DESIGN OF DICLOFENAC SODIUM TRANSDERMAL FILMS: PREPARATION, PHYSICAL CHARACTERIZATION, *EX VIVO* AND *IN VIVO* STUDIES

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NIGERIA

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NIGERIA

JUNE, 2014

DECLARATION

I declare that the work in this thesis entitled 'Design of diclofenac sodium transdermal films: preparation, physical characterization, *ex vivo* and *in vivo* studies' has been carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, under the supervision of Dr. T.S. Allagh, Prof. A.R. Oyi and Prof. K.C. Ofokansi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This thesis entitled 'DESIGN OF DICLOFENAC SODIUM TRANSDERMAL FILMS: PREPARATION, PHYSICAL CHARACTERIZATION, *EX VIVO* and *IN VIVO* STUDIES' by Nkechi Ngozi Ogwu meets the regulations governing the award of the degree of Master of Science (Pharmaceutics) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

The work is dedicated to my parents Prof. and Prof. (Mrs) D. Ogwu.

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ABSTRACT

Diclofenac sodium is a non steroidal anti-inflammatory drug, which undergoes potential problems such as substantial hepatic first pass effect and gastrointestinal irritation when given through the oral route, and this necessitates an alternative choice of route for the administration of such drugs. The aim of this study was to formulate diclofenac sodium into a transdermal film using blends of Eudragit RS 100 (a hydrophobic polymer) and Hydroxypropylmethylcellulose HPMC (a hydrophilic polymer) as matrices, and to evaluate the formulation. The films were prepared by the solvent casting technique using diclofenac sodium as the drug of choice, plasticizer (PEG 8000), permeation enhancer (Tween 80), and varying the combinations of film forming polymers (Eudragit RS 100 and HPMC). The formulated films were subsequently evaluated for any possible interaction which may occur between the drug and the excipients using the Fourier Transform Infrared Spectroscopy (FT-IR) and the Differential Scanning Calorimetry (DSC) Technique. The physicochemical characteristics of the formulated films were evaluated with reference to their physical appearance, weight uniformity, thickness uniformity, folding endurance, moisture uptake, moisture loss, flatness and drug content. *Ex vivo* studies were also carried out using a modified Franz diffusion cell, and the films that showed the best pattern in terms of permeation rate were further studied for their *in vivo* anti inflammatory activities. Skin irritation studies were carried out, as well as stability studies at room temperature under a relative humidity of 75%. The FT-IR and DSC spectroscopy revealed that the drug was compatible with the excipients used in the formulation, while the physicochemical properties showed that films with a higher proportion of HPMC gave good mechanical and flexibility properties. The *ex vivo* permeation studies revealed that formulation F1 and F7 with a greater proportion of HPMC, gave a faster rate of permeation, with a cumulative

amount of drug permeation of 57.10 % and 45.61 % respectively, while formulation F5 with a high proportion of Eudragit RS 100 gave the least rate of permeation of 18.79 %. The *in vivo* anti inflammatory studies indicated that the edema volume for the test group remained well below that of the control group, and percentage inhibition was found to be 79 % and 88 % respectively for F1 and F7. The result of the skin irritation test revealed no erythema and no edema with the formulated films F1 and F7. The data obtained from the stability studies showed no change in the physical appearance and drug content of the formulated films, indicating that they are relatively stable. The result obtained indicates the feasibility for a transdermal delivery of diclofenac sodium using Eudragit RS 100 and HPMC polymer blends as matrices.

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Abbreviations, Definitions, Glossaries and Symbols

CPE	Chemical penetration enhancers
DBT	Dibutyl phthalate
DMSO	Dimethyl sulfoxide
DS	Diclofenac sodium
DSC	Differential scanning calorimetry
EC	Ethyl cellulose
ERL 100	Eudragit RL 100
FT-IR	Fourier transform infrared spectroscopy
HPMC	Hydroxypropylmethyl cellulose
KTF	Ketotifen fumarate
NSAIDs	Non steroidal anti-inflammatory drugs
PEG	Polyethylene glycol
PVP	Polyvinyl pyrollidone
TDDS	Transdermal drug delivery system

CHAPTER ONE

1.0 INTRODUCTION

1.1 Brief History of Transdermal Drug Delivery Systems

Research and development activities are becoming far more vigorous in the field of novel drug delivery systems, than in the search for newer drugs. These novel drug delivery systems are partly developed by the application of the concepts and techniques of controlled release drug administration which can extend the potent life of an existing drug (Ramteke *et al*., 2012).

The route of administration of a particular drug is essential in eliminating some undesired properties of the drug. The most common form of drug delivery is the oral route, which has been utilized for centuries and most recently, through tablets and capsules. This route of administration has notable advantages and also significant drawbacks like first pass metabolism, drug degradation in gastrointestinal tract due to enzymes, pH etc (Latheeshjlal *et al*., 2011). Injectables came into being approximately 130 years ago, but have only become acceptable since the development of proper sterilization techniques. Topical application has also been used for centuries; the majority of these licensed preparations applied to the skin are aimed at delivering the drug for local, rather than systemic actions. These preparations tend to be relatively simple semi-solids such as creams, gels and ointments (Morrow *et al.*, 2007). In recent years, the transdermal route is now as successful an innovative area in drug delivery as the oral route, with about 40 % of drug delivery candidate products under clinical evaluation related to transdermal or dermal system (Kulkarni *et al.*, 2011). The potential of using intact skin as the route of drug administration has been known for several years. The inspiration of using skin for delivery of drug was from the ancient times. Ebers papyrus used the husk of castor oil plant bark imbibed with water placed on the

head for the relief of headache. The medicated plaster can be viewed as the first development of transdermal drug delivery as it became very popular in Japan as over the counter pharmaceutical dosage form (Aquil *et al.,* 2010).

Transdermal patches were developed in the 1970's, and the first was approved by the US Food and Drug Administration (USFDA) in 1979 for the treatment of motion sickness. It was a three-day patch that delivered scopolamine. In 1981, patches for nitroglycerin were approved for the management of angina pectoris (Patel *et al*., 2012).

Subsequently, patches containing clonidine, fentanyl, buprenorphine, levonorgestrel, lidocaine, norethisterone, estradiol, oxybutynin, testosterone and nicotine have received approval. Transdermal products containing granisetron, human growth hormone, insulin, parathyroid hormone and rotigotine are currently undergoing clinical trials (Morrow *et al.,* 2007).

A Transdermal Drug Delivery System (TDDS) can be defined as a self contained discrete dosage form, which when applied to the intact skin, delivers the drug(s) through the skin at a controlled rate to the systemic circulation with the aid of a patch (Vaseeha *et al.,* 2010). Transdermal absorption occurs through a slow process of diffusion driven by the gradient between the high concentration in the delivery system and the zero concentration prevailing in the skin. Thus, the delivery system must be kept in continuous contact with the skin for a considerable time (hours to days) (Kumar and Philip, 2007).

TDDS has gained a lot of interest during the last decade, as it offers many advantages over the conventional dosage forms and oral controlled release delivery systems. It avoids hepatic first pass effect, involves less frequency of administration, enjoys

reduction in gastrointestinal side effects and improvement in patient compliance (Patel *et al.,* 2009). For transdermal products the goal of dosage design is to maximize the flux through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin (Nagesh *et al*., 2011).

The transdermal delivery system is a growing market that is expected to expand in the near future with the discovery of new drug treatment applications and technologies (Patel *et al*., 2012).

1.1.1 Advantages of the transdermal drug delivery systems

i. Peaks and troughs in plasma level as experienced with oral drug delivery can be avoided, which reduces the risk of side effects (Eseldin *et al.,* 2010).

ii. Drugs that require consistent plasma levels are very good candidates for transdermal drug delivery. In addition, if toxicity were to develop from a drug administered transdermally, the effects could be terminated by removing the patch (Eseldin *et al.,* 2010).

iii. Transdermal delivery can increase the therapeutic value of many drugs by avoiding specific problems usually associated with some of the drugs e.g. gastro-intestinal irritation, low absorption, decomposition due to hepatic "first - pass" effect, formation of metabolites that cause side effects, short half life necessitating frequent dosing etc (Shahida *et al.,* 2012).

iv. The simplified medication regimen leads to improved patient compliance, improved adherence and reduced inter and intra patient variability (Nagesh *et al.,* 2011).

v. Self administration is possible with these systems (Arunachalam *et al.*, 2010).

vi. Controlled drug release can be achieved by TDDS which can deliver medicines via the skin portal to systemic circulation at a predetermined rate over a prolonged period of time (Rajendra and Kavitha, 2011).

vii. Allows continued drug administration, permitting the use of a drug with short biological half-life.

viii. Transdermal drug delivery can be used as an alternative delivery system for patients who cannot tolerate oral dosage forms or who are nauseated or unconscious.

1.1.2 Disadvantages of the transdermal drug delivery systems

i. One of the greatest disadvantages of transdermal drug delivery is the possibility of local irritation at the site of application. Erythema, itching, and local edema can be caused by the drug, the adhesive, or other excipients in the patch formulation. For most patients, site rotation can minimize irritation. However, some patients develop severe allergic reactions to transdermal patches, and in these cases therapy must be discontinued (Eseldin *et al.*, 2010).

ii. The barrier function of the skin changes from one site to another on the same person, from person to person and with age (Arunachalam *et al.*, 2010).

iii. Patches tend to be relatively complex systems, expensive to develop and manufacture (Morrow *et al.*, 2007).

1.2 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the oldest drugs. Despite the fact that NSAIDs are very well prescribed and are generally over-the-counter medications, new structures and formulations have been introduced by various research groups and pharmaceutical industries (Gulgun *et al.,* 2010).

NSAIDs have been the first line of choice for transdermal drug delivery system for a long time due to their improved localized effects and possibility of avoiding the gastro-irritating effects (Shahida *et al.,* 2012).

NSAIDs play an important role in reducing inflammation and pain. However, NSAIDs are associated with gastrointestinal side effects and generally cause gastritis, peptic ulceration due to inhibition of cyclooxygenase-2 and in some cases cyclooxygenase-1, which are essential for the production of prostaglandins and thromboxane associated with pain and inflammation (Shokri *et al.,* 2012).

1.2.1 Mechanism of action

Most non-steroidal anti-inflammatory drugs, act as nonselective inhibitors of the enzyme cyclooxygenase (COX), inhibiting both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes. COX catalyzes the formation of prostaglandins and thromboxane from arachidonic acid (itself derived from the cellular phospholipid bilayer by phospholipase A2). Prostaglandins act (among other things) as messenger molecules in the process of inflammation (Sean *et al*., 2009).

1.2.2 Diclofenac sodium

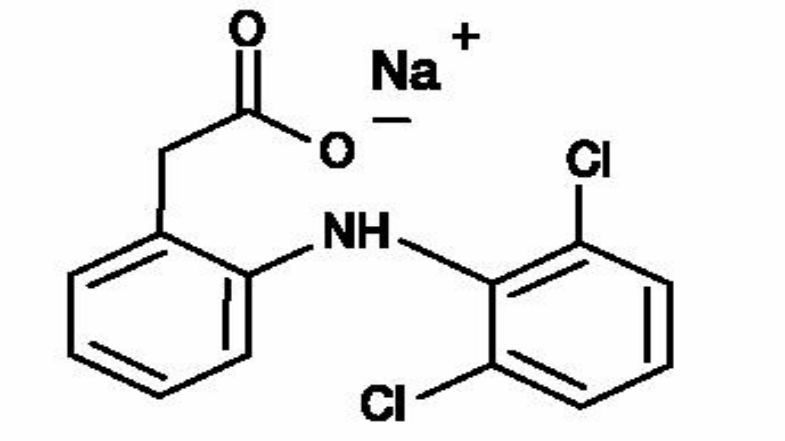


Figure 1.1: Diclofenac Sodium

Diclofenac sodium is a phenyl acetic acid derivative as shown in Figure 1.1. It is a non-steroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic and anti-pyretic activities in both animals and human beings (Syed *et al.,* 2010).

Diclofenac sodium is a white to off-white, hygroscopic, crystalline powder, sparingly soluble in water; soluble in alcohol, practically insoluble in chloroform and in ether, but is freely soluble in methyl alcohol. The pH of a 1 % solution in water is between 7.0 and 8.5. Its molecular weight is 318.14 (Sean *et al*., 2009).

1.2.2.1 Pharmacokinetic parameters of diclofenac sodium

Diclofenac sodium given orally is almost completely absorbed, it is subject to first-pass metabolism so that only about 50% of the drug reaches the systemic circulation in the unchanged form. It is also absorbed percutaneously. At therapeutic concentrations, it is more than 99% bound to plasma proteins. Diclofenac sodium penetrates the synovial fluid where concentrations may persist even when plasma concentrations fall. Small amounts are distributed into the breast milk. The terminal plasma half life is about 1- 2 hours.

Diclofenac sodium is metabolized to 4-hydroxydiclofenac, 5-hydroxydiclofenac, 3^1 – hydroxydiclofenac and 4^1,5-dihydroxydiclofenac. It is excreted in the form of glucuronide and sulfate conjugates mainly in the urine (about 60%) but also in the bile (about 35%), less than 1% is excreted as unchanged diclofenac (Sean *et al*., 2009).

1.2.2.2 Therapeutic information

Diclofenac sodium is indicated in the following conditions:

i. Inflammatory and degenerative forms of rheumatism: rheumatoid arthritis, ankylosing spondylitis, osteoarthritis.
ii. Post-traumatic and postoperative pain, inflammation, and swelling, e.g. following dental or orthopaedic surgery.
iii. Painful and/or inflammatory conditions in gynaecology, e.g. primary dysmenorrhoea (Sean *et al*., 2009).

1.2.2.3 Adverse effects

The commonest adverse effects associated with NSAIDs are the gastrointestinal disturbances. Occasionally, a patient may experience epigastric pain and other gastro-intestinal disorders like nausea, vomiting, diarrhea, and rarely gastro-intestinal bleeding, peptic ulcer. In isolated cases, peptic ulcer with perforated lower gut disorders (e.g. non-specific haemorrhagic colitis and exacerbations of ulcerative colitis), constipation and pancreatitis occur (Sean *et al*., 2009)

1.2.2.4 Dosing

For the symptomatic treatment of acute chronic rheumatoid arthritis the dose is 150 mg daily administered in divided doses of 75 mg (delayed release tablets only) twice daily or 50 mg three times daily (Sean *et al*., 2009).

1.3 Statement of Problem

Conventional systems of medications require multi dose therapy with a potential to cause numerous problems and complications. The design of tablets, or an injectable, to deliver the right amount of medicine at the right target site becomes complex if each medication were to be delivered in an optimal and preferred manner to the individual patient.

The administration of most NSAIDs by oral route is associated with potential disadvantages such as peptic ulceration and gastric irritation (Patel *et al.,* 2012). These severe drawbacks create a potential need for development of transdermal films of NSAIDs.

Diclofenac sodium also undergoes substantial hepatic first-pass metabolism when administered through the oral route and only about 50% of administered dose reaches systemic circulation, necessitating an alternative choice of route of administration for such drugs (Gattani *et al*., 2009).

1.4 Justification of the Study

The skin being the largest organ of the body plays an important role by offering selective entrance of molecules and preventing access of noxious matters through it. Therefore, drug delivery through the skin has gained recognition as an effective and suitable drug delivery system which will deliver drugs in a desired manner with reduced toxicity and improved compliance.

This route of drug delivery has become very attractive to many researchers due to the fact that, it does not cause pain or tissue damage, compared to those given intravenously

and intramuscularly. This helps alleviate fear from the patient and makes it much easier to administer.

1.5 Aim of Present Study

The aim of the present study is to formulate diclofenac sodium into a transdermal film using blends of Eudragit RS 100 (a hydrophobic polymer) and HPMC (a hydrophilic polymer) as matrices, and to evaluate the formulation.

1.6 Objectives

i. To formulate transdermal films using blends of Eudragit RS 100 and HPMC as polymers.

ii. To investigate the possible interactions between the drug and the various transdermal film excipients, using Fourier Transform Infrared Spectroscopy (FT-IR) analysis.

iii. To investigate drug-excipient compatibility using Differential Scanning Calorimetry (DSC).

iv. To characterize the formulated films using their physical appearance, weight uniformity, thickness uniformity, folding endurance, moisture uptake, moisture loss, flatness and drug content.

v. To investigate the *ex vivo* drug permeation characteristics of the formulated transdermal films using a Franz diffusion apparatus.

vi. To assess the degree of skin irritation of the formulated films.

vii. To evaluate the *in vivo* anti-inflammatory properties of the formulated films using an animal model.

viii. To investigate the stability properties of the various formulated films.

1.7 Research Hypothesis

1.7.1 Null hypothesis

Diclofenac sodium cannot be delivered transdermally when formulated into a transdermal film using Eudragit RS 100 and HPMC as polymer blends.

CHAPTER TWO

2.0 THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Anatomy and Physiology of the Human Skin

The skin is one of the most extensive organs of the human body covering an area of about 2 m^2 in an average human adult. This multilayered organ receives approximately one third of all blood circulating through the body (Harunusman *et al*., 2012). The human skin surface is known to contain, on an average, 10-70 hair follicles and 200-250 sweat ducts on every square centimeters, of the skin area (Eseldin *et al.,* 2010).

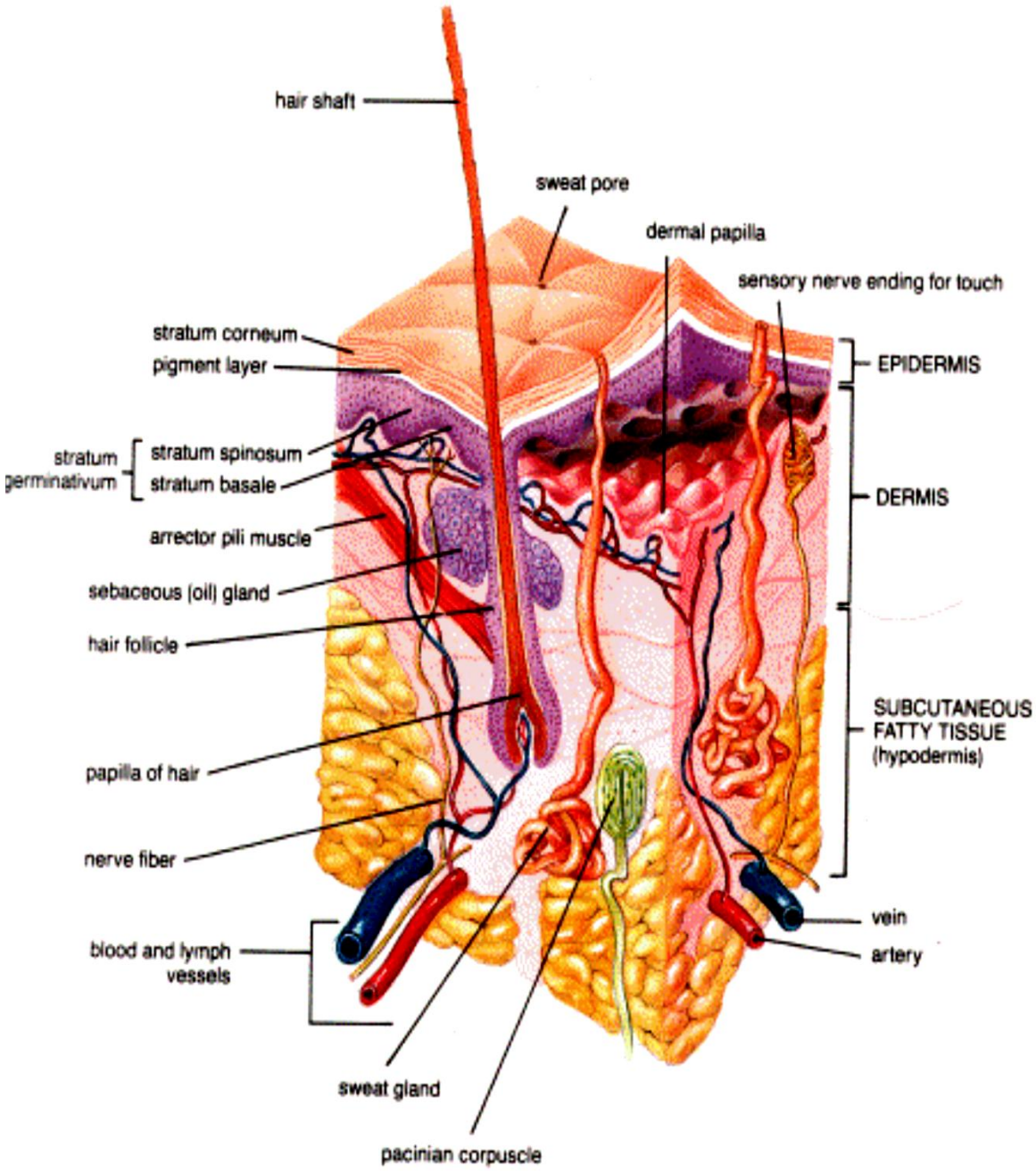


Figure 2.1: Structure of the Human Skin (Latheeshjlal *et al*., 2011)

Human skin comprises of three distinct but mutually dependent tissues as shown in Figure 2.1:

i. The stratified, vascular, cellular epidermis
ii. Underlying dermis of connective tissues
iii. Subcutaneous layer or hypodermis

Each layer has its own function and importance in maintaining the integrity of the skin and thereby the whole body structure.

2.1.1 The epidermis

Epidermis results from an active epithelial basal cell population and is approximately 150 µm thick. It is the outermost layer of the skin and the process of differentiation results in migration of cells from the basal layer towards the skin surface (Harunusman *et al*., 2012).

The epidermis is further divided into two parts, the non-viable epidermis (Stratum corneum) and the viable epidermis. The viable epidermis is divided into four layers, viz., stratum lucidium, stratum granulosum, stratum spinosum and stratum germinativum (Harunusman *et al*., 2012) (Figure 2.1).

2.1.2 Stratum corneum

The stratum corneum, or horny layer, is the outermost layer of the skin and has been identified as the principal barrier for percutaneous absorption (Latheeshjlal *et al*., 2011). The horny layer represents the final stage of epidermal cell differentiation. The thickness of this layer is typically 10 µm, but a number of factors, including the degree of hydration and skin location, influence it. For example, the stratum corneum on the palms and soles can be, on average, 400-600 µm thick, whilst hydration can result in a

4-fold increase in thickness (Morrow *et al.,* 2007). The stratum corneum consists of 10-25 rows of dead keratinocytes (corneocytes) embedded in a lipid matrix (Nikhil *et al*., 2011).

The corneocytes are flattened, elongated, dead cells, lacking nuclei and other organelle (Ramteke *et al.,* 2012). The cells are joined together by desmosomes, maintaining the cohesiveness of this layer (Morrow *et al*., 2007; Inayat and Mallikarjuna, 2009).

The main lipids located in the stratum corneum are ceramides, fatty acids, cholesterol, cholesterol sulphate and sterol/wax esters. These lipids are arranged in multiple bilayers called lamellae (Morrow *et al*., 2007). Phospholipids are largely absent, a unique feature for a mammalian membrane. The ceramides are the largest group of lipids in the stratum corneum, accounting for approximately half of the total lipid mass and are crucial to lipid organization of the stratum corneum (Morrow *et al*., 2007).

The architecture of horny layer may be modeled as a wall-like structure. In this model, the keratinized cells function as protein “bricks” embedded in lipid “mortar” The lipids are arranged in multiple bilayers. There is sufficient amphiphilic material in the lipid fraction, such as polar free fatty acids and cholesterol, to maintain a bilayer form (Nikhil *et al.,* 2011; Ramteke *et al.,* 2012). The epidermis contains no vascular elements. The cells receive their nourishment from the capillary beds located in the papillary layers of the dermis by diffusion of plasma and serum components (Latheeshjlal *et al*., 2011).

2.1.3 Viable epidermis

This is situated beneath the stratum corneum and varies in thickness from 0.06 mm on the eyelids to 0.8 mm on the palms. Going inwards, it consists of various layers as stratum lucidum, stratum granulosum, stratum spinosum and the stratum basal. In the

basal layer, mitosis of the cells constantly renews the epidermis and this proliferation compensates the loss of dead horny cells from the skin surface. As the cells produced by the basal layer move outward, they alter morphologically and histochemically, undergoing keratinization to form the outermost layer of the stratum corneum (Nikhil *et al*., 2011).

2.1.4 Dermis

Dermis is 3 to 5 mm thick layer and is composed of a matrix of connective tissue, which contains blood vessels, lymph vessels and nerves. The cutaneous blood supply has essential function in regulation of body temperature. It also provides nutrients and oxygen to the skin while removing toxins and waste products. Capillaries are within 0.2 mm of skin surface and provide sink conditions for most molecules penetrating the skin barrier (Harunusman *et al*., 2012).

2.1.5 Hypodermis

The hypodermis or subcutaneous fat tissue supports the dermis and epidermis. It serves as a fat storage area. This layer helps to regulate temperature, provides nutritional support and mechanical protection. It carries principal blood vessels and nerves to skin and may contain sensory pressure organs.

For transdermal drug delivery, drug has to penetrate through all these three layers and reach into systemic circulation while in case of topical drug delivery only penetration through stratum corneum is essential and then retention of drug in skin layers is desired (Sharma *et al*., 2011).

2.2 Drug Penetration Pathways

There are critically three ways in which a drug molecule can cross the intact stratum corneum.

i. Skin appendages (Shunt Routes)

ii. Intercellular lipid domains

iii. Transcellular route

A particular drug is likely to permeate by a combination of these routes, with the relative contributions of these pathways to the gross flux governed by the physicochemical properties of the molecule.

