprofen across a human skin-like synthetic membrane was found to be greater for all three emulgels as compared to AGFD. From this study, it can be concluded that emulgels serve as a better vehicle for ibuprofen than gels. However, the presence of undissolved ibuprofen suggests the use of a higher

proportion of oil phase/solubilizing agents sufficient to fully dissolve the ibuprofen. This may significantly alter the properties of the emulgels. Also, the use of a combination of penetration enhancers in F3 resulted in a better permeation as compared to F1 and F2. This suggests that the use of penetration enhancers, which work by different mechanisms may have been helpful in improving the overall permeation of ibuprofen in F3.

Chapter 7

Future Studies

Certain variations in experimental parameters can shed further light upon the observations from this research work. Varying the pH of the formulation and the pH of the receptor fluid in the Franz cells can help understand the role of pH in the drug transport across the skin. Particularly, both symmetric conditions, i.e., same pH of formulation and receptor fluid, and asymmetric conditions, i.e., different pH of formulation and receptor fluid, should be studied. This will be helpful in investigating the effect of the pH of the vehicle, the skin and the blood on the permeation and release of ibuprofen. Furthermore, using freshly excised pig ear skin in Franz cell studies will help elucidate the transport pathways and retention of ibuprofen in the different strata of skin. It would also be useful to evaluate whether deep subcutaneous delivery of ibuprofen to the underlying musculature can be successfully achieved using different classes of penetration enhancers. Such a study would require using human cadaveric skin, which will further shed light on transport pathways across the skin. Using a different polymer in the gel phase will help evaluate the effect of the polymer on the drug release and permeation.

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