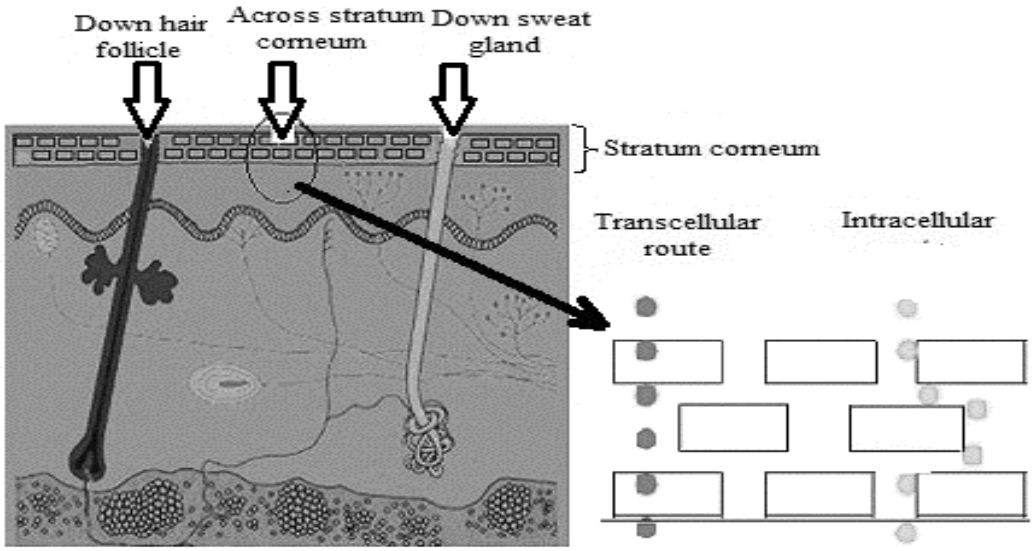


Figure 2.2: Permeation Pathways through the Skin (Morrow *et al*., 2007)

2.2.1 The appendageal route

Skin appendages provide a continuous channel directly across the stratum corneum barrier. However, their influence on drug penetration is hindered by a number of factors. The surface area occupied by hair follicles and sweat ducts is small (typically 0.1% of skin's surface area), therefore limiting the area available for direct contact of the applied drug formulation (Harunusman *et al*., 2012).

Sweat ducts are either empty or actively secreting an aqueous salt solution. Although an aqueous pathway across the skin is considered desirable for many drugs, permeation may be limited as sweat is travelling against the diffusion pathway of the permeating molecule. Sebaceous glands are filled with a lipid rich sebum, which may present a barrier to hydrophilic drugs (Morrow *et al.,* 2007).

Although the contribution of the appendgeal route is generally considered to have little influence on flux, it has been proposed that this route may be important for large polar molecules and ions (Morrow *et al.,* 2007).

2.2.2 Transcellular route

This is the most direct route. Here drug crosses the skin by directly passing through both the phospholipid membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. Although this is the path of shortest distance, the drugs encounter significant resistance to permeation. This is because the drugs must cross the lipophilic membrane of each cell, then the hydrophilic cellular contents containing keratin, and then the phospholipid bilayer of the cell one more time. This series of steps is repeated numerous times to traverse the full thickness of the stratum corneum (Sharma *et al.,* 2011).

2.2.3 Intercellular route

The more common route is through the intercellular pathway. It involves drug diffusing through the continuous lipid matrix (Morrow *et al.,* 2007). This route has some significant obstacles for two reasons:

i. Here drug has to pass through small spaces between the cells of the skin, from the ‘bricks and mortar’ model of the stratum corneum, the inter-digitating nature of the corneocytes yields a tortuous pathway for

R

intercellular drug permeation, which is in contrast to the relatively direct path of the transcellular route (Sharma *et al.*, 2011).

ii. The intercellular domain is a region of alternating structured bilayers. Consequently, a drug must sequentially partition into and diffuse through repeated aqueous and lipid domains (Harunusman *et al.*, 2012). This route is generally accepted as the most common path for small uncharged molecules penetrating the skin (Harunusman *et al*., 2012; Morrow *et al*., 2007).

2.3 Kinetics of Transdermal Permeation

For a systemically active drug to reach a target tissue from the skin, it has to possess some physico-chemical properties which facilitate the sorption of the drug through the skin, and also the uptake of the drug by the capillary network in the dermal papillary layer (Bhargava *et al.*, 2011). Various events governing percutaneous absorption are shown in Figure 2.3.

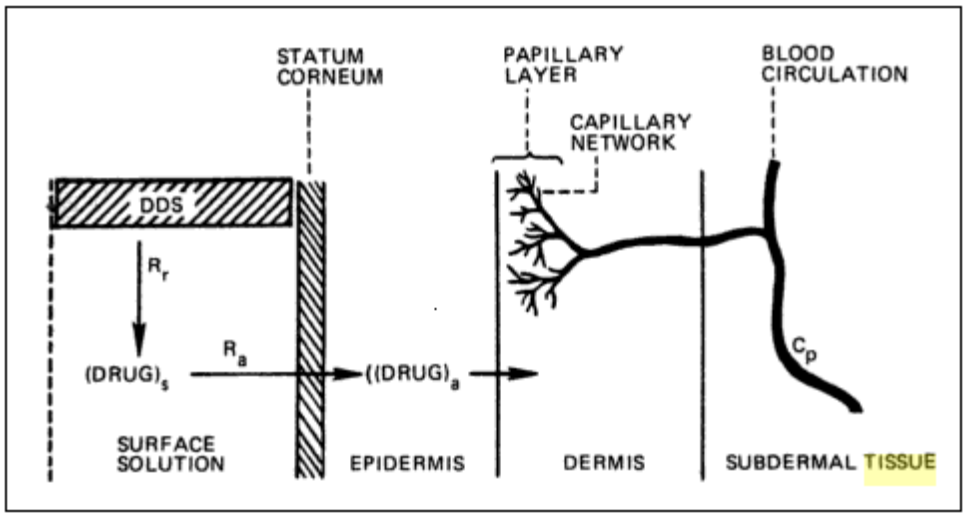


Figure 2.3: Diagrammatic Illustration of the Relationship between the Rate of Drug Release ( ) from a Transdermal Drug Delivery (Arunachalam *et al.*, 2010)

The rate of permeation, , across various layers of skin tissues can be expressed as

(1)

Where, and are respectively, the concentrations of drug penetrating through the skin in the donor phase (stratum corneum) and the receptor phase (systemic circulation), and

is the overall permeability coefficient of the skin and is defined by

(2)

Where = Partition coefficient of the penetrant

= Apparent diffusivity of penetrant

= Thickness of skin.

Thus, permeability coeffiefficient ( ) may be constant since , and terms in equation (2) are constant under the given set of conditions. A Constant rate of drug permeation is achieved, if , then the equation (1) may be reduced to

(3)

And the rate of skin permeation ( ) becomes a constant, if the value remains fairly constant throughout the course of skin permeation. To maintain the at a constant value, it is critical to make the drug to be released at a rate ( ) which is always greater than the rate of skin uptake ( ), i.e

So, the drug concentration on the skin surface (is maintained at a level which is always greater than the equilibrium (or saturation) solubility of the drug in the stratum Corneum ( ), i.e., and a maximum rate of skin permeation ( ) m, as expressed by equation (4), is thus reached:

(4)

Apparently, the magnitude of ( ) m is determined by the skin permeability coefficient ( ) of the drug and its equilibrium solubility in the stratum corneum ( ). (Shaik *et al.*, 2011; Arunachalam *et al*., 2010).

2.4 Factors Affecting Transdermal Drug Delivery

These factors can be grouped into three namely, physicochemical properties of the substance permeating, physicochemical properties of the drug delivery system and the physiological factors.

2.4.1 Physicochemical properties of substances permeating through the skin

2.4.1.1 Partition coefficient

For molecules with intermediate partition coefficient (log K of 1 to 3) and highly lipophilic molecules (log K > 3), the intercellular route will be almost the pathway used to traverse the stratum corneum. However, for these molecules, a further consideration is the ability to partition out of the stratum corneum into the aqueous viable epidermal tissues. For more hydrophilic molecules (log K < 1), the transcellular route probably predominates (Harunusman *et al*., 2012).

2.4.1.2 Molecular size

A second major factor in determining the flux of a material through human skin is the size of the molecule. However, for simplicity, the molecular weight is generally taken as an approximation of molecular size. It has been suggested that an inverse relationship exist between transdermal flux and molecular weight of the molecule (Vinod *et al*., 2010).

2.4.1.3 Solubility/melting point

It is well known that most organic materials with high melting points have relatively low aqueous solubility at normal temperature and pressure. The lipophilic molecules tend to permeate through the skin faster than more hydrophilic molecules. However, while lipophilicity is a desired property of transdermal candidates, it is also necessary for the molecule to exhibit some aqueous solubility since topical medicaments are generally applied from an aqueous formulation (Harunusman *et al*., 2012).

2.4.1.4 Ionization

According to pH-partition hypothesis, only the unionized forms of the drug can permeate through the lipid barrier in significant amounts (Vinod *et al*., 2010).

2.4.1.5 Drug concentration

Transdermal permeability across mammalian skin is a passive diffusion process and this depends on the concentration of molecule permeating the surface layer of the skin (Ramteke *et al*., 2012).

2.4.1.6 Diffusion coefficient

Penetration of drug depends on diffusion coefficient of the drug. At a constant temperature, the diffusion coefficient of drug depends on properties of the drug, diffusion medium and interaction between them (Aulton, 2007).

2.4.1.7 Other factors

Apart from the factors mentioned above, there are other molecular properties that can affect drug delivery through the skin. Drug binding is a factor that should be borne in mind when selecting appropriate candidates (Harunusman *et al*., 2012).

Interactions between drug substances and the tissue can vary from hydrogen bonding to weak Van der Waals forces and the effect of drug binding (if any) on flux across the tissue will vary, depending on the permeating substance, for example with a poorly water soluble drug in an aqueous donor solution, significant binding to the stratum corneum may completely retard drug flux. Consequently, there will be a delay between applying a drug to the surface of the tissue and its appearance in a receptor solution (*in vitro*) or the blood (*in vivo*). Depending on the type of formulation selected, other factors may be important in a transdermal delivery system. For instance, if the drug is suspended, then the particle size may become a key regulator of flux.

2.4.2 Physicochemical properties of the drug delivery system

2.4.2.1 Release characteristics

Solubility of the drug in the vehicle determines the release rate. The mechanism of drug release depends on the following factors:

i. Whether the drug molecules are dissolved or suspended in the delivery systems.
ii. The interfacial partition coefficient of the drug from the delivery system to the skin tissue.
iii. pH of the vehicle.

2.4.2.2 Composition of the drug delivery systems

The composition of the drug delivery systems, example boundary layers, thickness, polymers and vehicles, not only affects the rate of drug release, but also the permeability of the stratum corneum by means of hydration, or other sorption promoting effects, e.g. benzocaine permeation decreases with PEG of low molecular weight.

2.4.2.3 Enhancement of transdermal permeation

Majority of drugs will not penetrate the skin at rates sufficiently high for therapeutic efficacy. In order to allow clinically useful transdermal permeation of most drugs, the penetration can be improved by the addition of a permeation promoter into the drug delivery systems.

2.4.3 Physiological factors

Some of the physiological factors affecting the transdermal drug delivery include:

2.4.3.1 Skin barrier properties in neonate and young infants

The skin of newborns is known to be relatively susceptible to irritants. Other variables related to stratum corneum function such as pH and stratum corneum hydration may enhance the irritant potential in the newborn skin. Skin surface pH values in newborns are significantly higher in all body sites than those in adult skin, but stabilized at values similar to adults within the first month. There are also significant changes in the metabolic capacity of infants, whether full or preterm and adult levels of cutaneous enzyme activity are not observed until two months or even six to twelve months of age which may additionally account for the sensitivity of baby skin to irritants. The skin surface of the newborn is slightly hydrophobic and relatively dry and rough when compared to that of older infants. Stratum corneum hydration stabilizes by the age of three months (Aulton, 2007).

2.4.3.2 Skin barrier properties in the aged skin

There are changes in the physiology of aged skin (greater than sixty five years). The corneocytes are shown to increase in surface area which may have implications for stratum corneum function due to the resulting decreased volume of inter-corneocyte space per unit volume of stratum corneum. The moisture content of human skin

decreases with age. There is a flattening of the dermoepidermal junction and, consequently, the area available for diffusion into the dermis is diminished (Aulton, 2007).

2.4.3.3 Race

Racial differences between black and white skins have been shown in some anatomical and physiological functions of the skin although data is relatively sparse. In black skin, increased intracellular cohesion, higher lipid content and higher electrical skin resistance levels compared to whites have been demonstrated. Black skin appears to have a decreased susceptibility to cutaneous irritants, but this difference is not detected in stripped skin, suggesting the stratum corneum modulates the different racial response to irritants. Black skin responds with a decrease in blood flow and hence is less erythematic than Hispanics or Caucasians (Aulton, 2007).

2.4.3.4 Body site

It is readily apparent that skin structure varies to some degree over the human body. However, the relative permeability of different skin sites is not simply a function of stratum corneum thickness as different permeants exhibit varied rank orders through different skin sites. It is apparent that genital tissue usually provides the most permeable site for transdermal drug delivery. The skin of the head and neck is also relatively permeable compared to other sites of the body such as the arms and legs (Harunusman *et al*., 2012).

2.4.3.5 Skin temperature

The human body maintains a temperature gradient across the skin from around 37 ºC to 32 ºC at the outer surface. Since diffusion through the stratum corneum is a passive process, elevation of the skin temperature can induce structural alterations within the

stratum corneum, and these modifications can also increase diffusion through the tissue (Aulton, 2007).

2.4.3.6 Skin condition

The intact, healthy skin is a tough barrier but many agents damage it. Vesicants like acids and alkalis, many solvents like chloroform and methanol, damage the skin cells and promote penetration. Diseased state of patient alters the skin conditions. The intact skin is better barrier but the above mentioned conditions affect penetration (Harunusman *et al.,* 2012).

2.4.3.7 Blood flow

Changes in the peripheral circulation could affect transdermal absorption, theoretically; an increased blood flow could reduce the time a permeating substance remains in the dermis and also raise the concentration gradient across the skin. Usually, the effect is not clinically important (Aulton, 2007).

2.4.3.8 Skin metabolism

Skin metabolizes steroids, hormones, chemical carcinogens and some drugs. Such metabolism affects the efficacy of drug permeated through the skin (Aulton 2007).

2.5 Approaches to Development of a Transdermal Drug Delivery System (TDDS)

Several technologies have been successfully developed to provide a rate control over the release and the transdermal permeation of drugs. These technologies can be classified into four approaches as follows namely, membrane permeation controlled systems, adhesive dispersion systems, matrix diffusion controlled systems and micro- reservoir controlled system (Dipen *et al.,* 2011; Nikhil *et al*., 2011).

2.5.1 Membrane permeation controlled systems

In this system, the drug reservoir is embedded between an impervious backing layer and a rate controlling membrane. The drug releases only through the rate controlling membrane, which can be micro-porous or non-porous. In the drug reservoir compartment, the drug can be in the form of a solution, suspension, or gel or dispersed in solid polymer matrix (Figure 2.4). The rate of drug release from this type of transdermal drug delivery system can be tailored by varying the polymer composition, permeability coefficient and thickness of the rate controlling membrane. (Singhal *et al.*, 2012; Kapoor *et al.*, 2011*).* Scopolamine, for three days protection of motion sickness and nitroglycerin for once a day medication of angina pectoris are examples of this type of system.

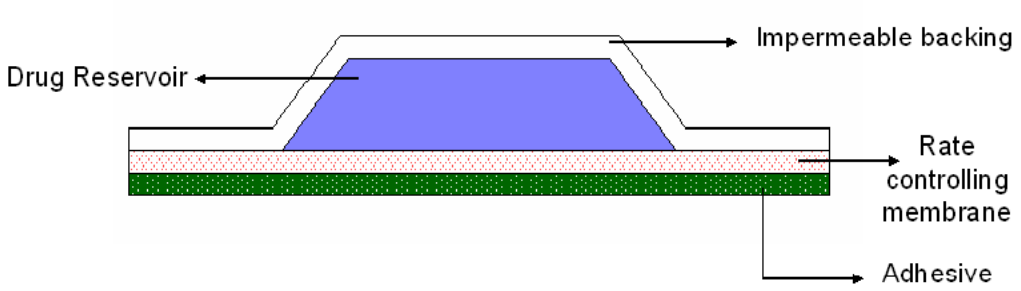


Figure 2.4: Polymer Membrane Permeation Controlled system (Singhal *et al.*, 2012).

2.5.2 Adhesive dispersion systems

The drug reservoir is formed by dispersing the drug in an adhesive polymer and then spreading the medicated polymer adhesive by solvent casting or by melting the adhesive (in case of hot-melt adhesives) onto an impervious backing layer (Figure 2.5). The drug reservoir layer is then covered by a non-medicated rate controlling adhesive polymer of constant thickness to produce an adhesive diffusion controlling drug delivery system rim (Singhal *et al.*, 2012; Kapoor *et al.*, 2011*).* Example is nitroglycerin for once a day medication of angina pectoris.

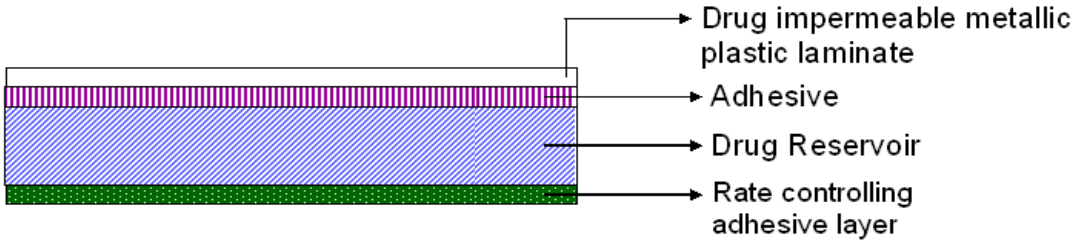


Figure 2.5: Adhesive Diffusion Controlled System (Singhal *et al.*, 2012).

2.5.3 Matrix diffusion controlled system

The drug is dispersed homogeneously in a hydrophilic or lipophilic polymer matrix. This drug containing polymer disk is then fixed onto an occlusive base plate in a compartment fabricated from a drug impermeable backing layer (Figure 2.6). Instead of applying the adhesive on the face of the drug reservoir, it is spread along the circumference to form a strip of adhesive rim (Singhal *et al*., 2012; Kapoor, *et al.*, 2011*)*.

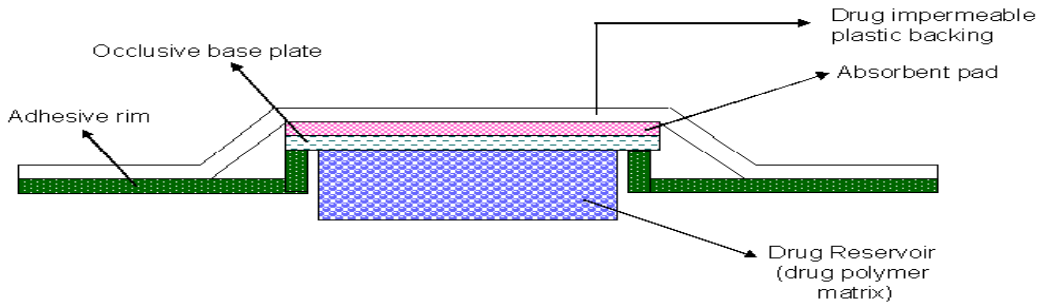


Figure 2.6: Matrix Diffusion Controlled System (Singhal *et al.*, 2012).

2.5.4 Microreservoir controlled system

This drug delivery system is a combination of reservoir and matrix-dispersion systems. The drug reservoir is formed by first suspending the drug in an aqueous solution of water-soluble polymer and then dispersing the solution homogeneously in a lipophilic polymer to form thousands of microscopic spheres of drug reservoirs (Singhal *et al.*, 2012) (Figure 2.7). The thermodynamically unstable dispersion is stabilized quickly by immediately cross linking the polymer *in situ* (Kapoor *et al.*, 2011).

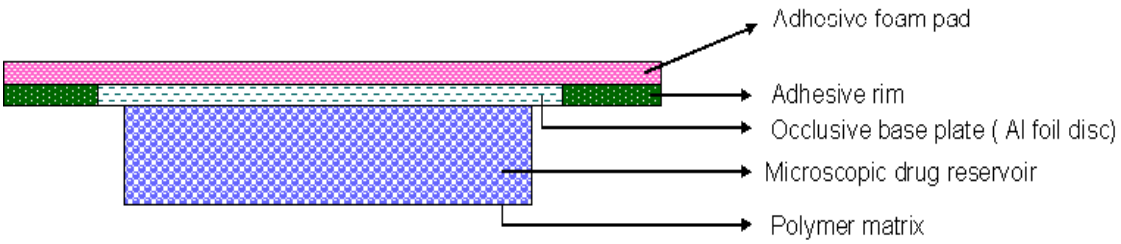


Figure 2.7: Micro Reservoir Controlled System (Singhal *et al.*, 2012)

2.6 Basic Components of Transdermal Films

The basic components of a transdermal film include, polymer matrix, drug, permeation enhancer, plasticizers and solvents.

2.6.1 Polymer matrix

Polymers are the backbone of a transdermal drug delivery system, which control the release of the drug from the device. Polymer matrix can be prepared by dispersion of drug in liquid or solid state synthetic polymer base (Ahmed *et al.*, 2011; Arunachalam *et al*., 2010).

Systems for transdermal delivery are fabricated as multilayered polymeric laminates in which a drug reservoir or a drug-polymer matrix is sandwiched between two polymeric layers, an outer impervious backing layer that averts the loss of drug through the backing surface and an inner polymeric layer that functions as an adhesive and/ or rate-controlling membrane (Kevin *et al*., 2009).

The polymers utilized for TDDS can be classified as natural polymers, e.g. cellulose derivatives, zein, gelatin, shellac, waxes, gums, natural rubber and chitosan *etc*. Synthetic elastomers**,** e.g. polybutadiene, hydrin rubber, polyisobutylene, silicon rubber, nitrile, acrylonitrile, neoprene, butylrubber *etc*. Synthetic polymers: e.g. polyvinyl alcohol, polyvinylchloride, polyethylene, polypropylene, polyacrylate, polyamide, polyurea, polyvinylpyrrolidone, polymethylmethacrylate etc. The polymers like cross

linked polyethylene glycol, eudragits, ethylcellulose, polyvinylpyrrolidone and hydroxypropyl-methyl-cellulose are used as matrix formers for TDDS. Other polymers like silicon rubber and polyurethane are used as rate controlling membrane.

Polymer selection and design must be considered when striving to meet the diverse criteria for the fabrication of effective transdermal drug delivery systems. The main challenge is in the design of a polymer matrix, followed by optimization of the drug loaded matrix not only in terms of release properties, but also with respect to its adhesion-cohesion balance, physicochemical properties, compatibility and stability with other components of the system as well as with skin (Harunusman *et al.*, 2012).

Polymer must possess the following properties:

i. Molecular weight and chemical functionality should be such that specific drug diffuses properly and gets released through it.
ii. Stable, non reactive with the drug, easily manufactured and inexpensive
iii. Polymer and its degradation products must be non toxic.
iv. Mechanical properties should not deteriorate excessively when large amounts of active ingredient are incorporated in it (Bhargava *et al.*, 2011).

2.6.1.1 Two Commonly Used Polymers

1) Hydroxypropyl methylcellulose (HPMC): It is an odourless and tasteless, white to slightly off white, fibrous or granular, free-flowing powder that is a synthetic modification of the natural polymer, cellulose. Specifically, it is a modification of alkali cellulose, which is produced when purified wood pulp is treated with 18 % sodium hydroxide solution (Kajal *et al*., 2011).

Methyl and hydroxypropyl ether groups are introduced into the molecule by reacting the alkali cellulose with methyl chloride and propylene oxide, respectively. The degree of substitution (DS) of commercial HPMC with these methoxy and hydroxyl groups will vary, depending on the commercial use and properties desired. These added groups confer on the molecule, its unique properties of being cold-water soluble, while at the same time exhibiting reversible gelation when heated and recooled (Kajal *et al*., 2011).

The reason for its widespread acceptance includes:

i. Solubility characteristics of the polymer in gastrointestinal fluid, organic and aqueous solvent systems.
ii. Noninterference with tablet disintegration and drug availability.
iii. Flexibility, chip resistance and absence of taste and odor.
iv. Stability in the presence of heat, light, air or reasonable levels of moisture.
v. Ability to incorporate color and other additives into the film without difficulty.

Verma *et al*. (2008) investigated the possibility of using different concentrations and polymeric grades of hydroxypropylmethylcellulose (HPMC) for transdermal delivery of methotrexate, an immunosuppressive drug for rheumatic arthritis. Results of the study indicate that they have good film forming properties.

2) Eudragit RS 100: This is a trade name of copolymers derived from acrylic acids, methacrylate acids, whose properties are determined by functional groups. Eudragit grades differ in their proportion of neutral, alkaline or acid groups, resulting in different physicochemical properties and offer excellent choice for

formulating compounds.

The glass transition temperature (Tg) of soluble polymethacrylate grade (e.g. Eudragit E or NE) is 8^0 C, enteric grade (e.g. Eudragit L) is over 160^0 C, and insoluble ones (e.g. Eudragit RL/RS) is about 55^0 C. To obtain a proper degree of suppleness, 10 % plasticizer is needed for insoluble grade and 40 % plasticizer for enteric grade, but no plasticizer is needed for gastrosoluble grade methacrylate polymer. Enteric type methacrylate polymers such as Eudragit L/S coatings provide a better barrier against drug release in the stomach and enabled controlled release in the intestine. Enteric type FS is employed for controlled release of drugs in the colon. Eudragit RS and RL types are widely used in controlled release coating. The amount of quanternary ammonium groups in RS type is between 4.5 % and 6.8 % and in RL type is between 8.8 % and 12 %. Both RS and RL types are insoluble at physiological pH values and capable of swelling. Eugragit RS 100 and RL 100 are pH independent cationic polymers. Eudragit RL films are more permeable than Eudragit RS films. The skillful use of combination of different Eudragit polymers offers ideal solutions for controlled drug release in various pharmaceutical and technical applications.

Rajabalaya *et al.* (2010) studied the release and permeation of transdermal therapeutic systems with chlorpheniramine maleate (CPM). The patches were prepared with Eudragit RS 100 and RL 100 with polyvinyl pyrrolidone (PVP) and dibutyl phthalate (DBP) in different compositions. Chemical enhancers like L- menthol, oleic acid and phospholipon 80 were added to compare the release patterns of the drug. They reported that the percentage release and permeation from the Eudragit RL 100 formulations were greater than the corresponding

values of the Eudragit RS 100 formulations, with the other physical properties like patch thickness and tensile strength being slightly lower.

Gottipati *et al.* (2012), developed transdermal patches of valsartan with hydrophilic and hydrophobic polymers containing the drug reservoir, by solvent evaporation method. The polymers used were ethylcellulose, Eudragit RS 100 and Eudragit RL 100, along with HPMC combination were used to achieve controlled release of the drug. They concluded that HPMC and Eudragit RS 100 showed better release than other polymer ratio for the development of TDDS of valsartan.

Umesh *et al*. (2009), prepared transdermal films of carvedilol by using Eudragit RL 100 (ERL 100), either alone or in combination with Eudragit RS 100 (ERS 100), Hydroxypropyl methylcellulose K 15 (HPMC) and ethyl cellulose (EC). They concluded that polymeric composition of transdermal films affects its pharmacotechnical properties, and that among the various polymeric compositions, A5, comprising of polymers Eudragit RL 100 (ERL 100), ERS 100, HPMC and EC in the ratio 7:1:1:1, fulfils the requirement for good TDDS.

2.6.2 Drug

The most important criteria for TDDS are that the drug should possess the right physicochemical and pharmacokinetic properties (Saroha *et al.,* 2011). The selection of drug for transdermal drug delivery depends upon various physicochemical and biological factors (Patel *et al.,* 2011).

Physicochemical properties include:

i. The drug should have some degree of solubility in both oil and water (ideally greater than 1 mg/ml).

ii. The substance should have melting point less than 93°C. Concentration gradient across the membrane is directly proportional to the log solubility of drug in the lipid phase of the membrane, which in turn is directly proportional to the reciprocal of the melting point.

iii. Substances having a molecular weight of less than 1000 units are suitable.

iv. A saturated aqueous solution of the drug should have a pH value between 5 and 9. Drugs highly acidic or alkaline in solution are not suitable for TDD, because they get ionized rapidly at physiological pH.

v. Hydrogen bonding groups should be less than 2.

vi. Partition coefficient (Log P) should be between 1 and 3.

vii. Skin permeability coefficient should be less than 0.5×10^{-3} cm/ h (Patel *et al.,* 2011; Dhiman *et al.*, 2011)

Biological properties include:

i. Drug should be very potent, i.e. it should be effective in few milligrams per day (ideally less than 25 mg/day).

ii. The drug should have short biological half life.

iii. The drug should be non-irritant and non-allergic to human skin.

iv. The drug should be stable when in contact with the skin.

v. The drug should not stimulate an immune reaction to the skin.

vi. Tolerance to drug must not develop under near zero order release profile of transdermal delivery.

vii. The drug should not get irreversibly bound in the subcutaneous tissue.

viii. The drug should not get extensively metabolized in the skin.

ix. Drugs, which degrade in the GIT or are inactivated by hepatic first-pass effect, are suitable candidates for transdermal delivery. Drugs, which have to be administered for a long period of time or which cause adverse effects to non-target tissues can also be formulated for transdermal delivery.

viii. Therapeutic index should be low (Patel *et al*., 2011; Dhiman *et al*., 2011; Jain *et al*., 2011)

2.6.3 Permeation enhancers

These compounds are useful to increase permeability of stratum corneum by interacting with structural components of stratum corneum *i.e.*, proteins or lipids to attain higher therapeutic levels of the drug. They alter the protein and lipid packaging of stratum corneum, thus chemically modifying the barrier functions leading to increased permeability (Karande *et al.,* 2005). The enhancement in absorption of oil soluble drugs is apparently due to the partial leaching of the epidermal lipids by the chemical enhancers, resulting in the improvement of the skin conditions for wetting and for transepidermal and transfollicular penetration. The miscibility and solution properties of the enhancers used could be responsible for the enhanced transdermal permeation of water soluble drugs (Arunachalam *et al.,* 2010)

1) Passive penetration enhancers

These techniques include saturated drug solutions, microemulsions, liposomes, prodrugs, drug eutectic mixtures and chemical penetration enhancers.

2) Physical penetration enhancers

Physical enhancers induce the skin permeability by using physical forces such as magnetic field, electric current, vibration etc. These innovative techniques are capable of enhancing the diffusivity of a wide range of drugs through the tough barrier ‘stratum

corneum'. Ionized and high molecular weight drugs (insulin, therapeutic proteins, peptides etc) can be transferred effectively when they are assisted with iontophoresis and phonophoresis. The technique remains costly and very technical. Various techniques are used for physically enhancing TDDS, such as the structure-based enhancement techniques, electrically-based enhancement techniques and velocity based enhancement techniques.

3) Chemical penetration enhancers

Chemical penetration enhancers (CPE) are the substances that facilitate the absorption of penetrant through the skin by temporarily diminishing the impermeability of the skin. Ideally, these materials should be pharmacologically inert, non-toxic, nonirritating, non-allergic, compatible with the drug and excipients, odourless, tasteless, colorless and inexpensive and have good solvent properties. No single penetration enhancers can possess all the required properties. However many CPE's exhibit some of these attributes. The use of CPE significantly enhances the number of candidates suitable for transdermal delivery (Inayat and Mallikarjuna, 2009). Penetration enhancers can be classified into a wide variety of chemical classes as shown in the Table 2.1.

Table 2.1: Chemical Classes of Penetration Enhancers (Inayat and Mallikarjuna 2009).

Chemical Class	Examples
Fatty acids	Oleic acid, Undecanoic acid
Alcohols, Fatty alcohols	Ethanol, Octanol, Nonanol, Propylene glycol
Terpenes	Menthol, Thymol, Limonene, Cineol, Nerodilol
Sulfoxides	Dimethyl sulfoxide, dodecyl methyl sulfoxide
Anionic surfactants	Sodium lauryl sulphate
Cationic surfactants	N,N-bis (2 hydroxy ethyl) oleylamine
Non ionic surfactants	Polyoxyethylene (20) sorbitan mono oleate
Zwitterionic surfactants	Dodecyl dimethyl ammoniopropane sulphate
Polyol	Propylene glycol, Polyethene glycol
Amide	n,n- dimethyl-m-toluamide
Ureas	Urea
Lactams	Laurocapram (Azone)
Sugars	Cyclodextrins

2.6.4 Plasticizers

Plasticizers are generally non-volatile organic liquids or solids with low melting temperature and when added to polymers, they cause changes in definite physical and mechanical characteristics of the material (Bharkatiya *et al.,* 2010, Felton, 2007, Meier *et al.,* 2004).

2.6.4.1 Properties of plasticizers

A plasticizer is firstly expected to be compatible with the polymer substance. This means that, it can fully mix with the polymer and can remain permanently in the polymer.

Tendency for migration, exudation, evaporation or volatilization of the plasticizers employed in a polymeric system must be low (Felton, 2007, Harper, 2006).

Other properties expected from an ideal plasticizer are its workability, its ability to provide desired thermal-electrical and mechanical characteristics to the end product, its durability at high and low temperature values, its being effective over a wide temperature range not being affected by ultraviolet radiation, its cost being low and its conformity to the health and safety arrangements (Rahman and Brazel, 2004).

2.6.4.2 *Plasticizers in transdermal drug delivery systems*

Many of the polymers used in pharmaceutical formulations are brittle and require the addition of a plasticizer into the formulation. Plasticizers are added to pharmaceutical polymers to ease the thermal workability, modify the drug release from polymeric systems and improve the mechanical properties and surface properties of the dosage form (Felton, 2007; Lin *et al*., 2000; Zhu *et al.*, 2002). The list of frequently used plasticizers in pharmaceutical formulations is given in Table 2.2.

Table 2.2: Different Groups of Plasticizers (Wypch, 2004).

Group	Hydrophilic/Lipophilic	Plasticizer
Glycerol and esters	Hydrophilic	Glycerine, Glycerine triacetate, Glyceryltributyrate
Glycol derivatives	Hydrophilic	Propylene glycol, Polyethylene glycol
Phthalic acid esters	Lipophilic	Dibutyl phthalate, Diethyl Phthalate
Sebacic acid esters	Lipophilic	Dibutyl sebacate, Diethyl sebacate
Oleic acid esters	Hydrophilic	Oleil oleate
Sugar alcohols	Hydrophilic	Sorbitol
Citric acid esters	Hydrophilic	Triethyl citrate, Tributhyl citrate
Tartaric acid esters	Lipophilic	Diethyl tartarate

It was observed that the plasticizers added to transdermal therapeutic systems are mostly used in the proportions between 5-20 %. It has been reported that, plasticizers such as glycerine, sorbitol and polyethylene glycol can change release rate of the therapeutic components contained in the formula of transdermal drug delivery systems. Release rate of the drug can be adjusted by changing the type and concentration of the plasticizer (Lin *et al.,* 2000; Wypch, 2004).

It was also found that the effect of the plasticizer on drug transport is related to the physicochemical properties of the permeant, in particular, to its solubility in the plasticizer (Padula *et al.*,2010).

Kumar *et al*. (2007) carried out a study in which polyvinyl alcohol and polyvinyl alcohol-xanthan gum mixture were used as polymer, propylene glycol and dibutyl phthalate as plasticizer. It was observed that, addition of xanthan gum and dibutyl phthalate to the films prepared with only polyvinyl alcohol decreased the tensile strength and increased the percentage elongation. On the other hand, tensile strength was found to be higher in polyvinyl alcohol films prepared with propylene glycol. Besides, *in vitro* release of the terbutaline sulphate, which was the active substance, was found to be higher in the propylene glycol-containing films.

In transition studies conducted with diltiazem hydrochloride and indomethacin, diffusion of the drug from plasticized transdermal films was found to follow the order, polyethylene glycol 600 > dibutyl phthalate > propylene glycol. It was concluded that, permeation of the drugs and mechanical properties of the film were affected by the choice of suitable plasticizer and its concentration (Rao and Diwan, 1997).

Amgaokar *et al.* (2011) prepared transdermal films of budesonide. In the films, Eudragit RL 100: Eudragit RS 100 and ethylcellulose-polyvinylpyrrolidone (in proportions of 7:3 and 7:2, respectively) were used as polymers and polyethylene glycol 400 as plasticizer. While drug release was proportional to the polymer concentration, increase in the plasticizer amount caused an increase in the weight of the film. It was also observed that, increase in the plasticizer concentration caused an increase in the humidity absorption of the transdermal films. It was therefore reported that, permeation of budesonide was the highest when polyethylene glycol 400 was used in proportion of 10 %.

Irfani *et al.* (2011) used different combinations of hydoxypropylmethylcellulose, Eudragit RS 100 and polyvinylpyrrolidone, to prepare transdermal films of the active substance, valsartan. Different proportions (10 %, 20 % and 30 %) of glycerin were tried as plasticizer. It was found that, increasing plasticizer concentration increased the diffusion rate of the active substance. When the polymer combination of Eudragit RS 100 and hydroxypropylmethylcellulose was used with 30% glycerine, an increase in the diffusion rate of valsartan was observed.

2.7 Evaluation of Transdermal Films

2.7.1 *In vitro* skin permeation and release kinetics

The design and development of transdermal drug delivery systems is greatly aided by *in vitro* studies. *In vitro* studies can help in investigating the mechanism of skin permeation of drug before it can be developed into a transdermal therapeutic system. The methodology used in the *in vitro* study is relatively easy to follow and generally affords the investigator better control over the experimental conditions than is possible *in vivo* (Ahmed *et al*., 2011).

The factors that require consideration when selecting an *in vitro* system include:

i. The rate limiting process such as drug solubilization or diffusion in the vehicle, partitioning from the vehicle, diffusion through the test membrane or partitioning and removal by the receptor phase.

ii. The intrinsic diffusivity of the drug penetrating and apparent diffusivity.

iii. The predominating route of diffusion during the experiment (i.e the membrane to be used) and the relative drug binding and metabolism, occurring in the membrane.

iv. The predominating route of diffusion during the experimentation and the relative extents of drug binding.

v. The intrinsic barrier potential of the membrane and the effects that vehicle components may have on retardation properties (Arunachalam *et al.*, 2010).

Hydration of the membrane and the presence of penetration enhancers may be important here. The kinetics of skin permeation can be more precisely analyzed by studying the time course for the permeation of drug across a freshly excised skin mounted on a diffusion cell, such as the Franz diffusion cell (Figure 2.8). Some diffusion cells are designed to hold the skin at a vertical position between donor and receptor chambers. A more recent example is the valia, Chien cell, which is superior to similar earlier models in that it does not expose both, the donor and the receptor phases to the same temperature, and does not allow solvent loss from either phase. Moreover, the design overcomes another inadequacy of the Franz cell, namely, the susceptibility of its donor phase to the changes in ambient temperature (Arunachalam *et al.*, 2010). Finally, the donor compartment contents may be stirred which makes the cell suitable for transdermal drug delivery from solutions and suspensions.

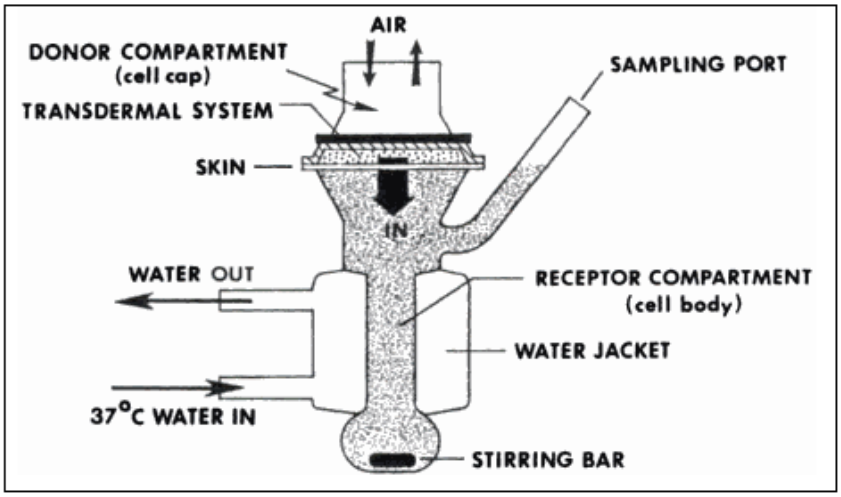


Figure 2.8: Franz Diffusion Cell Apparatus (Arunachalam *et al.*, 2010)

Majority of *in vitro* experiments are conducted on animal skin i.e. hairless mouse, guinea pig, rabbit etc. Although there are a number of similarities, no animal skin completely mimics the penetration characterization of a human skin (Aulton, 2007).

2.7.2 *In vivo* Studies

In vivo evaluations are the true depiction of the drug performance. The variables which cannot be taken into account during *in vitro* studies can be fully explored during *in vivo* studies. *In vivo* evaluation of TDDS can be carried out using both the animal models and the human volunteers.

i. Animal models

The most common animal species used for evaluating transdermal drug delivery systems are mouse, hairless rat, hairless dog, hairless rhesus monkey, rabbit, guinea pig etc.

ii. Human models

The final stage of the development of a transdermal device involves collection of pharmacokinetic and pharmacodynamic data following application of the patch to

human volunteers. Clinical trials have been conducted to assess the efficacy, risk involved, side effects, patient compliance of the patches produced (Yadav *et al*., 2011).

2.8 Applications of Transdermal Patches

i. The highest selling transdermal patch in the United States is the nicotine patch, which releases nicotine in controlled doses to help with cessation of tobacco smoking (Yadav *et al*., 2010).

ii. Fentanyl and buprenorphine, used to provide round the clock relief for severe pain are often prescribed in patch form.

iii. Estrogen patches are sometimes prescribed to treat menopausal symptoms as well as post menopausal osteoporosis. Other transdermal patches for hormone delivery include the contraceptive patch.

iv. Nitroglycerin patches are sometimes prescribed for the treatment of angina in lieu of sublingual pills.

v. The anti-hypertensive drug clonidine is available in transdermal patch form.

vi. Transdermal form of the monoamine oxidase inhibitor (MAOI) selegiline, became the first transdermal delivery agent for an antidepressant.

vii. Transdermal delivery agent for the Attention Deficit Hyperactivity Disorder (ADHD) is also available (Singhal *et al*., 2012; Dipen *et al*., 2012).

2.9 Review of Previous Works on Various Drugs Formulated Using Different Ratios of Polymer Blends

Bheisare and Tilloo (2012), studied transdermal films of diclofenac sodium, by using *Hyptis suaveolens* seeds mucilage as film forming agent in various concentrations. It

was concluded that there was no significant alteration of drug, when the films were formulated using *Hyptis suaveolens* seed mucilage as polymer.

Gajanan *et al.* (2011) formulated and evaluated the transdermal drug delivery system of clopidogrel bisulfate using various polymers such as HPMC, PVP (polyvinyl pyrolidone) and ethylcellulose by solvent evaporation technique for improvement of bioavailability of the drug and reducing toxic effects. The prepared transdermal drug delivery system showed promising results from all the evaluated parameters.

Gattani *et al.* (2012) worked on transdermal films of diclofenac sodium (DS) using different polymer combinations such as hydrophilic (Polyvinyl alcohol: Polyvinyl pyrollidone), and combination of hydrophilic-lipophilic polymers (Ethyl cellulose: Polyvinyl pyrollidone), to study the effect of plasticizers such as dibutyl phthalate and propylene glycol using Keshary-Chein diffusion cell. It was reported that the polymeric matrix-type transdermal films of DS prepared with different grades and ratios of polymers had potential for transdermal delivery.

Kelvin *et al.* (2009) carried out a study on monolithic matrix transdermal systems containing tramadol HCl, using various ratios of the polymer blends of hydroxypropyl methyl cellulose (HPMC) and Eudragit S 100 (ES) with triethylcitrate as a plasticizer. They concluded that the selection of a particular blend formulation can vary the diffusion of the drug significantly. HPMC/ES polymer blends could have potential in the formulation of TDDS as they have good film forming property and mechanical strength.

Shivaraj *et al.* (2010) developed and evaluated matrix-type transdermal therapeutic system containing ketotifen fumarate (KTF) with different ratios of hydrophilic and

hydrophobic polymeric combinations by the solvent evaporation method. They reported that controlled release TDDS patches of KFT can be prepared using the polymer combinations, hydroxypropylmethylcellulose E5 (HPMC E5): ethylcellulose (EC) (5:5) with dibutyl phthalate (DBT) and dimethyl sulfoxide (DMSO) as plasticizer and enhancer, respectively. The release rate of the drug through the patches increased when the concentration of hydrophilic polymer was increased.

Nagesh *et al.* (2011) prepared and evaluated matrix type transdermal films of lisinopril dehydrate. The *in-vitro* skin permeation data show that drug release from the patch, was affected by type and concentration of the polymer used, thus it was concluded that the molecular diffusion through the polymer matrix, is an effective, simple and reliable means to achieve sustained/ controlled release of a variety of active agents from a transdermal therapeutic system.

Naseera *et al.* (2012) developed a matrix-type transdermal therapeutic system containing simvastatin with different ratios of hydrophilic and hydrophobic polymeric combinations by the solvent evaporation technique. Based on the observations, it was concluded that HPMC: Eudragit RL100 showed better release than other polymer blends for the development of TDDS for simvastatin.

Parthasarathy *et al*. (2011) designed a transdermal drug delivery system of naproxen with ethylcellulose and hydroxypropylmethyl cellulose polymer in various concentrations. The transdermal films were fabricated by matrix technique with various polymer proportions using dibutylphthalate as plasticizer. They concluded that the high proportion of hydrophobic polymer ethyl cellulose is responsible for sustaining the drug release.

Rajendra *et al.* (2011) developed and evaluated a matrix-type transdermal therapeutic system containing Lornoxicam with different ratios of hydrophilic and hydrophobic polymeric combinations by the solvent evaporation technique. They concluded that, controlled release TDDS patches of Lornoxicam can be prepared using the polymer combinations, HPMC E5: EC Ethyl cellulose (1:1) with PEG-400 and Tween-80 as plasticizer and enhancer, respectively. The release rate of the drug through the patches increased when the concentration of hydrophilic polymer was increased.

Shahida *et al.* (2012) investigated the effect of different polymers on the release profile of ibuprofen from the matrix type transdermal film. Transdermal films of ibuprofen using Eudragit L 100, Kollidon SR and their combinations were separately prepared by solvent casting method. The study showed that the matrix type transdermal film of ibuprofen can be prepared successfully using two different polymers and their combination by solvent casting method. From the *in vitro* drug release data it was concluded that controlled release of ibuprofen from the film could be achieved for a prolonged period.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

i. Anhydrous calcium chloride, potassium chloride, sodium chloride (Johnson Solomon Export Ltd London, England).

ii. Diclofenac sodium, polyethylene glycol, tween 80 (BDH Chemicals Ltd Poole, England).

iii. Eudragit Rs 100 (Evonik Pharma, Germany).

iv. Hydroxypropylmethylcellulose (HPMC), monobasic potassium phosphate, methanol (Sigma Aldrich, Germany).

v. Sodium hydroxide pellet (Merck, Germany).

3.1.2 Equipment

i. Differential Scanning Calorimeter (Perkin Elmer Pyris 6DSC Model, Germany).

ii. Digital Venier Caliper (Control company, 4455 Rex Road Friendswood, Tx 77546 USA).

iii. Electronic scale (Serial No: X0 27555, Denver Instruments, 6542 Fig Street, Arvada CO 80004, U.S.A).

iv. Fourier Transform infrared Spectroscopy (Shimadzu Corporation Model 8400, U.S.A).

v. Franz diffusion apparatus (Perme Gear, Inc 1815 Leithsville Road, Hellertown 18055 U.S.A).

vi. Helios Zeta UV-VIS Spectrophotometer (Thermo Fisher Scientific Inc., Cambridge, UK).

vii. 79-1 Magnetic Stirrer with water.

viii. Oaklon pH Meter (pH 1100 series) (Serial No. 378486, Eutech Instruments, in Singapore).

3.2 Methodology

3.2.1 Preparation of transdermal films

The prepared transdermal films were formulated by the solvent casting technique using diclofenac sodium as the drug of choice. Varying concentrations of film forming polymer blends (Eudragit RS100 and HPMC), plasticizer and permeation enhancer Polyethylene glycol (PEG 8000), and surfactant (Tween 80) respectively were employed. The diclofenac sodium (200 mg) and polymer blend (Eudragit RS 100:HPMC, 2:8) were dispersed in a beaker containing 10 ml of methanol: water (9:1). The mixture was stirred continuously followed by addition of PEG 8000 (100 mg) and Tween 80 (5 drops). This dispersion was properly stirred and poured into an aluminum foil-lined petri dish of defined area (8 cm^2). A funnel of suitable size was inverted over the petri dish to control solvent evaporation. The casting solvent was then allowed to evaporate for 48 h at room temperature to obtain dry films. The films were stored between sheets of wax paper in a desiccator until further use. The above procedure was repeated using other polymer blends of Eudragit RS 100 and HPMC (4:6,5:5, 6:4 and 8:2) as shown in Table 3.1.

Table 3.1: Composition of Various Formulations

Formulation	F1	F2	F3	F4	F5	F6	F7
Eudragit RS 100 (mg)	100	200	250	300	400	500	0
Hydroxypropylmethylcellulose (HPMC) (mg)	400	300	250	200	100	0	500
Polyethylene glycol(mg)	100	100	100	100	100	100	100
Tween 80 (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Diclofenac Sodium (mg)	200	200	200	200	200	200	200
Methanol: Water 9:1 (ml)	10	10	10	10	10	10	10

3.2.2 Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR was used to assess the interaction between the drug and transdermal film excipients. Sufficient quantity of pure diclofenac sodium, Eudragit RS 100, HPMC, PEG 8000 and the formulated transdermal films were scanned over a wave range of 4000 to 650 cm^{1}at a resolution of 4 cm^{-1} in a Shimadzu Corporation Model 8400 FT-IR instrument. The system was operated in the transmission mode. Samples were placed on the sample stage and 100 N force was applied for scanning (Shivaraj *et al.*, 2010).

3.2.3 Differential scanning calorimetry (DSC) analysis

A differential scanning calorimeter (Perkin Elmer Pyris 6DSC Model, Germany) was used for thermal analysis of drug and mixtures of drug and excipients. Pure drug, polymers, and the formulated films were analysed, in order to detect any possible incompatibility between drug and film excipients. Samples of 1–2 mg were weighed directly into aluminum sample pans. The thermal analyses were conducted in a flow of air at atmospheric pressure. Scans were carried out from 30–300°C at a heating rate of 10°C min^{-1} and thermograms obtained were observed for any interactions (Michael *et al.*, 2012)

3.2.4 Characterization of transdermal films

3.2.4.1 Physical Appearance

All the prepared films were inspected for colour, clarity, flexibility and smoothness (Shivaraj *et al.*, 2010).

3.2.4.2 Weight Uniformity

A specific area of the patch (1.53 cm^{2}) was cut from different parts of the film and weighed on an electronic balance. The average weight of three films and standard deviation values were calculated from the individual weights (Gajanan *et al.*, 2011).

3.2.4.3 *Thickness Uniformity*

The thickness of the formulated film was measured at three different points using a digital caliper and average thickness of three readings was calculated (Rajendra and Kavitha 2011).

3.2.4.4 Folding Endurance

Folding endurance was determined manually by folding a 1 cm^2 strip of the film repeatedly at the same place until a breaking point was reached. The number of times that the film could be folded at the same place without breaking or cracking was taken as the value for the folding endurance (Dangi *et al*., 2012).

3.2.4.5 Percentage Moisture Uptake

The films were weighed accurately and placed in a desiccator containing 100 ml of saturated solution of potassium chloride (84 % relative humidity). At 24 h intervals, the films were taken out and weighed until there was no observable change in weight. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight (Shahida *et al*., 2012).

_____ (5)

3.2.4.6 Percentage Moisture Loss

The films were weighed accurately and kept in a desiccator containing 500 g of anhydrous calcium chloride. At daily intervals, the films were taken out and weighed repeatedly until there was no observable change in weight. The percentage of moisture loss was then calculated as the difference between initial and final weight with respect to initial weight (Shahida *et al*., 2012).

_____ (6)

3.2.4.7 Flatness Determination

Three longitudinal strips were cut from each film at different portions, one from centre, another from left side and the other from the right side. The length of each strip was measured, then kept for 2 h and the variation in length because of nonuniformity in flatness, if any, was measured by determining percent constriction using formula given below, with 0 % constriction, equivalent to 100 % flatness (Rita and Mohammed 2011).

——— (7)

Where is the initial length of each strip, and is the final length.

3.2.4.8 Drug Content Uniformity

A stock solution containing 0.1 mg/ml of diclofenac sodium was prepared by dissolving 100 mg of diclofenac sodium in 1000 ml of phosphate buffer pH 7.4. Serial dilutions were prepared to yield solutions of concentration range from 2-10 µg/ml. The absorbance of each concentration was measured spectrophotometrically at 276 nm and plotted against the various concentrations to obtain the calibration curve for diclofenac sodium. The linear regression equation for the graph was obtained from the plot and used to calculate the amount of drug contained in the film and the amount of drug released with time.

The films (1.53 cm^2) were cut and transferred to a beaker containing 100 ml of phosphate buffer solution of pH 7.4. The content was agitated using a magnetic stirring device for 5 h. It was then filtered using a whatmann filter paper and the filtrate analysed for drug content, spectrophotometrically. The experiment was repeated three times to validate the result (Shivaraj *et al.,* 2010).

3.2.5 Ex Vivo Skin Permeation Studies

3.2.5.1 Preparation of Rat Abdominal Skin

Male albino rats were sacrificed with anesthesia and the abdominal skin of each rat excised. Abdominal hairs on the skin were removed with a clipper and the skin surgically removed. The dermis was then wiped with isopropyl alcohol to remove residual adhering fat. Heat separation technique was used to separate the epidermis. The technique involved soaking the entire abdominal skin in water at 60 °C for 1 min followed by careful removal of the epidermis from dermis using blunt forceps. The epidermis was washed with water, wrapped with aluminum foil and stored at –20 °C in a freezer until use. The stored epidermis was then allowed to thaw, cut into 5.4 cm^2 pieces and hydrated by placing in phosphate buffer saline (pH 7.4), overnight before use.

3.2.5.2 *Skin Permeation Studies*

Ex vivo skin permeation studies were performed using a modified Franz diffusion cell with effective diffusion area of 2.27 cm^2. The excised rat abdominal skin was mounted between the donor and receptor compartments of the diffusion cell. The formulated films were placed over the skin and covered with paraffin film in order to ensure that the films do not dislodge from the skin during the test. The receptor compartment of the diffusion cell was then filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer set up, and the solution in the receptor compartment continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37± 1 ºC. Samples were withdrawn at hourly intervals, up to the twelfth hour and analyzed for drug content spectrophotometrically at 276 nm. The receptor phase was replenished with equal volume of phosphate buffer pH 7.4 at each sample withdrawal

(Shivaraj *et al.,* 2010). The cummulative percentages of drug permeated per square centimeter of films was plotted against time.

3.2.5.3 Permeation Data Analysis

The flux (µg $cm^{-2}.min^{-1}$) of diclofenac sodium was calculated from the slope of the plot of the cummulative amount of diclofenac sodium permeated per cm^2 of skin at steady state against time using linear regression analysis. The permeation coefficients were obtained from the steady-state flux values making use of the following equations.

$J =$ (µg/cm^2.min)…… (8)

Where *Q* indicates the quantity of substances crossing the rat skin, *A* is the area of the rat skin exposed and *t* is the time of exposure.

$P = J / C_o$ (cm/ min)…… (9)

Where P is the permeation coefficient, C_o is the initial drug concentration in the drug compartment and J represents the steady-state flux obtained from equation 8.

3.2.6 Skin irritation studies

Skin irritation studies were performed on healthy rabbits (average weight: 1.75 to 2.25 kg). The dorsal surface of the rabbits was used. The hair was removed by shaving and the animals were observed for 24 h before application of the films. The skin was then cleaned with rectified spirit. Aqueous solution of formalin 0.8 % was used as the standard irritant. The films were then applied to the skin, held in place using a surgical adhesive tape. After 24 h the films were removed with the help of an alcohol swab. The skin was examined for erythema / edema and other skin reactions (Table 3.2) and the scores assigned from 0-4 (Jamakandi *et al.,* 2009).

Table: 3.2 Scores Assigned to Various Levels of Erythema and Edema Draize *et al*., (1994)

Serial Number	Erythema Formation	Edema Formation	Score Assigned
1	No erythema	No edema	0
2	Very slight erythema	Very slight edema	1
3	Well defined erythema	Slight edema	2
4	Moderate to severe erythema	Moderate edema	3
5	Severe erythema	Severe edema	4

3.2.7 *In Vivo* Anti-inflammatory Studies

The anti-inflammatory activity and sustaining action of the formulated films were evaluated using the ‘‘egg albumin-induced hind paw edema’’ method developed by Winter *et al.* (1962), in white albino rats. Young male rats, weighing 200–290 g, were randomly divided into three groups, each containing five rats. The rats were given free access to water and food, and observed for 24 h.

The dorsal region of the rats’ right thigh was shaved 12 h before application of the drug. Films were applied on the shaved dorsal region of all the animals (except the control group) half an hour before subplantar injection of egg albumin in the right paws. Paw edema was induced by injecting 0.1 ml of a 1 % w/v homogeneous suspension of egg albumin. A digital caliper was used to measure the size of the paw administered with the egg albumin, immediately at 0 min and at 1, 2, 3, 4, 5 and 6 h. The amount of paw swelling was determined, and expressed as percent edema relative to the initial (0 min) hind paw size.

The results obtained were compared with the anti-inflammatory effect of the control group. The mean percentage values were determined for each time interval. Percentage inhibition of edema produced by each film treated group was calculated against the respective control group using the following formula (Arora and Mukherjee 2002).

_____ (10)

3.2.8 Stability Study of the Formulated Transdermal Films

Stability studies were carried out on the various batches of the formulations. The transdermal films were stored at ambient temperature in a dessicator containing sodium chloride having a relative humidity of 75 ± 5 %. The films were withdrawn and

evaluated for drug content and other physical parameters every month for three months (Kevin *et al.*, 2009).

3.2.9 Statistical analysis

All experiments were prepared in triplicates for validity of statistical analysis. Results were expressed as mean ± standard deviation. The analysis of variance (ANOVA) was used to detect statistically significant differences at p-values <0.05 (Patel *et al*., 2009).

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Characterization of the Formulated Diclofenac Sodium Transdermal Films

Table 4.1: Physical Characteristics of the Various Batches of Formulated Diclofenac Sodium Films

Samples	Physical Characterization
F1	White, Translucent, Flexible, Smooth, Uniform
F2	White, Translucent, Flexible, Smooth, Uniform
F3	White, Translucent, Flexible, Smooth, Uniform
F4	White, Translucent, Brittle, Rough
F5	White, Translucent, Brittle, Rough
F7	White, Translucent, Flexile, Smooth, Uniform

4.2 Results of the Fourier Transform Infrared (FT-IR) Spectroscopy Analysis

4.2.1 FT- IR spectra of pure diclofenac sodium

The FT-IR spectra of diclofenac sodium are presented in Figure 4.1, and showed a broad peak of 3417 cm^{-1} (N-H stretching bonds) indicating an amine group; a peak at 1568 cm^{-1} (aromatic C=C stretching bonds) indicating an alkene group, with peaks in the range of 1484 cm^{-1}, 1392 cm^{-1} (C-H bending bonds) indicating an alkane group, another peak at 1298 cm^{-1} (C-H bending bonds) still indicating an alkane group. There was also a peak at 1077 cm^{-1} (C-O stretching bonds) indicating a carbonyl group, and peaks at 754 cm^{-1} and 852 cm^{-1} (C-Cl vibrations) indicating a halide group. The spectra obtained are comparable to those reported by Bharathi *et al.* (2011), confirming the purity of the drug.

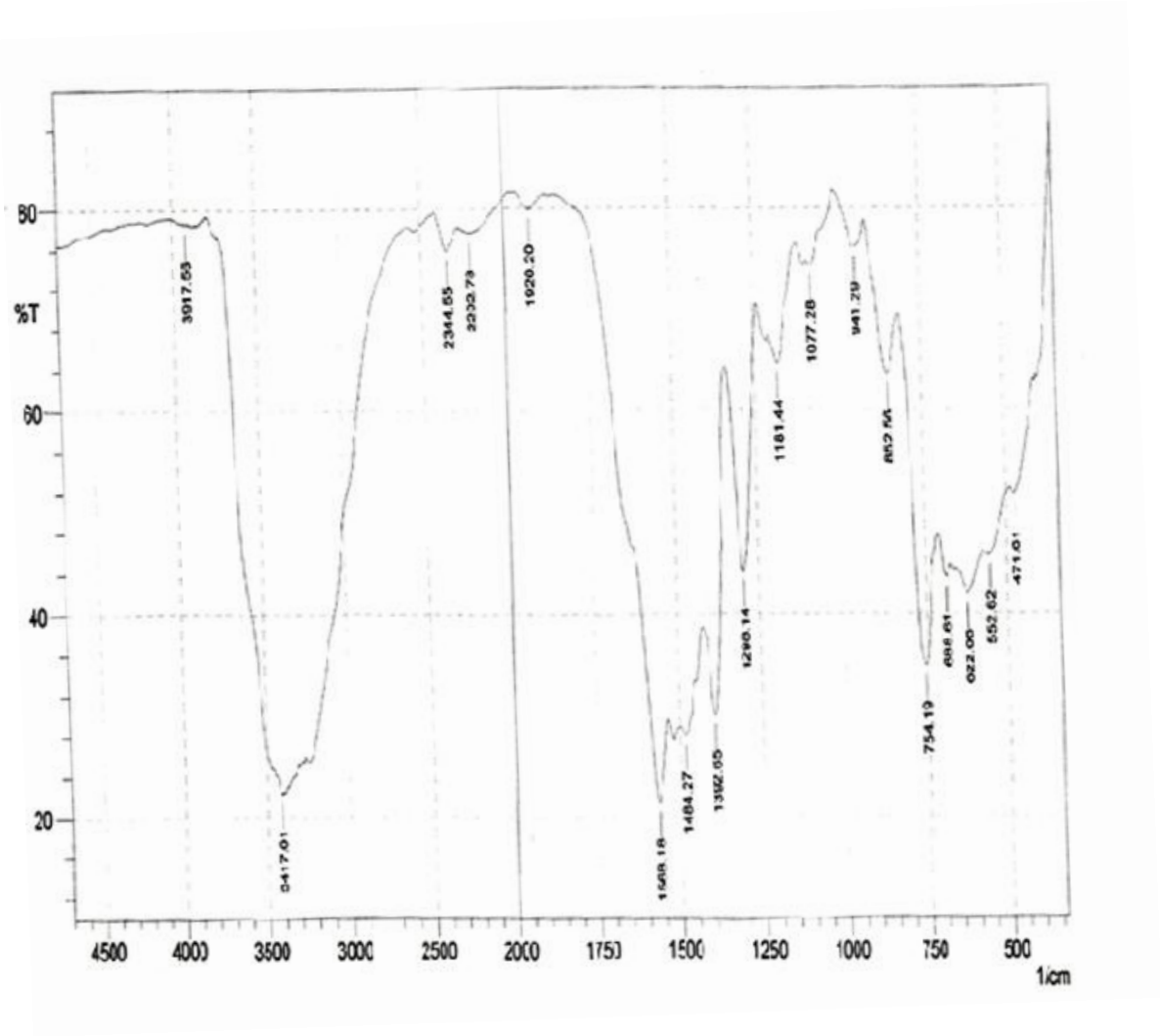


Figure 4.1: FT- IR Spectra of Pure Diclofenac Sodium

4.2.2 FT- IR spectra of Eudragit RS 100

The FT-IR spectra of Eudragit RS 100 alone are shown in Figure 4.2, and reveal characteristic peaks at 3612 $cm^{-1}$and 3339 cm^{-1} (–O-H stretching bonds) indicating a hydroxyl group, peaks at 3463 cm^{-1} (N-H stretching vibrations) indicating the presence of an amine group, peaks at 2973 cm^{-1} (-C-H stretching vibrations) indicating that an alkane group is present, peaks at 1732 cm^{-1} (-C=O stretching bonds) present in esters, peaks at 1463 cm^{-1} and 1380 cm^{-1} (-C-H bending bonds) also indicating the presence of an alkane group, and finally, peaks at 1280 cm^{-1} (C-O vibration bonds) present in esters. These peaks are similar to those reported by Rajabalaya *et al*. (2010) in the design and *in vitro* evaluation of chlorpheniramine maleate in different Eudragit based matrix patches.

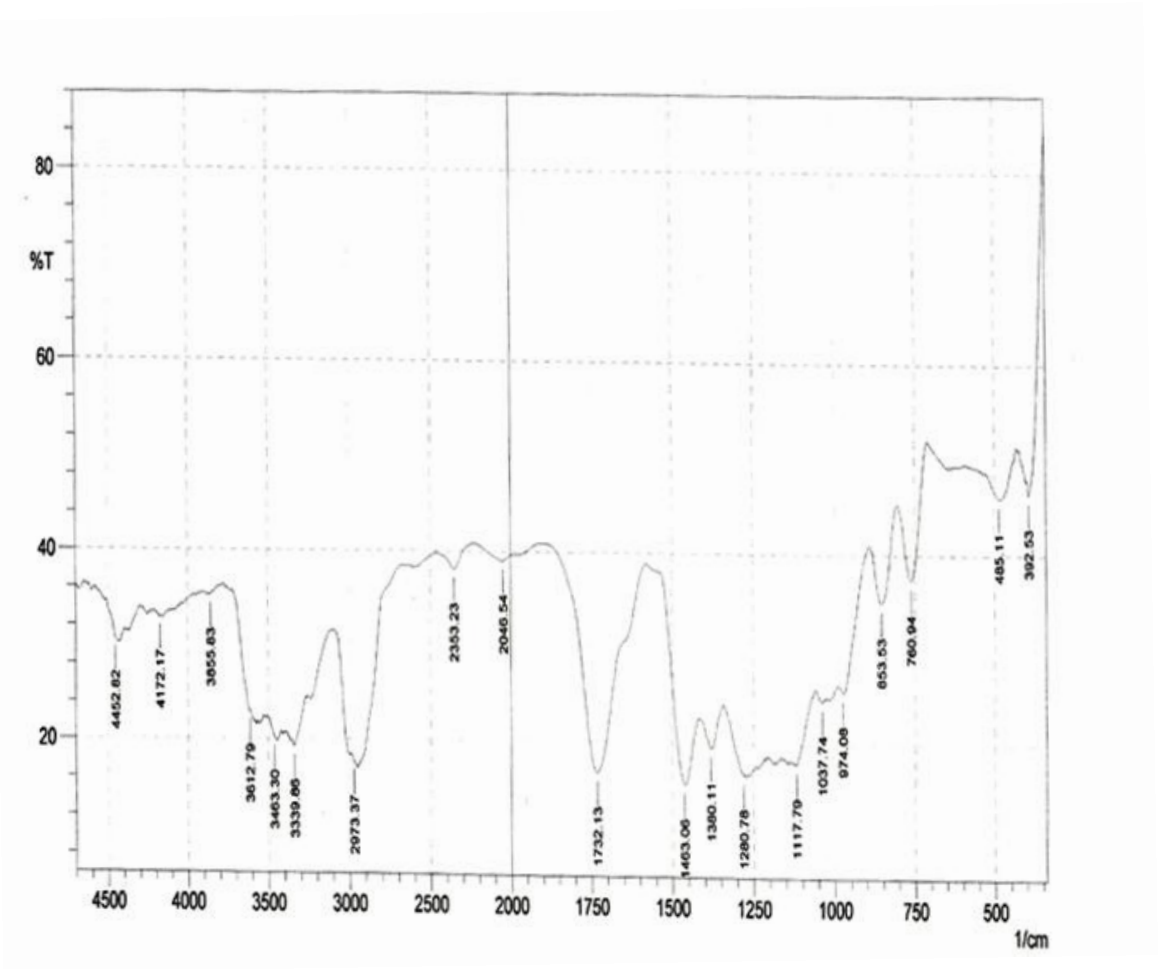


Figure 4.2: FT- IR Spectra of Eudragit RS 100

4.2.3 FT- IR spectra of HPMC

The FT-IR spectra of HPMC alone are shown in Figure 4.3, and reveal a broad peaks at 3493 cm^{-1} (O-H stretching bonds) indicating a hydroxyl group, peaks at 2911 cm^{-1} (-C-H stretching bonds) indicating an alkane group, peak at 1643 cm^{-1} (-C=O vibration bonds) indicating a carbonyl group, while peaks at 1448.5 cm^{-1} and 1368 cm^{-1} (C-H bending frequency) indicate an alkane group, and peaks at 1125 $cm^{-1}$and 1056 cm^{-1} (C-O vibration bonds) present in ether and alcoholic groups. The peaks are similar to those reported by Sahoo *et al.,* (2012), who carried out spectroscopic investigations on ciprofloxacin / HPMC muco- adhesive suspension.

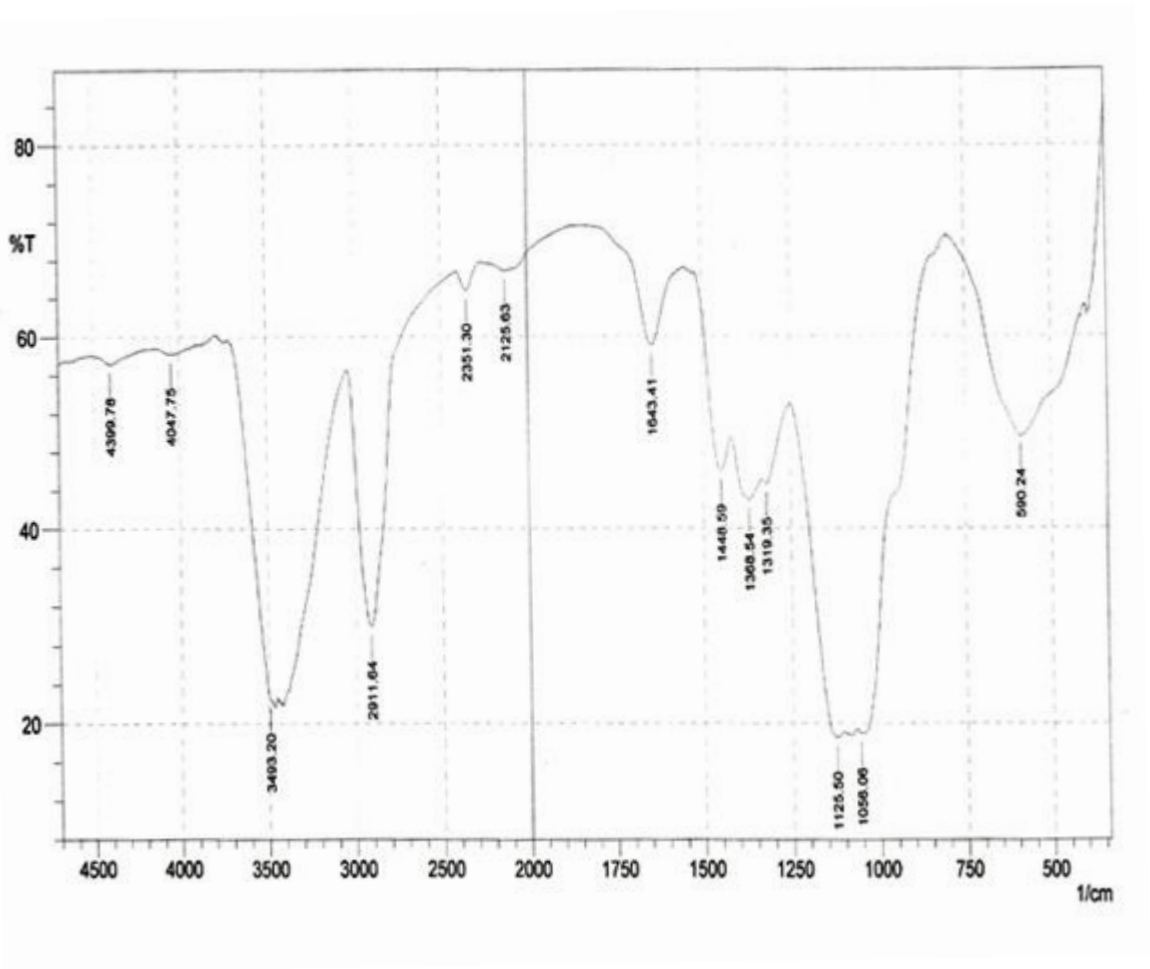


Figure 4.3: FT- IR Spectra of HPMC

4.2.4 FT- IR spectra of polyethylene glygol

The FT-IR spectra of polyethylene glycol alone as shown in Figure 4.4, reveals a peak at 3492 cm^{-1} (–O-H stretching bonds) indicating a hydroxyl group, peak at 2921 cm^{-1} (-C-H stretching bonds) indicating the presence of an alkane group, peak at 1635 cm^{-1} (-C=C stretching vibration) indicating the presence of an alkene group, while peaks in the range of 1467 cm^{-1} and 1356 cm^{-1} (-C-H bending vibration) indicates the presence of an alkane group; peaks at 1134 cm^{-1} and 1084 cm^{-1} (C-O vibration bonds) shows the presence of an alkane group and peaks at 949 $cm^{-1}$and 842 cm^{-1} (C-H vibration bonds) show a methylene chain $(CH_2)n$).

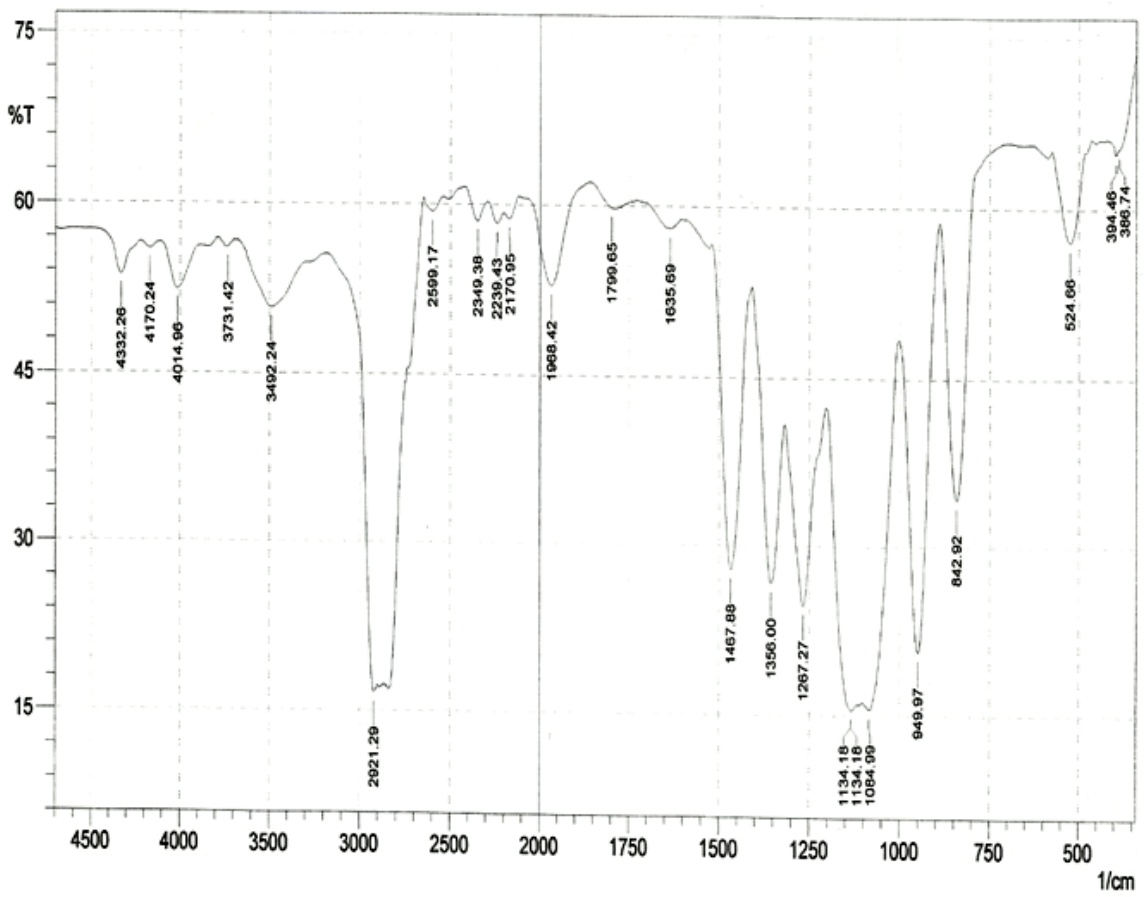


Figure 4.4: FT- IR Spectra of Polyethylene glygol

4.2.5 FT- IR spectra of diclofenac sodium films containing 100 mg of Eudragit RS 100 and 400 mg of HPMC (Formulation F1)

The FT-IR spectra of Formulation F1 Figure 4.5, and reveal some characteristic peaks as observed in the pure drug as well as additional peaks observed in the polymer combinations. The peak at 3448 cm^{-1} (N-H stretching bonds) indicate an amine group, peak at 2908 cm^{-1} (C-H stretching bonds) an alkane group, peak at 1728 cm^{-1} (C=O bonding vibration) a carboxylic acid group, peak at 1582 cm^{-1} (C=C stretching bonds) an aromatic ring, peaks at 1455 cm^{-1} and 1368 cm^{-1} (C-H bending bonds) the presence of an alkane group, while a peak at 1097 cm^{-1} (C-O bonds) show a carboxylic group, and peaks at 759 $cm^{-1}$and 853 cm^{-1} (C-Cl vibrations), the presence of a halide group.

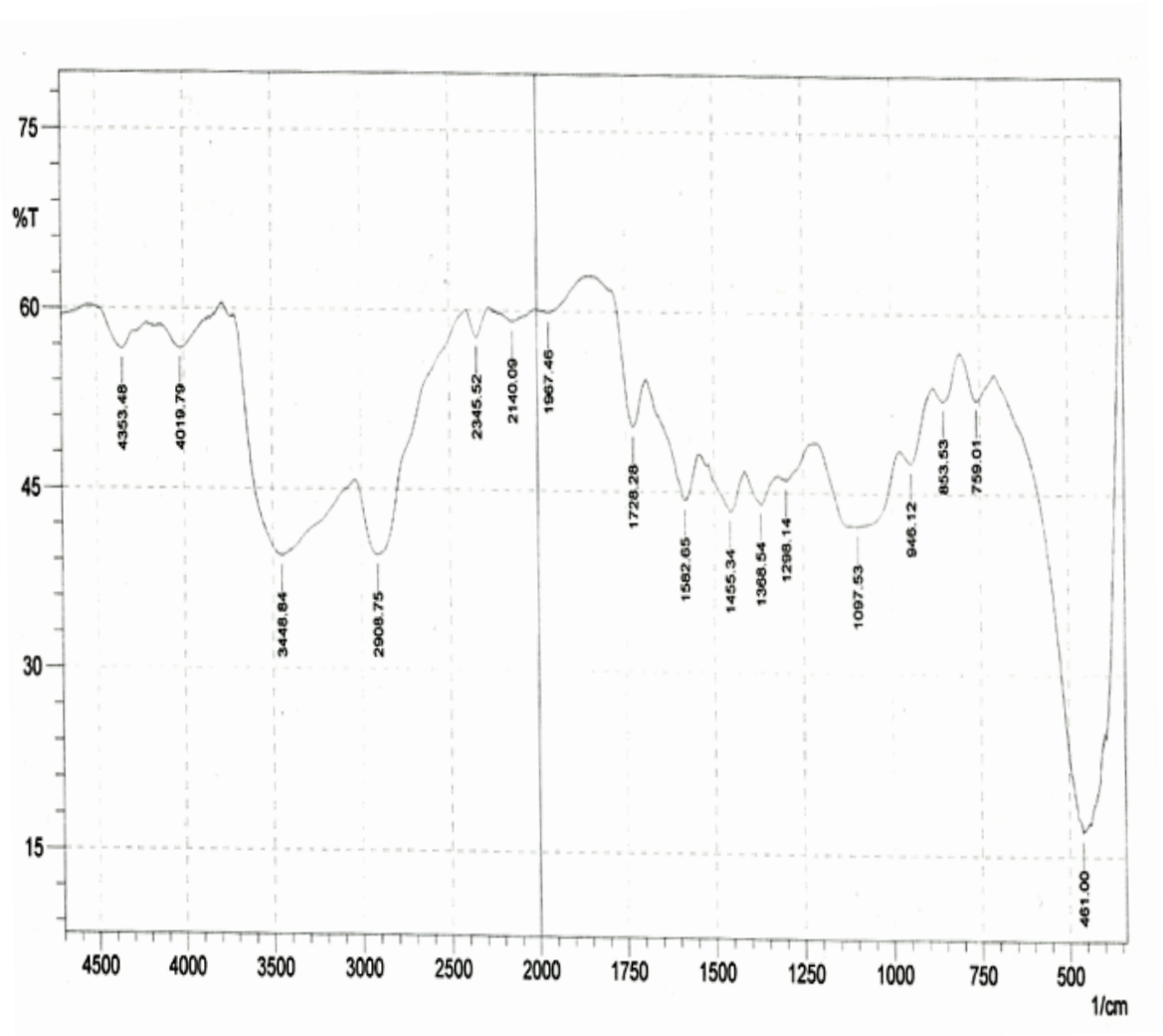


Figure 4.5: FT- IR Spectra of Diclofenac Sodium Films Containing 100 mg of Eudragit RS 100 and 400 mg of HPMC (Formulation F1)

4.2.6 FT- IR spectra of diclofenac sodium film containing 200 mg Eudragit RS 100 and 300 mg HPMC (Formulation F2)

The FT-IR spectra of Formulation F2 as shown in Figure 4.6, showed some peaks as observed in the pure drug and also additional peaks as a result of the combination of the different polymers used. The peak at 3448 cm^{-1} (N-H stretching bonds) indicates the presence of an amine group while a peak at 3221 cm^{-1} (-O-H stretching bonds) indicate a hydroxyl group, peaks at 2919 cm^{-1} (-C-H stretching vibrations) the presence of an alkane group and a peak at 1728 cm^{-1}(-C=O bonds) showing a carboxylic group, the peak 1582 cm^{-1} (C=C stretching vibrations) is present in an aromatic ring, peaks at 1455 $cm^{-1}$and 1366 cm^{-1} (-C-H bending bonds) indicating the presence of an alkane group , while peaks at 1293 cm^{-1} (C-H bending bonds) indicates the presence of an alkene group. Peaks at 1074 cm^{-1} (C-O bonding vibrations) shows the presence of a carboxylic acid and peaks at 761 $cm^{-1}$and 851 cm^{-1} (C-Cl bonds) indicate the presence of a halide group.

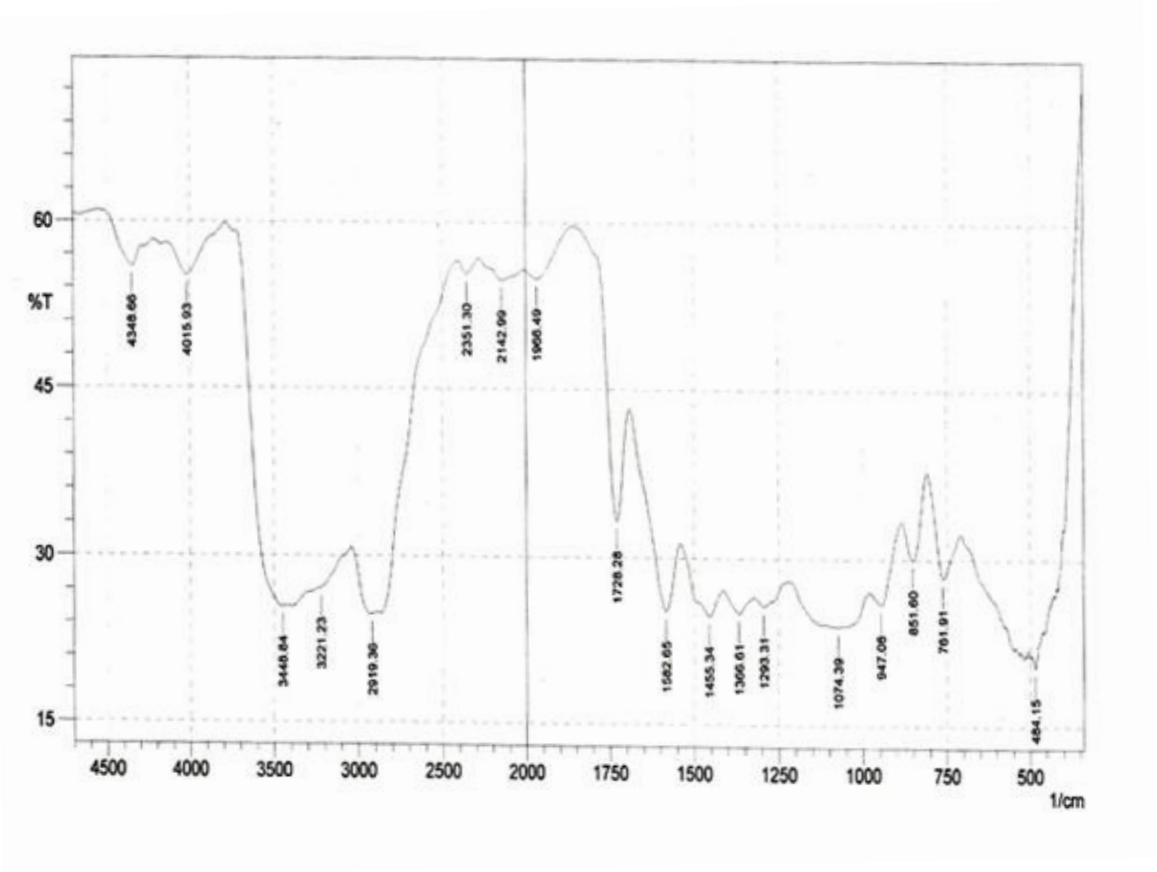


Figure 4.6: FT- IR Spectra of Diclofenac Sodium Film Containing 200 mg Eudragit RS 100 and 300 mg HPMC (Formulation F2)

4.2.7 FT- IR spectra of diclofenac sodium films containing 250 mg Eudragit RS 100 and 250 mg HPMC (Formulation F3)

The FT-IR spectra of Formulation F3 as shown in Figure 4.7, and reveal the characteristic peaks observed in the pure drug and additional peaks due to the combination of polymers present. Peak at (3613 cm^{-1}) (-O-H stretching bonds) indicate the presence of a hydroxyl group, peak at 2918 cm^{-1} (C-H stretching frequency) the presence of an alkane group, peak at 1728 cm^{-1}(-C=O bonding vibrations) the presence of carboxylic acid group, peaks at 1581 cm^{-1} (C=C stretching vibrations), the presence of an aromatic ring, and peaks at 759 $cm^{-1}$and 847 cm^{-1} (C-Cl vibrations) the presence of a halide group.

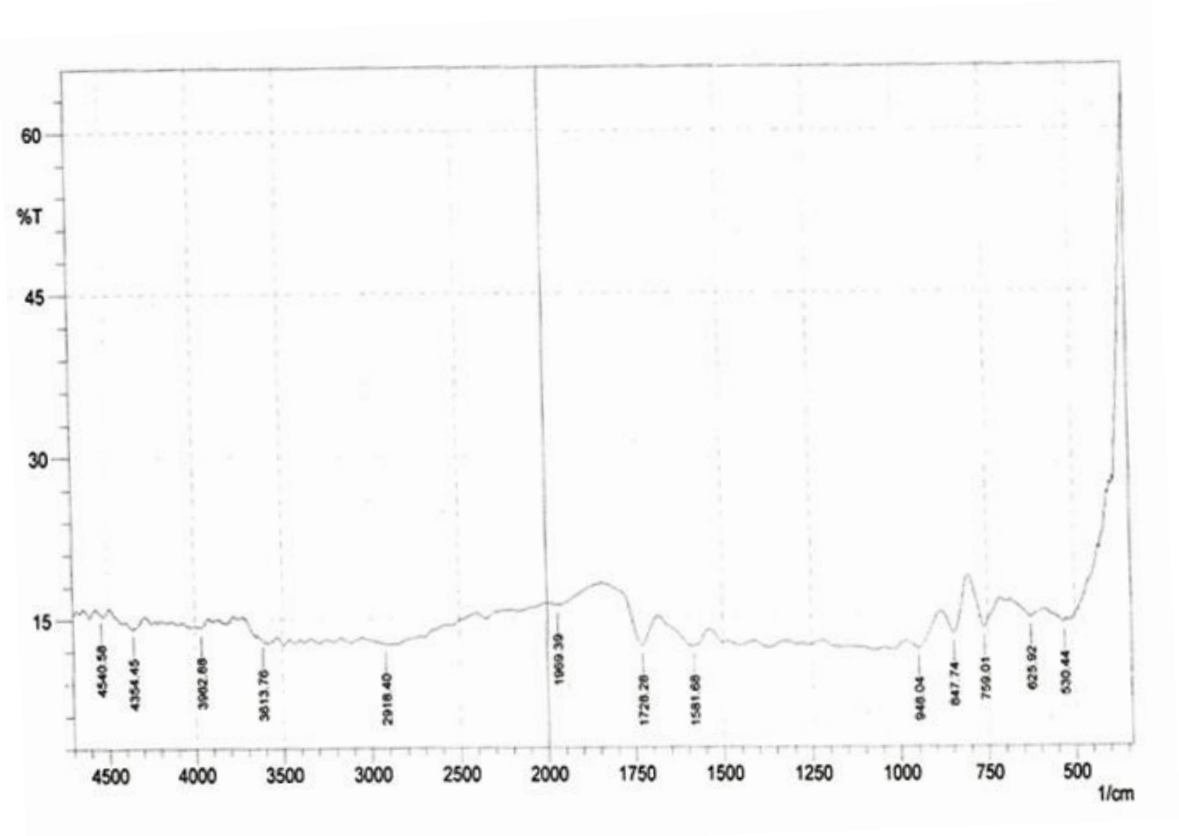


Figure 4.7: FT- IR Spectra of Diclofenac Sodium Films Containing 250 mg Eudragit RS 100 and 250 mg HPMC (Formulation F3)

4.2.8 FT- IR Spectra of diclofenac sodium films containing 300 mg of Eudragit RS 100 and 200 mg HPMC (Formulation F4)

Figure 4.8 shows the FT-IR spectra of Formulation F4, revealing major peaks observed in the pure drug as well as additional peaks as a result of the combination with the different polymers used in the study. The peak at 3596 cm^{-1} (N-H stretching bonds) indicates the presence of an amine group, while those at 3389 cm^{-1} and 3267 cm^{-1}(O-H stretching bonds) indicate the presence of a hydroxyl group. Peaks at 2816 cm^{-1} (C-H stretching bonds) indicates an alkane group, while peaks at 1731 cm^{-1}(C=O bonding vibrations) show a carboxylic acid group, and a peak at 1587 cm^{-1} (C=C stretching bonds) is indicative of an aromatic ring. Peaks at 1465 $cm^{-1}$and 1367 cm^{-1}(C-H bending vibrations) indicate the presence of an alkane group, peaks at 1260 cm^{-1}(C-H bending vibrations), an alkene group, peaks at 1031 cm^{-1} (C-O bonding vibrations) a carboxylic acid group and peaks at 761 $cm^{-1}$and 848 cm^{-1}(C-Cl bonding vibrations) indicative of a halide group.

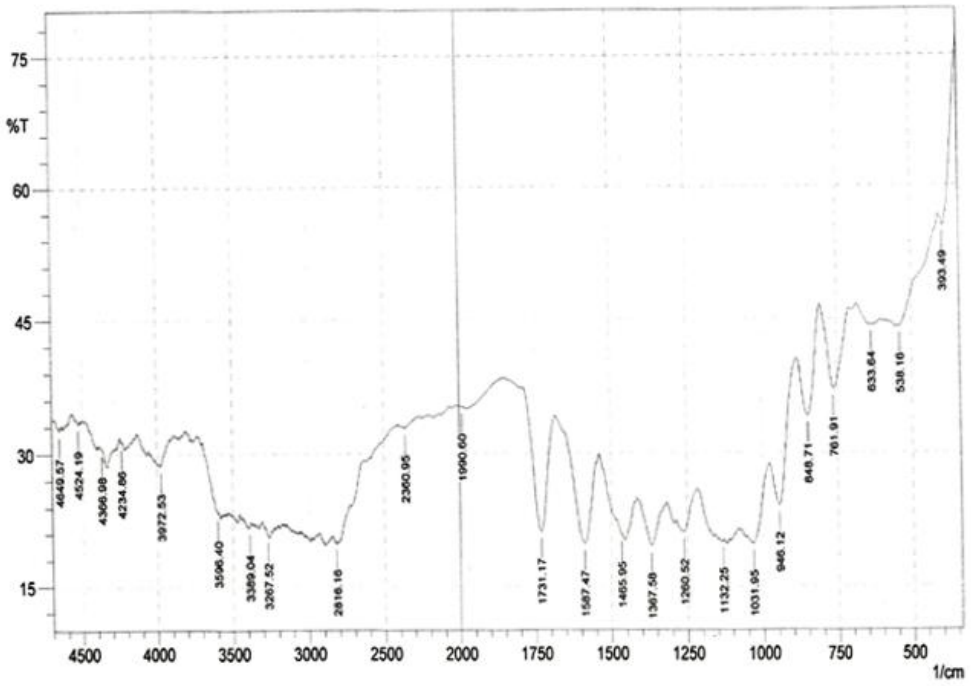


Figure 4.8: FT- IR Spectra of Diclofenac Sodium Films Containing 300 mg of Eudragit RS 100 and 200 mg HPMC (Formulation F4)

4.2.9 FT- IR spectra of diclofenac sodium films containing 400 mg of Eudragit RS 100 and 100 mg HPMC (Formulation F5)

The FT-IR spectra of Formulation F5 as shown in Figure 4.9, reveal some peaks as observed in the pure drug as well as additional peaks present as a result of the combination of drug and polymer. The peaks at 3434 cm^{-1} (N-H stretching bonds) indicate the presence of an amine group, the peak at 2893 cm^{-1} (C-H stretching bonds) indicate the presence of an alkane group, the peak at 1729 cm^{-1}(C=O bonding vibrations) a carboxylic acid group, peaks at 1583 cm^{-1} (C=C stretching vibrations) an aromatic nucleus, peaks at 1459 cm^{-1} and 1368 cm^{-1} (C-H bending vibrations) the presence of an alkane, peaks at 1262 cm^{-1} (C-H bending bonds) an alkene group, peaks at 1121 cm^{-1} (C-O bonding vibration) the presence in a carboxylic acid group, and peaks at 850 $cm^{-1}$and 756 cm^{-1} (C-Cl bonding vibrations) indicates a halide group present.

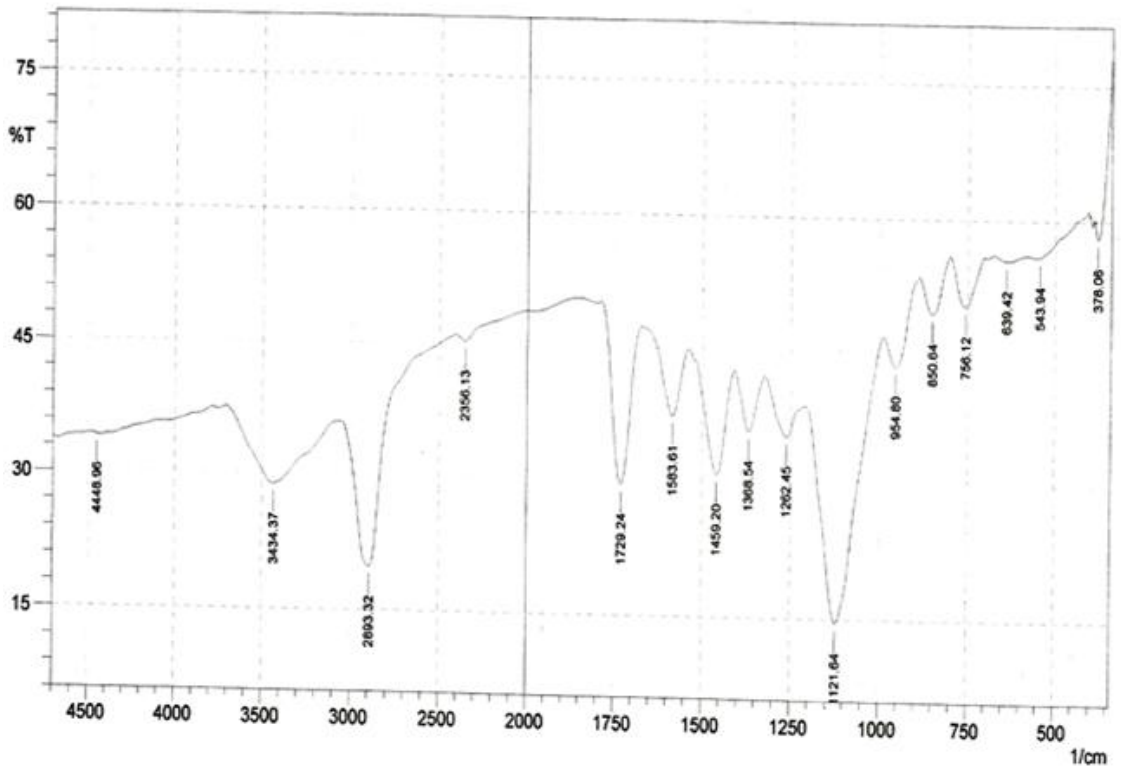


Figure 4.9: FT- IR Spectra of Diclofenac Sodium Films Containing 400 mg of Eudragit RS 100 and 100 mg HPMC (Formulation F5)

4.2.10 FT- IR spectra of diclofenac sodium films containing 500 mg of HPMC (Formulation F7)

The FT-IR spectra of Formulation F7 is shown in Figure 4.10, and reveal some peaks observed in the pure drug as well as additional peaks as a result of the combination of drug and polymer. The peaks at 3663 cm^{-1} and 3385 cm^{-1} (-O-H stretching bonds) show the presence of a carboxylic acid group, peak at 3538 cm^{-1} (- N-H stretching bonds) indicate the presence of an amine group, peaks at 2813 $cm^{-1}$and 2717 cm^{-1} (C-H stretching bonds) indicate an alkane group, peaks at 1734 cm^{-1} (-C=O bonding vibrations) the presence of a carboxylic acid group, peaks at 1596 cm^{-1} (C=C stretching vibrations) the presence of an aromatic nucleus, peaks at 1401 cm^{-1}(-C-H bending vibrations) the presence of an alkane group, peaks at 1278 cm^{-1} (C-H bending bonds) indicates the presence of an alkene group, peaks at 1139 cm^{-1} (C-O bonding vibrations) shows a carboxylic acid group, and peaks at 856 cm^{-1} and 760 cm^{-1} (C-Cl bonding vibrations) indicate the presence of a halide group.

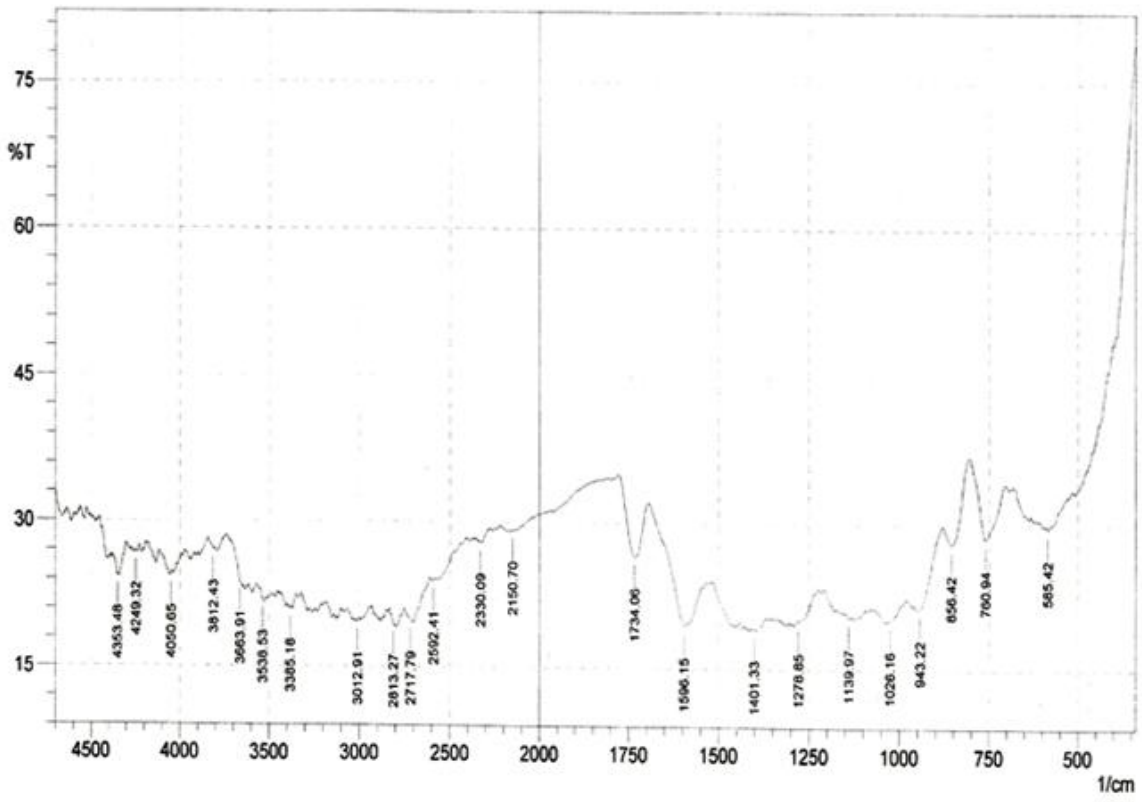


Figure 4.10: FT- IR Spectra of Diclofenac Sodium Films Containing 500 mg of HPMC Alone (Formulation F7)

4.3 Results of the Differential Scanning Calorimetry (DSC)

4.3.1 DSC thermogram of pure diclofenac sodium

The DSC thermogram of the pure drug (diclofenac sodium) displayed a broad endothermic peak at 64 ºC which may be due to water loss and a sharp endothermic peak at 284 ºC corresponding to its melting peak Figure 4.11. It also showed an exothermic peak at 293 ºC corresponding to the decomposition temperature of diclofenac sodium. The normal melting range of the drug is 283 ºC – 285 ºC (Sabyasachi *et al.,* 2011).

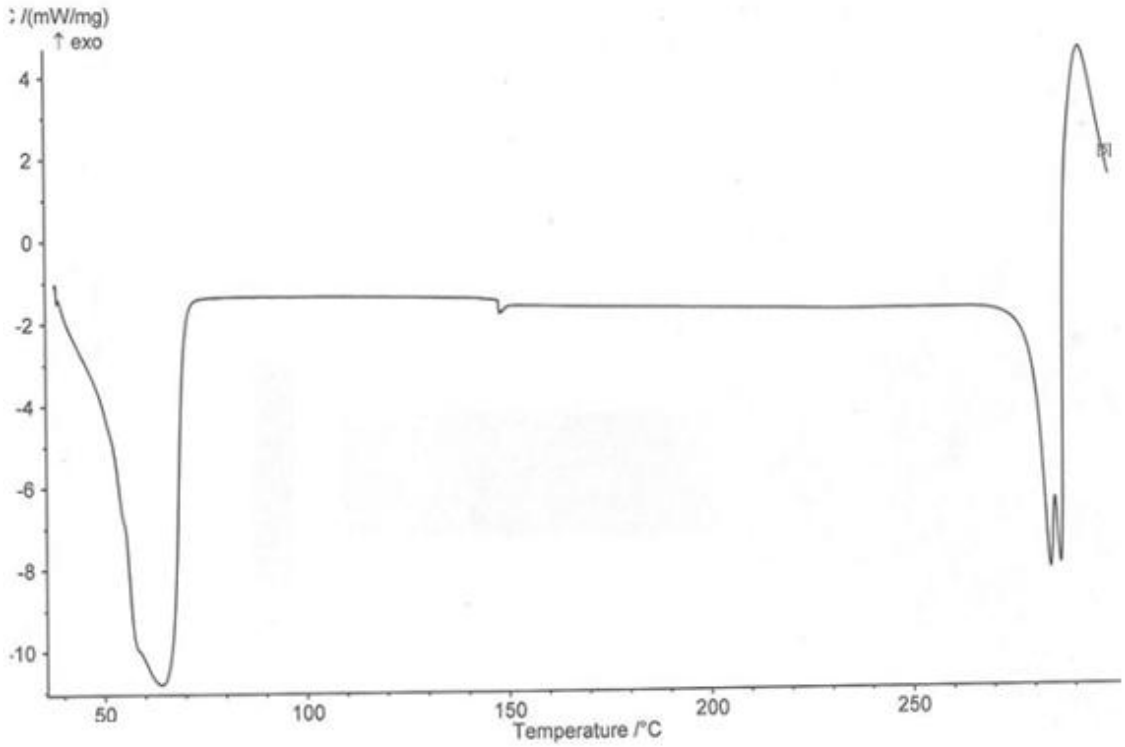


Figure 4.11: DSC Thermogram of Pure Diclofenac Sodium

4.3.2 DSC thermogram of pure polyethylene glycol

The DSC scan of polyethylene glycol alone as seen in Figure 4.12, shows a sharp endothermic peak at 66.3 ºC, corresponding to its melting peak.

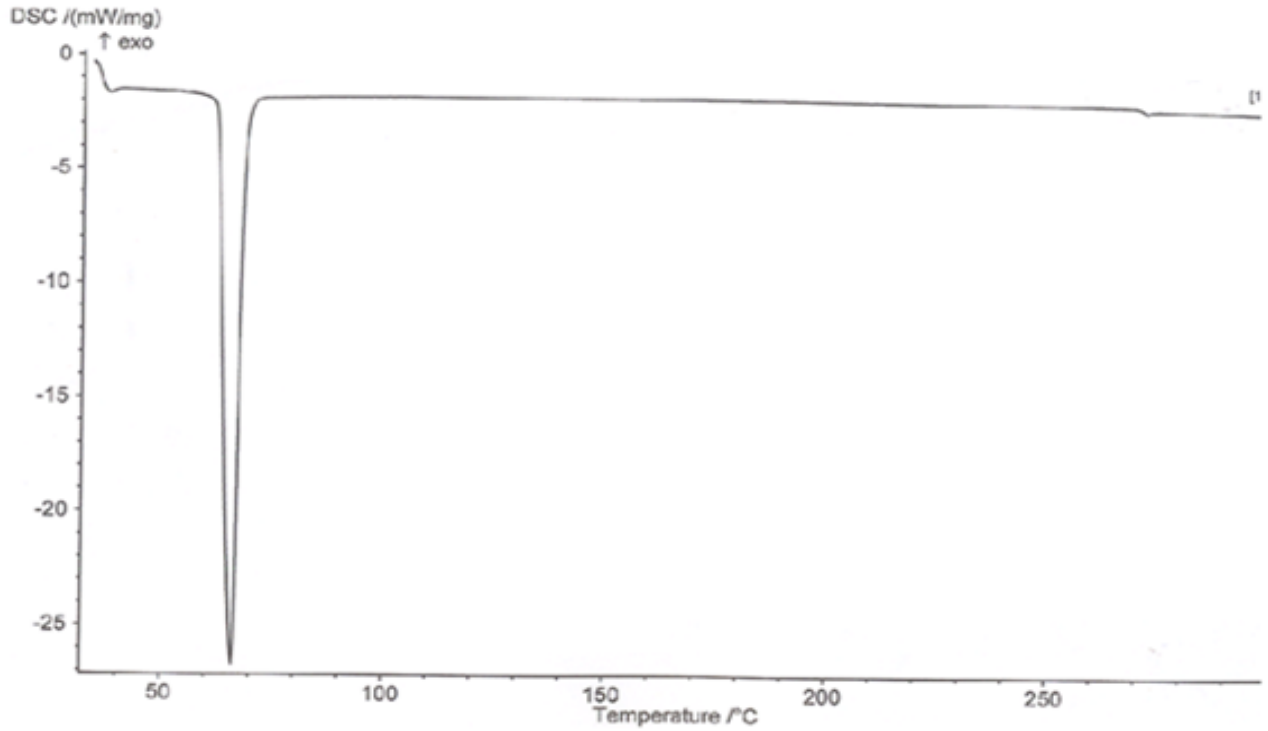


Figure 4.12: DSC Thermogram of Pure Polyethylene glycol

4.3.3 DSC thermogram of HPMC

The DSC scan of HPMC as seen in Figure 4.13, displays a broad endothermic peak at 53.2 ºC and 176.9 ºC, which corresponds to its melting peak.

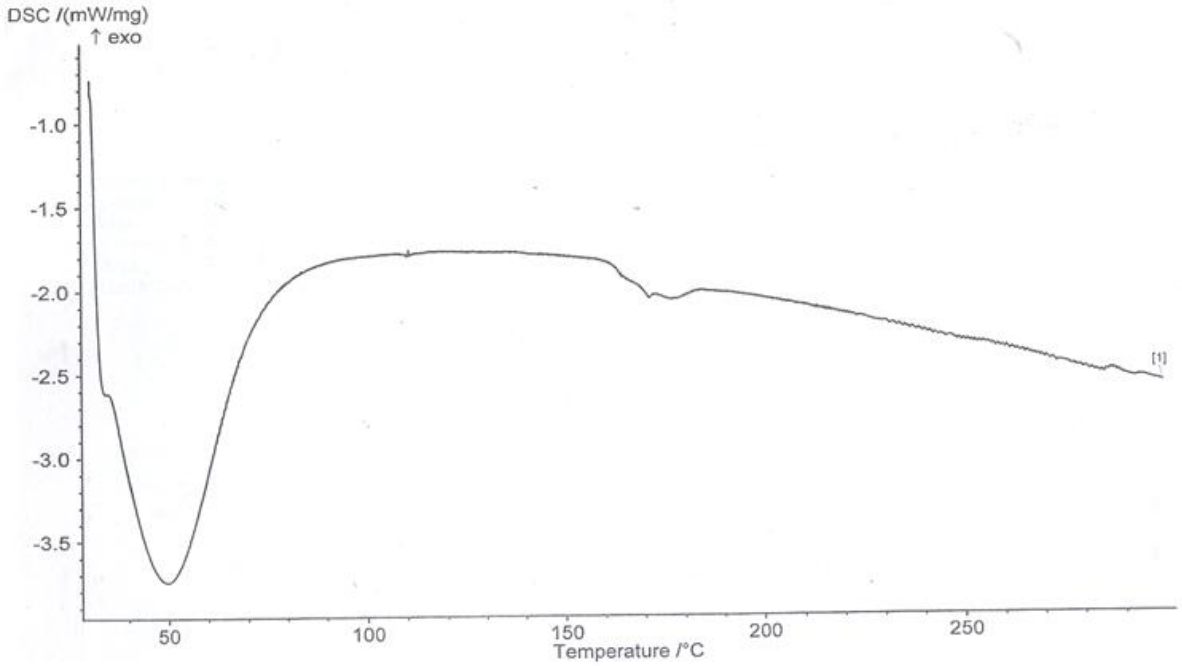


Figure 4.13: DSC Thermogram of HPMC

4.3.4 DSC thermogram of Eudragit RS 100

The DSC scan of Eudragit RS 100 alone as seen in Figure 4.14, shows a broad endothermic peak at, 190.9 ºC, and 204.1 ºC, which corresponds to its melting peak.

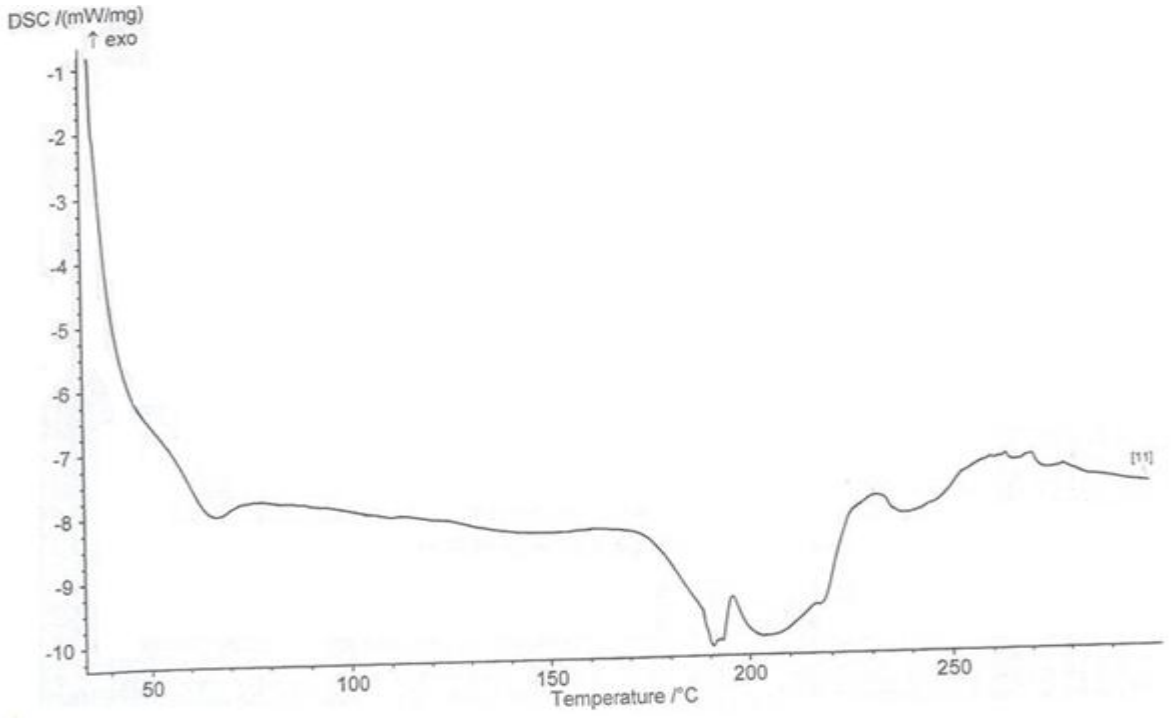


Figure 4.14: DSC Thermogram of Eudragit RS 100

4.3.5 DSC thermogram of diclofenac sodium films containing 100 mg of Eudragit RS 100 and 400 mg of HPMC (Formulation F1)

The DSC scan of Formulation F1 as seen in Figure 4.15, shows an endothermic peak at 64.1 ºC and 208.5 ºC and an exothermic peak at 244.7 ºC, which corresponds to its decomposition temperature.

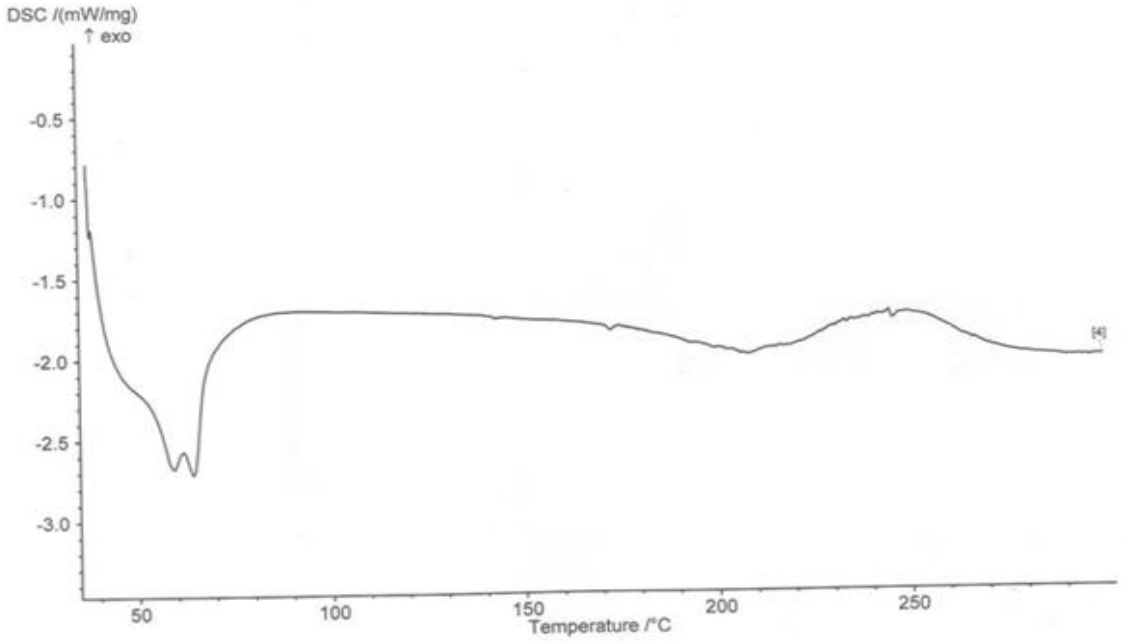


Figure 4.15: DSC Thermogram of Diclofenac Sodium Films Containing 100 mg of Eudragit RS 100 and 400 mg of HPMC (Formulation F1)

4.3.6 DSC thermogram of diclofenac sodium films containing 200 mg of Eudragit RS 100 and 300 mg of HPMC (Formulation F2)

The DSC scan of Formulation F2 is shown in Figure 4.16, displays endothermic peaks at 60.1 ºC and 163.7 ºC and an exothermic peak at 251.4 ºC, which corresponds to its decomposition temperature.

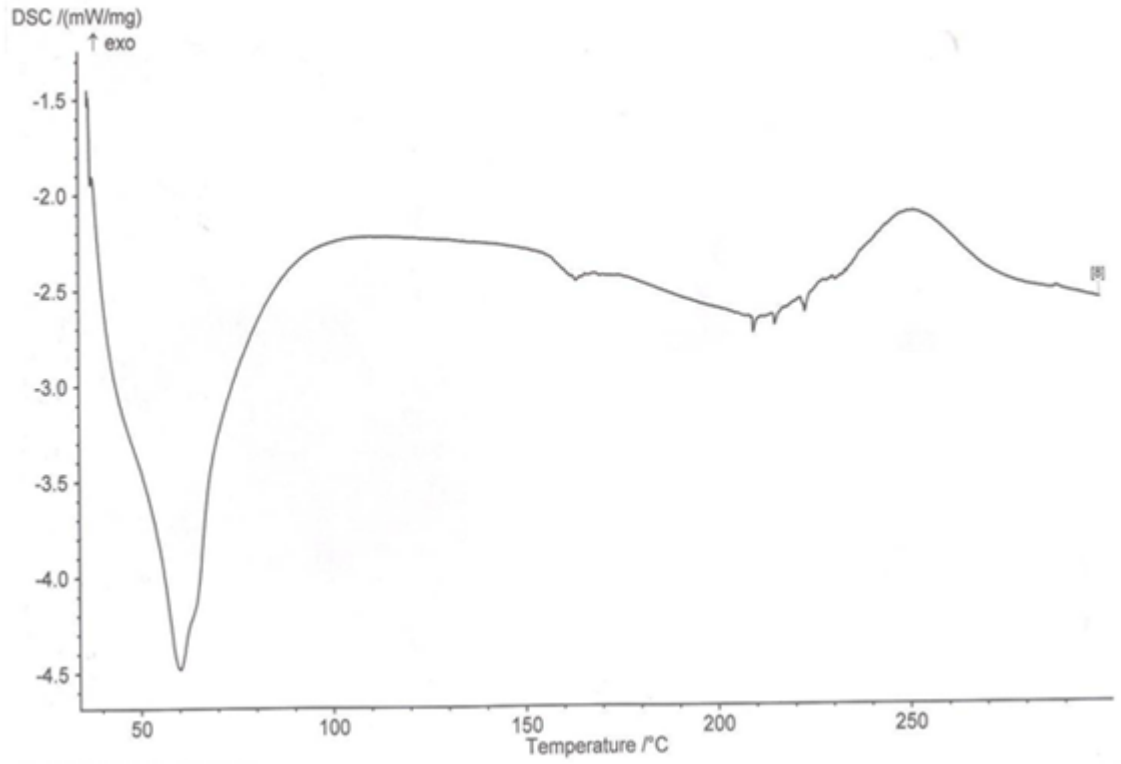


Figure 4.16: DSC Thermogram of Diclofenac Sodium Films Containing 200 mg of Eudragit RS 100 and 300 mg of HPMC (Formulation F2)

4.3.7 DSC thermogram of diclofenac sodium films containing 250 mg of Eudragit RS 100 and 250 mg of HPMC (Formulation F3)

The DSC scan of Formulation F3 as shown in Figure 4.17, displays endothermic peaks at, 59.2 ºC and 213.7 ºC and an exothermic peak at 249.4 ºC which is equivalent to its decomposition temperature.

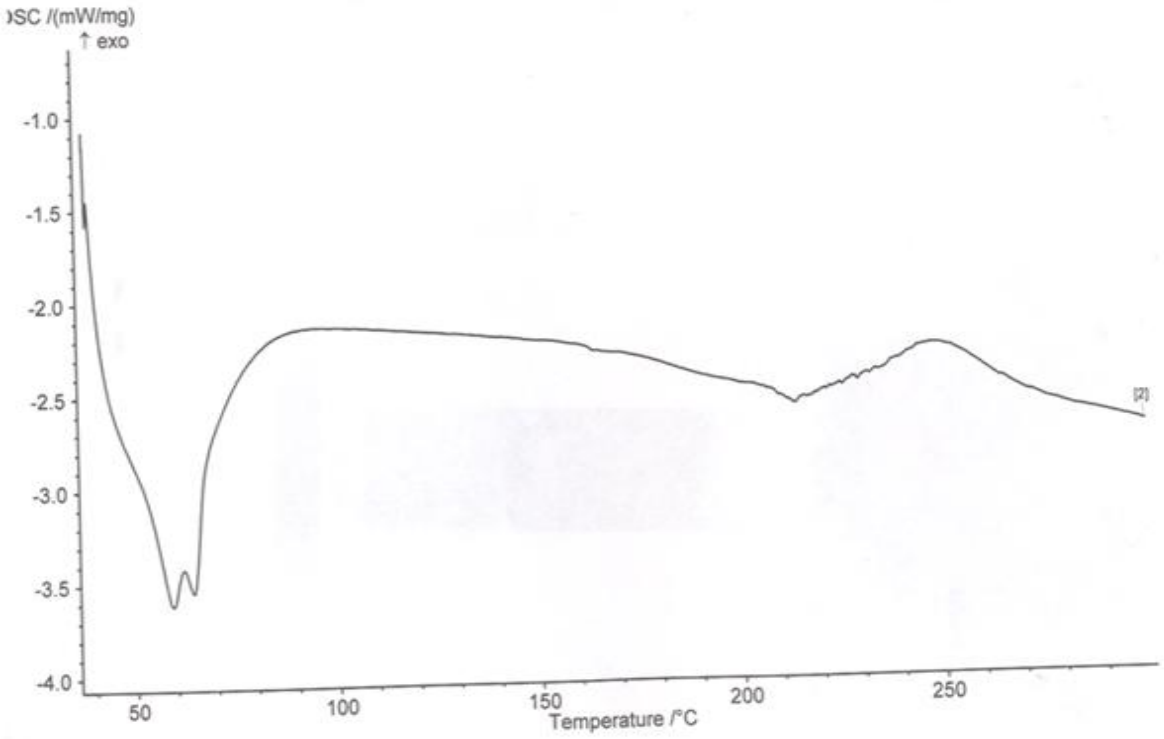


Figure 4.17: DSC Thermogram of Diclofenac Sodium Films Containing 250 mg of Eudragit RS 100 and 250 mg of HPMC (Formulation F3)

4.3.8 DSC thermogram of diclofenac sodium films containing 300 mg of Eudragit RS 100 and 200 mg of HPMC (Formulation F4)

The DSC scan of Formulation F4 as seen in Figure 4.18, shows an endothermic peak at 58.8 ºC and 213.9 ºC, and a broad exothermic peak at 251.4 ºC which is equivalent to its decomposition temperature.

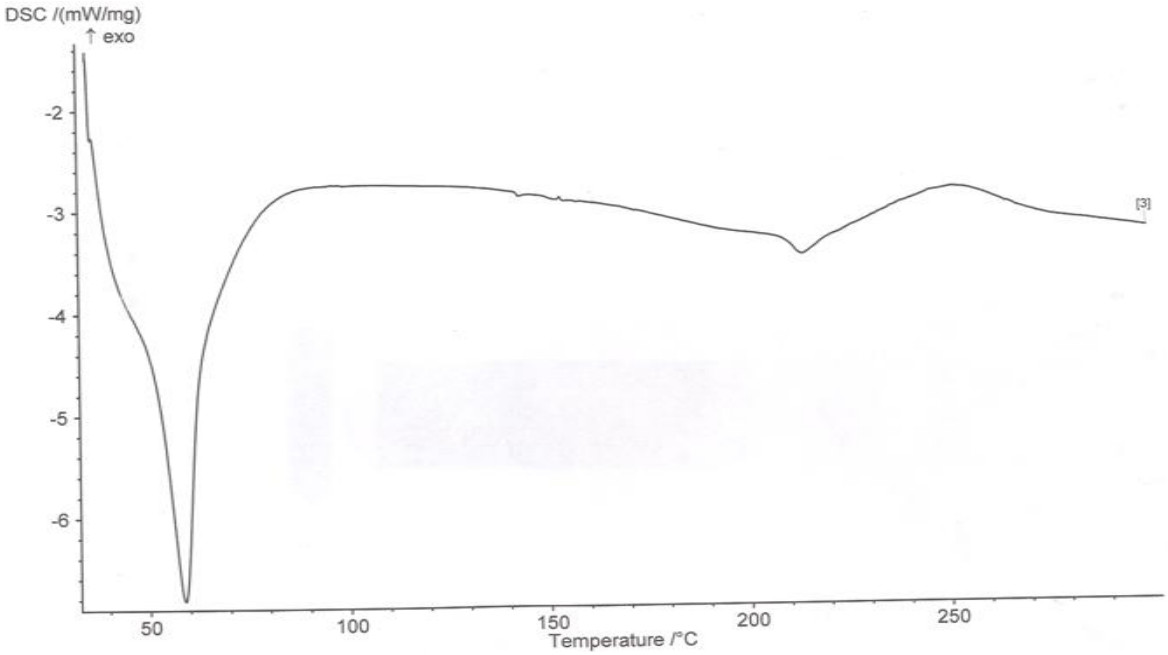


Figure 4.18: DSC Thermogram of Diclofenac Sodium Films Containing 300 mg of Eudragit RS 100 and 200 mg of HPMC (Formulation F4)

4.3.9 DSC thermogram of diclofenac sodium films containing 400 mg of Eudragit RS 100 and 100 mg of HPMC (Formulation F5)

The DSC scan of Formulation F5 as seen in Figure 4.19, shows endothermic peaks at 59.6 ºC and 220.0 ºC and an exothermic peak at 255.3 ºC which corresponds to its decomposition temperature.

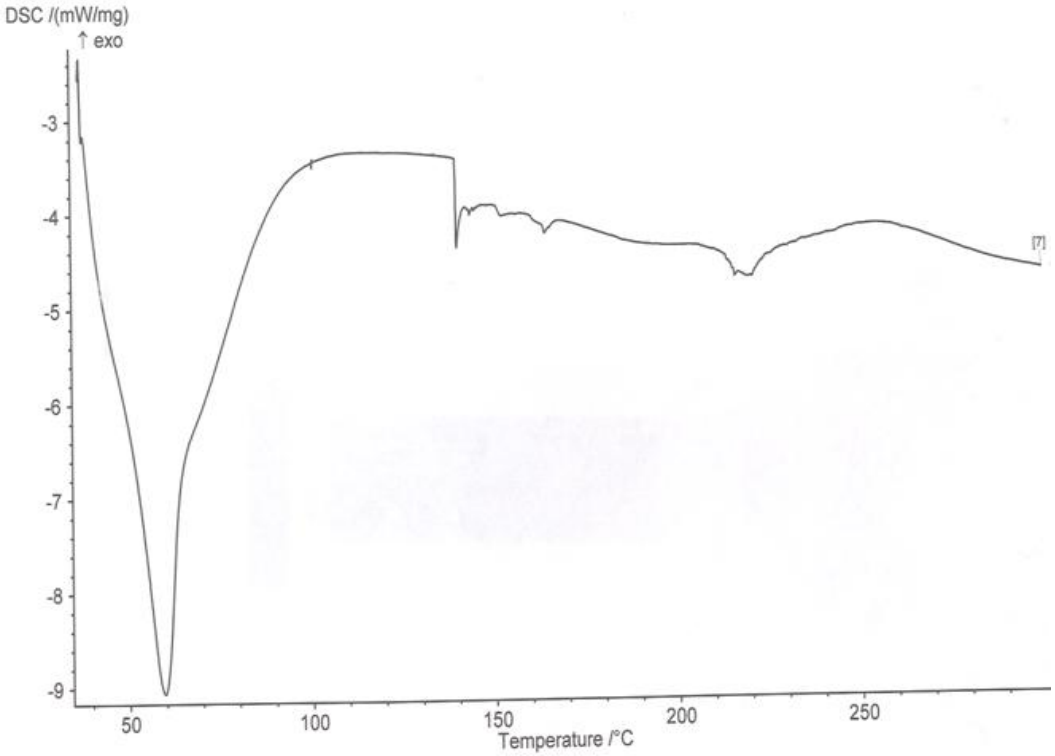


Figure 4.19: DSC Thermogram of Diclofenac Sodium Films Containing 400 mg of Eudragit RS 100 and 100 mg of HPMC (Formulation F5)

4.3.10 DSC thermogram of diclofenac sodium films containing 500 mg of HPMC (Formulation F7)

The DSC scan of Formulation F7 as seen in Figure 4.20, shows endothermic peaks at 63.8 ºC and 214.8 ºC and an exothermic peak at 253.2 ºC which corresponds to its decomposition temperature.

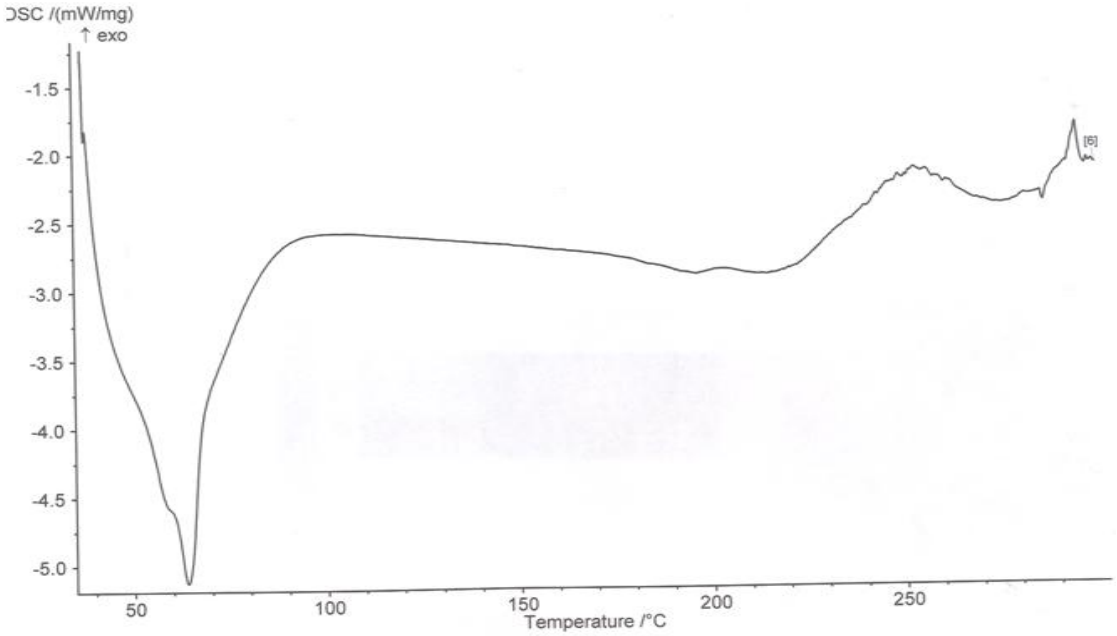


Figure 4.20: DSC Thermogram of Diclofenac Sodium Films Containing 500 mg of HPMC (Formulation F7)

4.4 Physicochemical Evaluation of the Formulated Transdermal Films

The results of the physicochemical evaluation of the formulated films are presented in Table 4.2. These films were observed to have similar weights of 0.030 ± 0.00 g, with the exception of formulation F4 and F5 which weighed 0.026 ± 0.005 g respectively.

The thickness of the films also varied, with formulation F1 having the greatest thickness of 0.130 ± 0.014 mm, while formulation F5 had the least thickness of 0.124 ± 0.007 mm. Folding endurance was greatest for formulation F1 which was able to fold without breaking up to 382 ± 0.578 times, while formulation F5 was only able to fold up to 2.33 ± 0.57 times. The result from the moisture uptake and loss studies, shows that the formulations containing higher proportions of Eudragit RS 100 did not show any apparent change in moisture uptake and loss while the formulation F7, with only HPMC gave the highest percentage moisture uptake and loss, (50.00 ± 0.00 % and 44.43 ± 19.25 % respectively), followed closely by F1, with a moisture uptake and loss of 33.33 ± 14.434 % and 44.43 ± 19.25 % respectively. All the films had 100 % flatness, meaning that there was a zero percent constriction. Theoretical drug content was calculated as 6.9 mg/ 1.53 cm^2 for all the formulations while the drug contents for all the formulations were in the range of 83.38 ± 0.5 to 89.23 ± 0.7 %, with formulations F4 and F5 containing higher proportions of Eudragit RS 100 having a higher range of 88.93 ± 0.5 and 89.23 ± 0.7 respectively.

Table 4.2: Physicochemical Evaluation of the Various Formulated Transdermal Films

Formulation Code	Weight (g)	Thickness (mm)	Folding Endurance	% Moisture Uptake	% Moisture Loss	Flatness Determination (%)	Theoretical Drug load in mg / 1.53 cm^2	Obtained Drug load in mg / 1.53 cm^2	Drug Content (%)
F1	0.030 ± 0.00	0.130 ± 0.01	382.0 ± 0.58	33.33 ± 14.43	44.43 ± 19.25	100	6.9	6.0262 ± 0.02	86.97 ± 0.2
F2	0.03 0 ± 0.00	0.127 ± 0.01	29.00 ± 1.00	33.33 ± 14.43	33.33 ± 0.00	100	6.9	6.0156 ± 0.03	86.52 ± 0.7
F3	0.030 ± 0.00	0.125 ± 0.01	14.33 ± 0.57	33.33 ± 14.43	33.33 ± 0.00	100	6.9	5.8166 ± 0.04	83.38 ± 0.5
F4	0.026 ± 0.01	0.125 ± 0.01	13.67 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	100	6.9	6.2044 ± 0.03	88.93 ± 0.5
F5	0.026 ± 0.01	0.124 ± 0.01	2.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	100	6.9	6.2253 ± 0.05	89.23 ± 0.7
F6	-	-	-	-	-	-	6.9	-	-
F7	0.030 ± 0.00	0.127 ± 0.01	86 ± 1.00	50.00 ± 0.00	44.43 ± 19.25	100	6.9	6.1205 ± 0.05	87.73 ± 0.7

All values are given as (mean ± SD) for n=3

4.5 *Ex Vivo* Permeation Study

It was observed that Formulation F7 gave the fastest rate of permeation of 57.10 % in 12 h, followed by formulation F1 with a rate 45.61 %, while formulation F5 gave the slowest rate of permeation of 18.79 % as depicted in Figure 4.21.

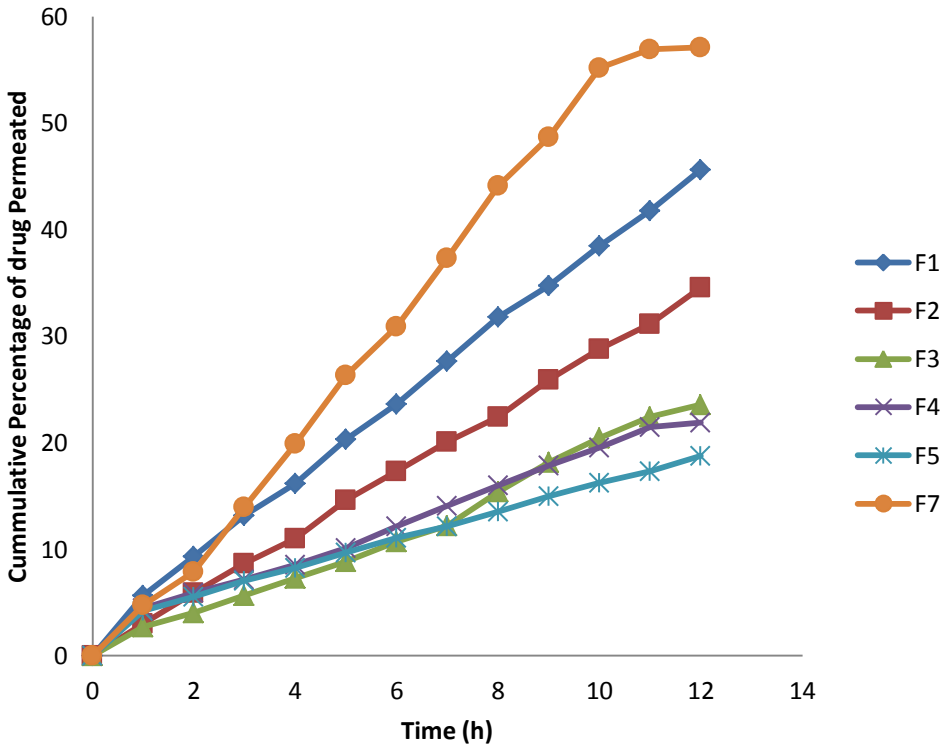


Figure 4.21: ***Ex vivo*** **Skin Permeation Profile of Diclofenac Sodium from the Different Formulated Transdermal Films against Time**

Key

F1 – (Eudragit RS 100 and HPMC 2:8)

F2 – (Eudragit RS 100 and HPMC 4:6)

F3 - (Eudragit RS 100 and HPMC 5:5)

F4 - (Eudragit RS 100 and HPMC 6:4)

F5 - (Eudragit RS 100 and HPMC 8:2)

F7 - (Eudragit RS 100 and HPMC 0:10)

4.5.1 Steady state flux (J) (µg/1.53 cm^2/hr)

From the result of the cumulative amount of the drug released from the films in Figure 4.22, steady state flux was calculated. It showed that formulation F7 produced the highest rate of flux in 1 h of 72.95 µg/1.53 cm^2/ h while F3 produced the lowest rate of flux in 1 h of 29.57 µg/1.53 cm^2/ h, which gradually decreased, before attaining a steady state for the remaining hours. This trend was observed in all the other formulations, with formulation F5 producing the least amount of flux after 12 h. Generally all the formulations achieved a sustained permeation rate.

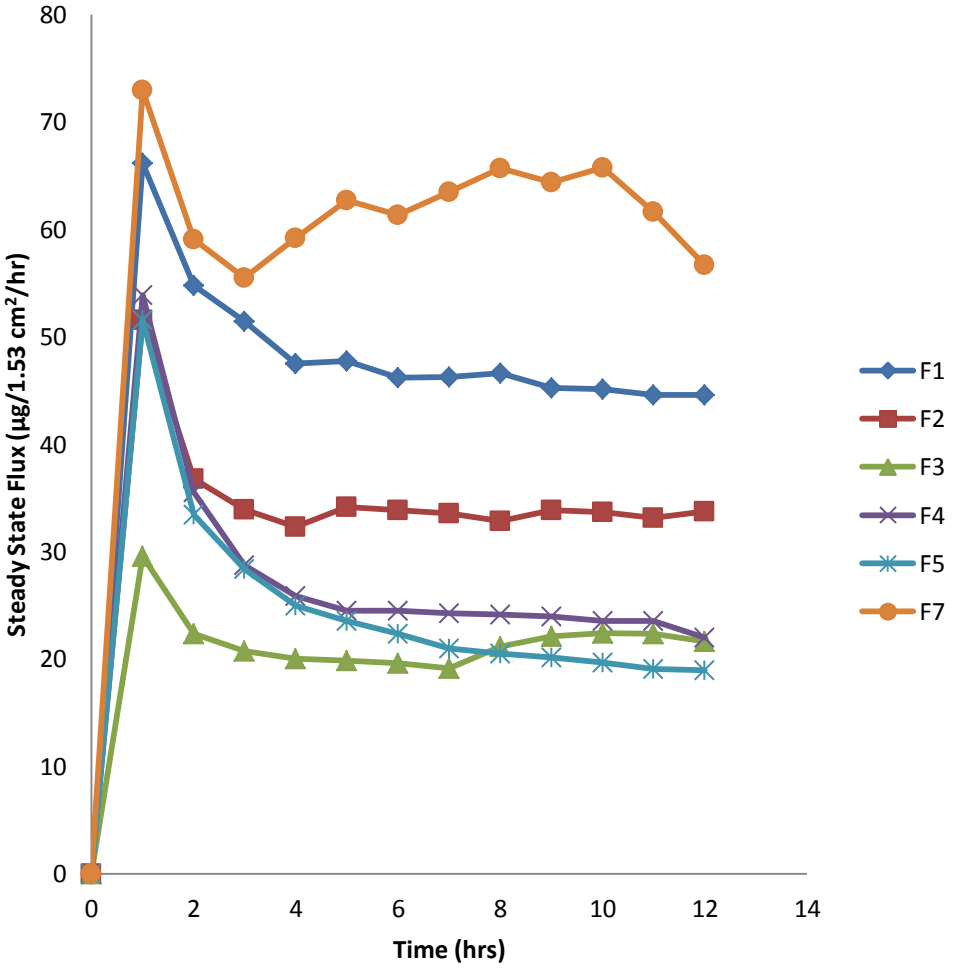


Figure 4.22: Steady State Flux of Various Diclofenac Sodium Film Formulations against Time

4.5.2 Permeation Coefficient (P) (cm / h)

From the result of the steady state flux, permeation coefficients for the various films formulated were calculated. The greater the flux produced, the larger the permeation coefficient as shown in Figure 4.23. The highest permeation coefficient was observed in formulation F7 of 0.01, while formulations F3, F4 and F5 gave a similar permeation coefficient of 0.004.

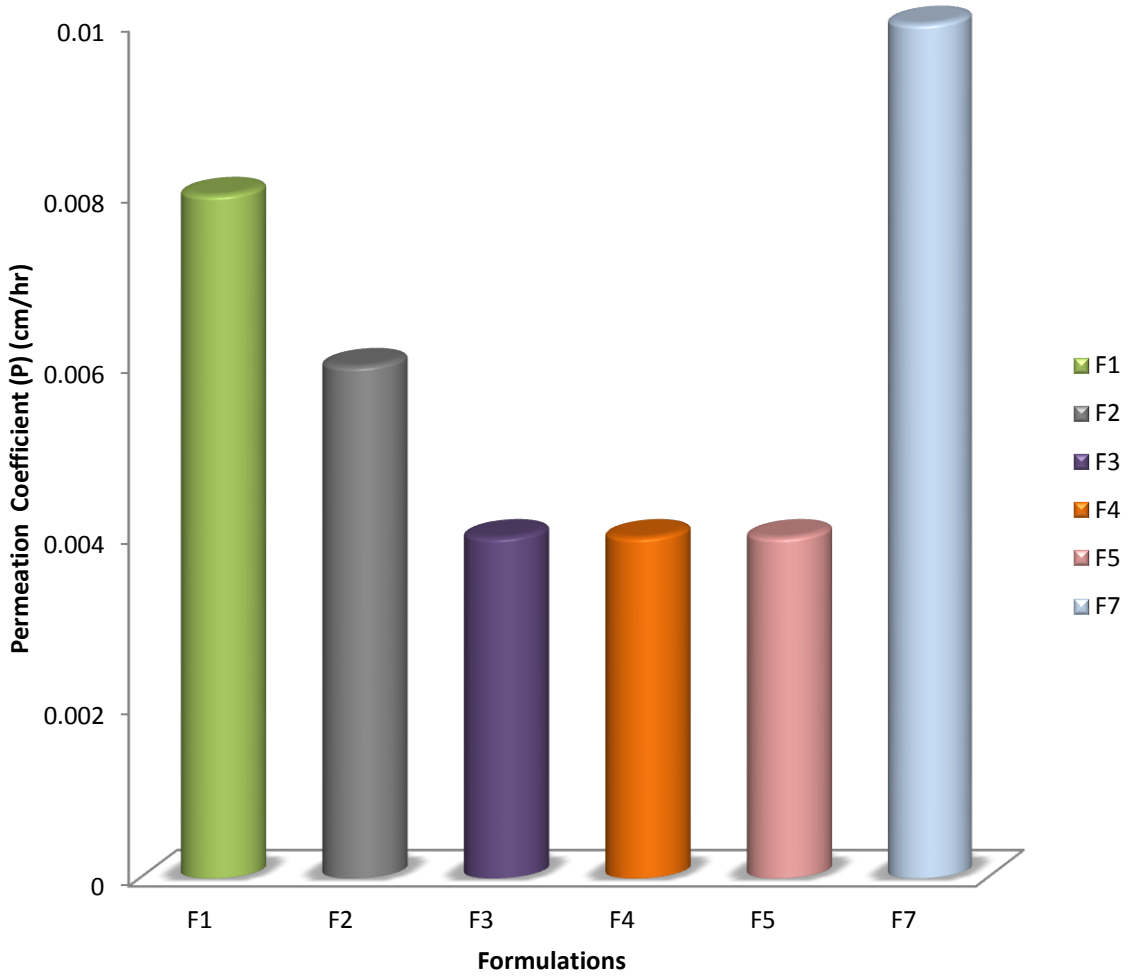


Figure 4.23: Permeation Coefficient (P) (cm / h) of the Various Diclofenac Sodium Film Formulations

4.6 *In Vivo* Anti Inflammatory Studies

From the result of the *ex vivo* study, formulations F1 and F7 were selected as the best films in terms of permeation rate, and were used to further carry out the animal studies. The result of egg albumin-induced paw edema test is shown in Figure 4.24. From the result, the mean increase in edema with respect to initial volume for Group A was 5.33 ± 0.75 mm after 1 h of administering the inflammatory agent (egg albumin). It was further increased to 6.43 ± 0.24 mm after 3 h, and gradually decreased to 4.47 ± 0.26 mm after 6 h, while formulations F1 and F7 showed a gradual increase to 4.97 ± 0.62 mm and 4.17 ± 0.51 mm respectively, after the administration of the inflammatory agent (egg albumin), which showed slight increase up to the third hour (5.17 ± 0.75 and 4.56 ± 0.58 mm). However, there was a reduction in the paw thickness after 6 h (1.78 ± 0.89 and 0.96 ± 0.62 mm) for formulations F1 and F7 respectively.

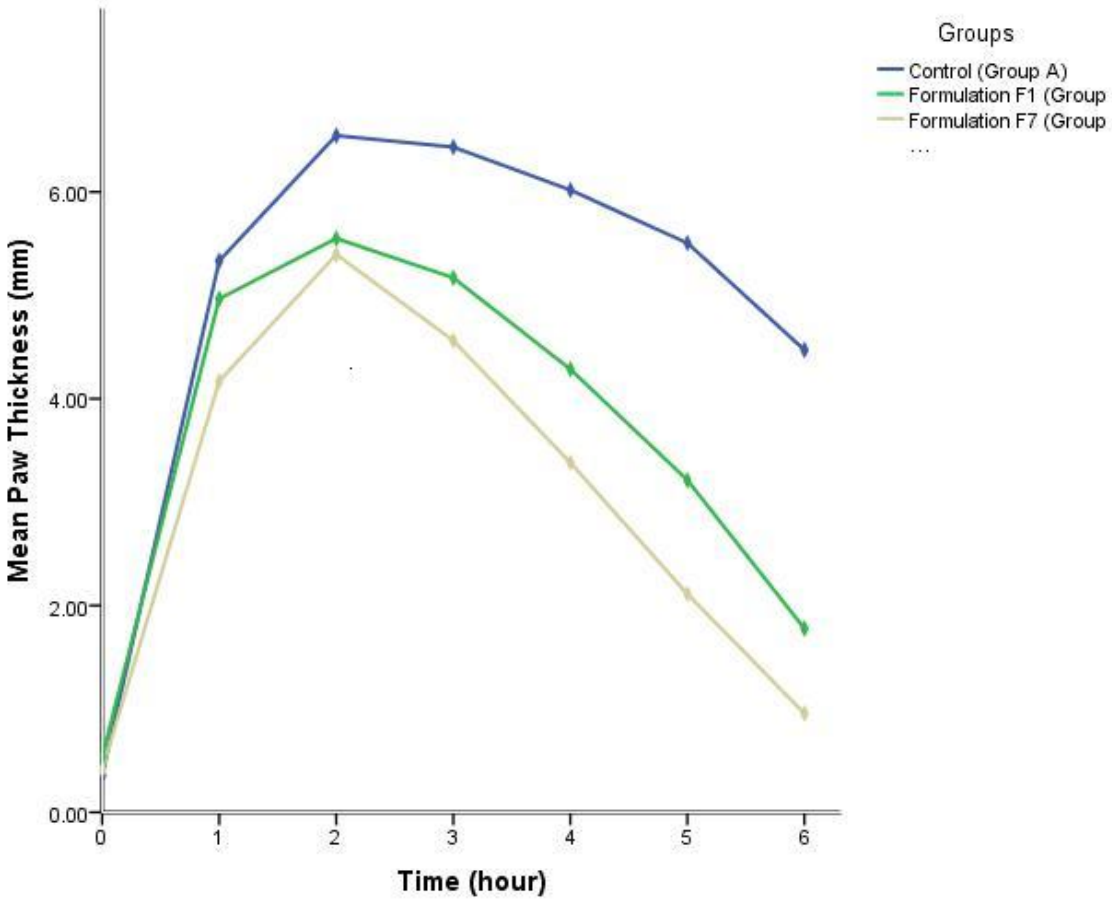


Figure 4.24: Mean Paw Thickness Produced After Administration of the Inflammatory Agent (Egg Albumin) in Various Groups against Time

4.7 Skin Irritation Test

The Formulations F7 and F1 administered to rabbit A and B as shown in Plates II and IV respectively, showed no visible signs of erythema or edema, and were subsequently scored a zero according to Draize *et al*., (1994) scoring method, as shown in Table 4.3 below, while the standard irritant (formalin) was administered to Rabbit C as shown in Plate VI, after a period of 24 h there was pronounced erythema and edema observed, it was then given a score of four according Draize *et al*. (1994) scoring method.

Table 4.3 Skin Reaction Scores Assigned to Transdermal Film Formulations F7 and F1, and the Standard Irritant (formalin) the During Skin Irritation Test.

Rabbit (Male)	Formulation/ Agent	Score After 24 h
A	F7	0
B	F1	0
C	Standard Irritant (0.8% Formalin)	4



Plate I: Hair of Rabbit A, Shaved 24 h before Application of the Formulated Film



Plate II: Rabbit A, After Application of Formulation F1 for 24 h



Plate III: Hair of Rabbit B, Shaved 24 h before Application of the Formulated Film



Plate IV: Rabbit B, After Application of Formulation F7 for 24 h



Plate V: Hair of Rabbit C, Shaved 24 h before Application of the Formulated Films



Plate VI: Rabbit C, After Application of Formaldehyde 0.8 % for 24 h

4.8 Stability Studies

Upon storage for a period of three months, the films retained their color, texture, flexibility and uniformity. Drug content after storage for three months showed no significant difference from that obtained upon preparation (Table 4.4).

Table 4.4: Stability Studies on the Formulated Films for a Period of Three Months.

Time	**Drug Content mg/ 1.53 cm^2**					
	F1	**F2**	**F3**	**F4**	**F5**	**F7**
Day 0	6.03± 0.02	6.02± 0.03	5.82 ± 0.04	6.20 ± 0.03	6.23 ± 0.05	6.12 ± 0.05
Day 30	6.02 ± 0.03	6.01 ± 0.03	5.80 ± 0.03	6.18 ± 0.02	6.22 ± 0.04	6.09 ± 0.01
Day 60	6.01 ± 0.04	5.98 ± 0.03	5.77 ± 0.01	6.15 ± 0.05	6.17 ± 0.03	6.08 ± 0.02
Day 90	5.97 ± 0.03	5.95± 0.03	5.76 ± 0.03	6.14 ± 0.03	6.14 ± 0.03	6.06 ± 0.02
F-ratio	4.066	1.748	2.362	2.981	2.732	1.478
P-value	0.487	0.234	0.147	0.214	0.114	0.292

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical Appearance of the Formulated Films

Results of the physical examination of the prepared films are shown in Table 4.1. Films F1, F2, F3 and F7, were white, translucent, flexible, smooth, and uniform, showing that films containing higher proportions of HPMC gave good mechanical properties, while F4 and F5, with higher concentrations of Eudragit RS 100, appeared white, translucent, brittle, and rough. Formulation F6 with Eudragit RS 100 alone did not form a transdermal film, probably because Eudragit RS 100 is not a good film forming polymer. It was however observed that, even with the addition of plasticizers (Polyethylene glycol 8000) to all the films produced, films with higher proportions of Eudragit RS 100, still produced brittle and rough films which were difficult to remove from the aluminum foil used in casting the mixture. Films appeared to be translucent, suggesting that the drug was not completely solubilized but rather uniformly dispersed/ suspended in the matrix.

5.2 Analysis by Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy detects the vibration characteristics of chemical functional groups in a sample. When an infrared light interacts with the matter, chemical bonds will stretch, contract and bend. Therefore, existence of any interaction can be exhibited in order to investigate drug excipient compatibility (Gulgun *et al*., 2010).

Figures 4.1- 4.9 show that the principal peaks of the pure drug appeared in the spectra of all the formulated films. Additional peaks appeared in some of the formulations. For Formulation F1, additional peaks were observed at 2908 cm^{-1} (C-H stretching in alkane), and 1728 cm^{-1} (C=O bonds in carboxylic acid). For Formulation F2, it was

observed at 3221 cm^{-1} (O-H stretching bonds), 2919 cm^{-1} (C-H stretching bonds), 1728 cm^{-1} (C=O bonding in carboxylic group), and 1582 cm^{-1} (aromatic C=C stretching bonds). Formulation F3 showed additional peaks at 2918 cm^{-1} (C-H stretching bands), and 1728 cm^{-1} (C=O bonding frequency). F5 showed additional peaks at 2893 cm^{-1} (C-H stretching bands), and 1729 cm^{-1} (C=O vibration bonds) while F7 also showed additional peaks at 3663 cm^{-1}, 3385 cm^{-1} (O-H stretching bonds), 2813 cm^{-1}, 2717 cm^{-1} (C-H stretching bonds), 1734 cm^{-1} (C=O vibration bonds), and these additional peaks occurred simply due to mixing of components independent of any chemical interactions. Since the main peaks of the pure drug were still present in the various formulations, it can be assumed that there was no drug polymer interaction.

5.3 Analysis by Differential Scanning Calorimetry (DSC)

DSC was further carried out to assess any interactions between the drug (diclofenac sodium), polymers (HPMC and Eudragit RS 100), and the plasticizer (polyethylene glycol) used in the formulation of the various films.

DSC in general, has gained popularity since it is an easy and quick way of detecting any interaction between drugs and excipients. It is based on the detection of endothermic and exothermic peaks that appear as a consequence of small changes in temperatures (Michael *et al.*, 2012). The number, location and shape of these peaks are used to identify a substance. When the substance undergoes a thermal event, the difference in the heat flow to a sample and to a reference is monitored against time or temperature, while the temperature is programmed in a specified atmosphere. As a result, energy associated with various thermal events (e.g., melting, glass transition temperature, crystallization, etc.) can be evaluated (Vueba *et al.*, 2012).

It has also been reported that the quantity of a material used, especially in drug-excipient mixture, affects the peak shape and enthalpy. Thus, these changes in the melting endotherm of drug could be due to the mixing of drug and excipients, which lowers the purity of each component in the mixture and may not necessarily, indicate potential incompatibility (Michael *et al.*, 2012).

From the results (Figures 4.10 – 4.20), Formulations F1-F7 showed a slight shift in their melting peaks. There was also broadening or elongation of both the exothermic and endothermic peaks.

Possible reasons for these changes could be the other excipients used (Eudragit RS 100, HPMC and Polyethylene glycol), which have lower melting points (endothermal event) compared to diclofenac sodium, suggesting that diclofenac sodium might dissolve in the melted excipients, thereby producing unexpected thermal events. Prior to reaching 284°C, the excipients might already have undergone a thermal decomposition (also shown as an endothermal change), interfering with the melting point of diclofenac sodium. Potential interactions could occur owing to the fact that the drug-excipient mixtures are exposed to extreme stress conditions (high temperature and small surface area). Therefore the occurrence of a physical or chemical interaction does not necessarily indicate an incompatibility (Vueba *et al.*, 2012).

5.4 Physicochemical Evaluation of the Formulated Transdermal Film

5.4.1 Weight variation

The weights ranged between 0.026 ± 0.005 g and 0.030 ± 0.00 g, which indicates that the different formulated films had relatively similar weights with the exception of formulation F4 and F5, showing that higher proportions of the polymer Eudragit RS 100 produced weaker films.

5.4.2 Thickness uniformity

From the result it can be seen that the films were fairly uniform in thickness, and varied from 0.124 ± 0.007 mm to 0.130 ± 0.014 mm. Formulation F1 showed the greatest thickness of 0.130 ± 0.014 mm, while formulation F5 had the least thickness of 0.124 ± 0.007 respectively, this may be attributed also to the polymer Eudragit RS 100, which is not a good film forming polymer.

5.4.3 Percentage moisture content

Moisture content is an important contribution in drug diffusion to the skin because it shows the extent of water uptake of the films, from body tissues as well as from the environment during the application period (Patel *et al.*, 2012). From the result of the percentage moisture uptake and loss, formulations with the highest concentration of HPMC gave the highest percentage moisture uptake and loss. Formulation F7, had the highest percentage moisture uptake of 50.00 ± 0.00 %, followed by formulation F1 with a percentage moisture uptake of 33.33 ± 14.43%, while the percentage moisture loss for F7 and F1 was 44.43±19.25 % each. Formulations F3 and F4 gave a moisture uptake and loss of (33.33 ± 14.434 % and 33.33 ± 0.00 %) respectively. It was observed that the formulated films F4 and F5 with the higher concentrations of Eudragit RS 100, had the least percentage moisture uptake and loss of 0.00 ± 0.00 % each, with no change observed even after three days of observation.

These findings agree with Arora and Mukherjee (2002), who observed that an increase in the hydrophilicity of a polymer, leads to a subsequent increase in percentage moisture uptake and loss.

5.4.4 Folding endurance

The result of the folding endurance, measured manually, showed that it was maximum for Formulations F1 (382 ± 0.578 folds) followed by F7 (86 ± 1.00 folds) whereas it was minimum for Formulation F5 (2.33 ± 0.57 folds). This shows that Formulations F1 and F7, would have the capacity to withstand mechanical pressure and exhibit good flexibility, thus maintaining their integrity when applied to the skin, meaning they could be placed on any part of the body involved in movement, and that part could be involved in folding movement for up to 382 and 86 times without breaking. The other Formulations, F3, F4 and F5 (14.33 ± 0.57, 13.67 ± 0.58, 2.33 ± 0.57 folds), with lower folding endurance, would easily break when applied to the skin. The folding endurance was higher in formulations containing higher proportions of HPMC than those of Eudragit RS 100, because Eudragit RS 100 is not a good film forming polymer.

5.4.5 Drug content

Drug content was found to be between 5.8166 ± 0.04 mg and 6.2253 ± 0.05 mg, while the theoretical drug content was calculated as 6.9 mg. Therefore, the percentage drug content was in the range of 83.38 ± 0.5 % - 89.23 ± 0.7 % for all the batches. Batches containing higher concentrations of Eudragit RS 100 gave higher percentage of drug content, probably because Eudragit RS 100 is hydrophobic, hence more drugs dissolved in the solution during mixing, while HPMC is hydrophilic so both drug and polymer would compete for the solution during the mixing process. The result indicates minimum batch variability, which demonstrates homogeneous distribution of the drug in the prepared formulations; it also shows that the process used in the preparation of the transdermal films is capable of giving films with uniform drug content.

5.4.6 Flatness determination

From the result obtained, there was no constriction of films. This was shown by the 100 % flatness observed. Thus, these formulations can maintain a uniform surface and would not constrict when they are administered onto the skin surface.

5.5 *Ex Vivo* Skin Permeation Studies

The results of the *ex vivo* cumulative permeation of diclofenac sodium from the transdermal films (Figure 4.21), showed a maximum value for Formulation F7 of 57.10 % and 45.61 % for Formulation F1 in 12 h. Drug permeation from the transdermal film was observed to have a value of 18.79 % for formulation F5.

These findings correlate with studies reported by Shinde *et al.,* 2010, Anasari *et al.*, 2011, and Denge *et al.,* 2012, stating that increasing the proportion of hydrophilic polymer (HPMC) in the polymer matrix increased the percentage cumulative drug permeation due to the highly hydrophilic nature of HPMC, which has less interactions with drugs. It absorbs water, leading to dissolution of the aqueous soluble fraction of polymer matrix, which causes swelling of the polymer, and results in more release of drug from gelatinous pores of the films because of adequate porosity and diffusivity. The formation of such pores leads to a decrease in the mean diffusion path length of the drug molecules into the diffusion medium causing a higher release and permeation rate respectively.

Formulation F5 containing the highest concentration of Eudragit RS 100, had the least permeation rate, which can be attributed to the relatively hydrophobic nature of the polymer and its cross linking ability, which retards the drug permeation from the polymer drastically (Gottipati *et al.*, 2012). Other formulations like F2, F3 and F4 also showed similar controlled permeation rates, due to the hydrophobic nature of Eudragit

RS 100 which acts as a better release retardant at higher concentrations than HPMC. The cumulative percentage of drug permeated from formulations is in the order F7> F1 > F2 > F3 > F4 > F5.

The flux of a given solute is simply defined as the mass or number of molecules moving through a given cross sectional area during a given period of time. Molecular transport or movement of molecules in solution across barriers is caused primarily by migration or diffusion. The most common biopharmaceutical use of flux studies is transport investigation of drug candidates or prodrug candidates across a barrier, while permeation coefficient obtained from permeation studies can be used to predict the bioavailability of a given drug substance.

The steady state flux and permeation coefficient data of all the formulated films are presented in Figures 4.22 and 4.23 respectively, with the highest flux and permeation coefficient observed in formulation F7 as 56.66 µg/1.53 cm^2/ h and 0.011 (1.53 cm^2/ h) respectively, while the lowest was 18.96 µg/1.53 cm^2/ h and 0.004 (1.53 cm^2/ h) respectively for formulation F5 with the highest concentration of Eudragit RS 100. Therefore, formulation F7 (containing HPMC alone) will be more readily bioavailable than the other formulations, due to its higher permeation coefficient. This rate of flux is due to the barrier nature of the skin for the transport of small molecules, but the addition of the penetration enhancer (Tween 80) may have had some influence on the rate of flux of all the formulated films. From the result of the steady state flux, it took 1 h for the formulations to reach their highest peak which correlates with an initial burst permeation rate observed for all the formulations, due to the drug that was saturated on the film surface during storage. The permeation rate decreased gradually, with time until

it attained a steady state, thereby providing controlled drug permeation for up to 12 h. This is in line with the findings of Rajendran and Ruckmani 2011.

5.6 *In Vivo* Anti Inflammatory Studies

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. It is trigged by the release of chemical mediators from the injured tissue and migrating cells (Sangita *et al*., 2012).

The formulated films which gave the best cumulative permeation data from the results in the *ex vivo* studies (Formulations F1 and F7), were further used to carry out the *in vivo* anti inflammatory test. From the result (Figure 4.24), it was evident that formulation F7 showed a faster reduction in inflammation than F1. This observation is similar to the trend observed in the *ex vivo* studies. It also indicates that formulations F1 and F7 are capable of readily inhibiting inflammatory reactions readily and that the films were effective from the first hour without any appreciable lag time. These findings are similar to the findings of Arora and Mukherjee 2002. Throughout the study, the percent increase in edema with respect to initial volume for test groups (B and C) remained well below that of the control group.

The group administered with formulations F1 and F7 showed 79 % and 88 % inhibition of edema respectively, after 6 h, indicating the formulations effectiveness as an anti inflammatory agent. Both formulations gave a significant anti-inflammatory activity, P-values were 0.038, 0.012, 0.001, <0.001, <0.001, <0.001, from the first hour to the sixth hour respectively.

5.7 Skin Irritation Test

Skin irritation is defined as a local, reversible inflammatory response of the skin to the application of an agent without the involvement of an immunological mechanism (Anilkumar *et al.,* 2010). The study carried out on the rabbit skin revealed that formulations, F7 and F1 of diclofenac sodium produced no signs of erythema and edema, rather, they reduced the inflammation and redness caused by the instrument during the shaving process, Plates II and IV. The scores assigned to the skin reactions are given in Table 4.3. Since no erythema or edema was observed on application of the formulated films F1 and F7, a score of zero was assigned to the various rabbits tested, implying that they are compatible with the skin. However, for the standard irritant Plate IV, severe erythema and edema were observed, hence a score of four was assigned to it. In general, all the excipients used in the preparation of the transdermal films have been widely used to formulate other dosage forms like the oral and topical forms of drug delivery and none has been associated with any form of irritation or allergic reactions, therefore the prepared films, should expectedly not be associated with any form of irritation, and therefore are suitable for application onto the skin.

5.8 Stability Studies

The formulations, analysed after a period of zero, thirty, sixty and ninety days for their physical appearance and drug content, retained their colour, flexibility, texture and translucent nature. The drug content during the study period is shown in Table 4.4, P-values from day zero to the third month were, 0.487, 0.234, 0.147, 0.105, 0.214, 0.114 and 0.292 respectively, indicating that there was no significant difference in drug content for all the formulations, even after three months of storage at a high relative humidity. This agrees with the findings of Kelvin *et al.*, (2009). The result of this study also corroborates the conclusion drawn in the DSC analysis.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Transdermal films of diclofenac sodium, were formulated by the solvent casting technique using two polymers, Eudragit RS 100 and HPMC in the ratios of 2:8, 4:6, 5:5, 6:4, 8:2, 0:10 and 10:0. A plasticizer (polyethylene glycol 8000), and a penetration enhancer (Tween 80), were added. The films with higher percentage of HPMC were white, translucent, flexible, smooth, and uniform, while those with higher concentrations of Eudragit RS 100 were white, translucent, brittle and rough. The FT-IR spectroscopy and DSC analysis showed no interaction between the drug and excipients used in the formulation. Physicochemical evaluation of the formulated films showed that they were relatively similar in weights thickness, high moisture uptake / loss was observed in formulations with high HPMC concentrations, while it was relatively low with formulations containing high concentrations of Eudragit RS 100. Folding endurance was greatest in formulations F1 and F7, while the drug content showed relatively minimum batch variability. The films showed 100 % flatness, indicating 0 % constriction.

The *ex vivo* permeation studies revealed that Formulations F1 and F7 with a greater proportion of HPMC, gave a faster rate of permeation, compared to other formulations. F5 with a higher proportion of Eudragit RS 100 gave the least permeation rate. From the *in vivo* anti inflammatory studies, it was observed that formulation F7 controlled edema faster than formulation F1. Both were able to control the edema volume for up to 6 h. The result of the skin irritation test revealed no erythema or edema with films F1 and F7. The data obtained from the stability studies showed no change in the physical

appearance and drug content of the formulated films, indicating that they are relatively stable.

6.2 Conclusion

i. Diclofenac sodium transdermal films can be formulated using HPMC and 8:2 polymer blends of HPMC and Eudragit RS 100.

ii. Eudragit RS 100, when used alone does not form films, and when used in larger concentrations along with HPMC, formed very weak and brittle films.

iii. The FT-IR spectra and DSC analysis showed that no drug-excipient interaction occurred.

iv. When higher proportions of HPMC were used, films of greater weight, thickness, folding endurance were produced, thereby improving the mechanical properties of the formulated films.

v. Drug permeation is faster in combinations with higher proportions of HPMC than Eudragit RS 100 as shown in the *Ex vivo* permeation studies.

vi. *In vivo* anti inflammatory studies for formulations F1 and F7 showed a significant anti-inflammatory activity ($P<0.05$) when compared to the control.

vii. Films formulated did not show any signs of irritation, thereby confirming their suitability and safety when placed on the skin.

viii. There was no significant change in drug content after three months of storage.

ix. Finally, it can be concluded that polymeric matrix transdermal films of diclofenac sodium prepared with higher concentrations of HPMC hold a stronger potential for formulation into a transdermal delivery system.

6.3 Recommendation

i. Formulations with Eudragit RS 100 can be evaluated further for its sustained release properties.

ii. HPLC as a method of analyzing the drug content, for the purpose of a more accurate result, can be performed.

iii. *Ex-vivo* studies using the skin of animals, like guinea pig and shed snake skin which are more closely related to the human skin should be carried out.

iv. Other naturally occurring penetration enhancers (e.g essential oils like eucalyptus, menthol, chenopodium), polymers (e.g gums like gum copal and gum damar, xantum gum, chitin and chitosan, zein, pectin, collagen, alginates, starch) and plasticizers (e.g sorbitol and glycerine) can be studied for further comparative purposes.

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APPENDICES

Appendix 1: Summary of the fourier transform infrared spectroscopy (FT-IR)

Sample		Wave Number Range	Functional Group
A)	Diclofenac Sodium	3417 cm^{-1}	N-H stretching frequency in Amine.
		1568 cm^{-1}	C=C stretching in aromatic ring
		1484 cm^{-1}, 1392 cm^{-1}	C-H bending frequency in alkane
		1298 cm^{-1}	C-H bending in alkene frequency
		1077 cm^{-1}	C-O stretching in carboxylic acid group
		754 cm^{-1}, 852 cm^{-1}	C-Cl bonding vibrations in a halide group
B)	Eudragit RS 100	3612 cm^{-1}, 3339 cm^{-1}	O-H stretching in esters
		3463 cm^{-1}	N-H stretching in amine
		2973 cm^{-1}	C-H stretching in alkane
		1732 cm^{-1}	C=O bonding vibration in ester
		1463 cm^{-1}, 1380 cm^{-1}	C-H bending frequency in alkane
		1117 cm^{-1}	C-O bonding vibrations in esters
C)	HPMC	3493 cm^{-1}	O-H stretching frequency in a hydroxyl group
		2911 cm^{-1}	C-H stretching in an alkane group
		1643 cm^{-1}	C=O bonding vibration in an ester group
		1448 cm^{-1}, 1368 cm^{-1}	C-H bending in alkane
		1125 cm^{-1}, 1056 cm^{-1}	C-O bonding vibrations in esters
D)	Polyethylene glycol	3492	O-H bonding frequency in hydroxyl group
		2921 cm^{-1}	C-H stretching in alkane group
		1635 cm^{-1}	C=C stretching frequency in alkene
		1467 cm^{-1}, 1356 cm^{-1}	C-H bending vibration in alkane
		1134 cm^{-1}, 1084 cm^{-1}	C-O bonding vibrations
		949 cm^{-1}, 842 cm^{-1}	C-H bonding vibration in

			methylene chain
E)	Formulation F1	3448 cm^{-1}	N-H stretching in amine
		2908 cm^{-1}	C-H stretching in alkane
		1728 cm^{-1}	C=O bonding vibration in carboxylic acid
		1582 cm^{-1}	C=C stretching in aromatic ring
		1455 cm^{-1}, 1368 cm^{-1}	C-H bending frequency in alkane
		1298 cm^{-1}	C-H bending frequency in alkene
		1097 cm^{-1}	C-O bonding vibration in carboxylic acid
		759 cm^{-1}, 853 cm^{-1}	C-Cl bonding frequency in halide group
F)	Formulation F2	3448 cm^{-1}	N-H stretching frequency in amine
		3221 cm^{-1}	O-H stretching frequency in carboxylic acid
		2919 cm^{-1}	C-H stretching frequency in alkane
		1728 cm^{-1}	C=O bonding in carboxylic acid
		1582 cm^{-1}	C=C stretching in aromatic ring
		1455 cm^{-1}, 1366 cm^{-1}	C-H bending frequency in alkane
		1293 cm^{-1}	C-H bending frequency in alkene
		1074 cm^{-1}	C-O bonding vibrations in carboxylic acid
		761 cm^{-1}, 851 cm^{-1}	C-Cl bonding vibrations in halide group
G)	Formulation F3	3613 cm^{-1}	O-H stretching frequency in carboxylic acid group
		2918 cm^{-1}	C-H stretching frequency in alkane
		1728 cm^{-1}	C=O bonding frequency in carboxylic acid group
		1581 cm^{-1}	C=C stretching in aromatic ring
		759 cm^{-1}, 847 cm^{-1}	C-Cl bonding vibration in halide group
H)	Formulation F4	3596 cm^{-1}	N-H stretching frequency in amine
		3389 cm^{-1}, 3267 cm^{-1}	O-H stretching frequency in carboxylic acid
		2816 cm^{-1}	C-H stretching frequency in alkane
		1731 cm^{-1}	C=O bonding vibration in

			carboxylic acid group
		1587 cm^{-1}	C=C stretching frequency in aromatic ring
		1465 cm^{-1}, 1367 cm^{-1}	C-H bending frequency in alkane
		1260 cm^{-1}	C-H bending frequency in alkene
		1031 cm^{-1}	C-O bonding vibration in carboxylic acid
		761 cm^{-1}, 848 cm^{-1}	C-Cl bonding vibration in halide group
I)	Formulation F5	3434 cm^{-1}	N-H stretching frequency in amine group
		2893 cm^{-1}	C-H stretching frequency in alkane group
		1729 cm^{-1}	C=O bonding vibration in carboxylic acid group
		1583 cm^{-1}	C=C stretching frequency in aromatic nucleus
		1459 cm^{-1}, 1368 cm^{-1}	C-H bending frequency in alkane
		1262 cm^{-1}	C-H bending frequency in alkene
		1121 cm^{-1}	C-O bonding vibrations in carbolic acid
		850 cm^{-1}, 756 cm^{-1}	C-Cl bonding vibration in halide group
J)	Formulation F7	3663 cm^{-1}, 3385 cm^{-1}	O-H stretching frequency in carboxylic acid group
		3538 cm^{-1}	N-H stretching frequency in amine
		2813 cm^{-1}, 2717 cm^{-1}	C-H stretching frequency in alkane
		1734 cm^{-1}	C=O bonding vibration in carboxylic acid.
		1596 cm^{-1}	C=C stretching frequency in aromatic ring
		1401 cm^{-1}	C-H bending frequency in alkane
		1278 cm^{-1}	C-H bending frequency in alkene
		1139 cm^{-1}	C-O bonding vibration in carboxylic acid group
		856 cm^{-1}, 760 cm^{-1}	C-Cl bonding vibration in a halide group

Appendix 2: Summary of the differential scanning calorimetry (DSC) analysis

Sample	Endothermic Peaks	Exothermic Peaks
Diclofenac Sodium	64 °C, 284 °C	293 °C
Polyethylene glycol	66.3 °C	-
HPMC	53.2 °C, 176.9 °C	-
Eudragit RS 100	66.8 °C, 190.9 °C, 204.1 °C	-
F1	64.1°C, 208.5 °C	244.7 °C
F2	60.1 °C, 163.7 °C	251.4 °C
F3	59.2 °C, 213.7 °C	249.4 °C
F4	58.8 °C, 213.9 °C	251.4 °C
F5	59.6 °C, 220.0 °C	255.3 °C
F7	63.8 °C, 214.8 °C	253.2 °C



Appendix 3: Diclofenac sodium films containing 100 mg of Eudragit RS 100 and 400 mg of HPMC (Formulation F1)



Appendix 4: Diclofenac sodium films containing 200 mg of Eudragit RS 100 and 300 mg of HPMC (Formulation F2)



Appendix 5: Diclofenac sodium films containing 250 mg of Eudragit RS 100 and 250 mg of HPMC (Formulation F3)



Appendix 6: Diclofenac sodium films containing 300 mg of Eudragit RS 100 and 200 mg of HPMC (Formulation F4)



Appendix 7: Diclofenac sodium films containing 400 mg of Eudragit RS 100 and 100 mg of HPMC (Formulation F5)



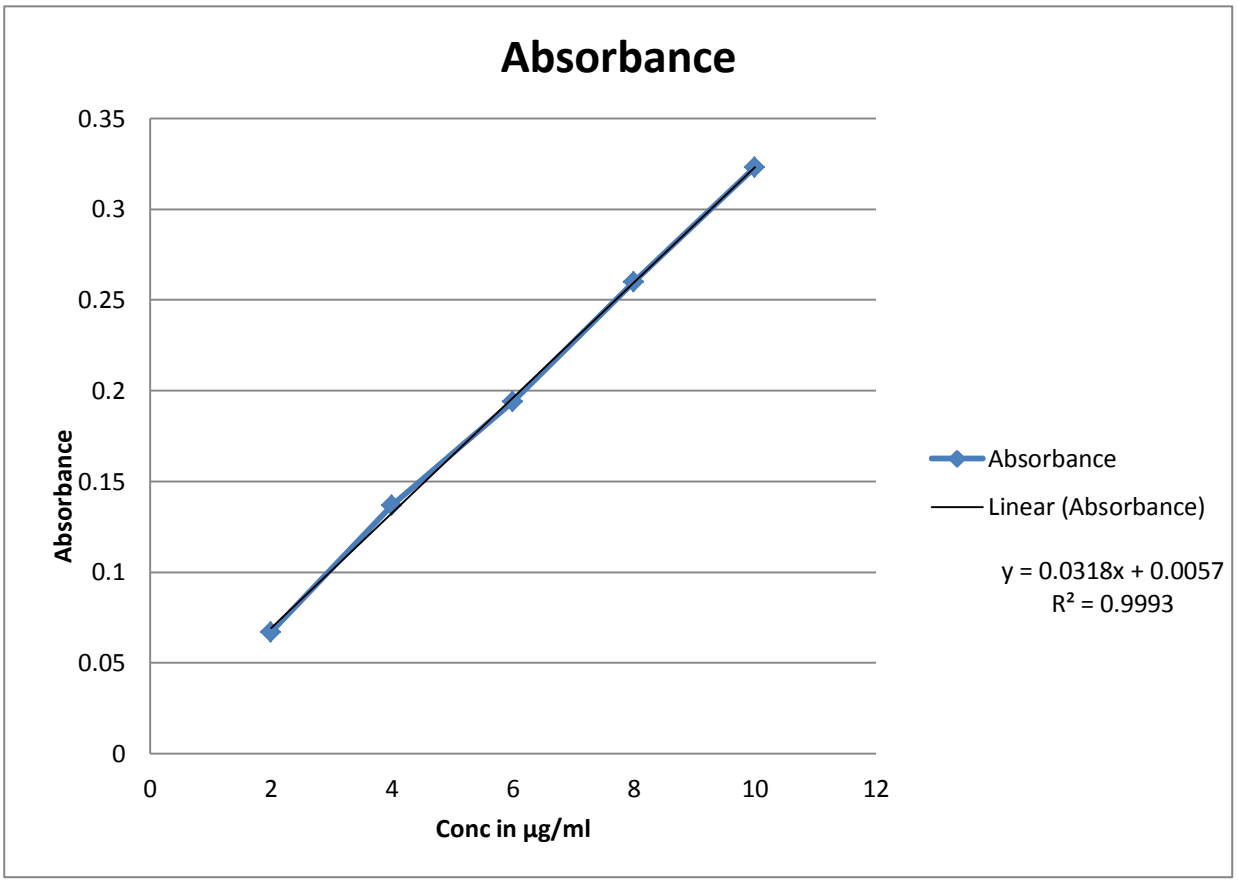
Appendix 8: Diclofenac sodium films containing 500 mg of HPMC alone (Formulation F7)



Appendix 9: Diclofenac sodium films, having an area of 1.53 cm^2

Appendix 10: Absorbance values for diclofenac sodium

Concentration (µ/ ml)	Absorbance (nm)
2	0.067
4	0.137
6	0.194
8	0.260
10	0.323



Appendix 11: Calibration Curve for Diclofenac Sodium

Appendix 12: Drug content values for the various formulations

Sample	Absorbance (nm)
F1	0.197 ± 0.001
F2	0.197 ± 0.001
F3	0.190 ± 0.001
F4	0.203 ± 0.001
F5	0.204 ± 0.002
F6	-
F7	0.200 ± 0.002

Appendix 13: Drug content values after Permeation through the rat abdominal skin for formulation F1 (nm)

Time (h)	Absorbance
1	0.115
2	0.185
3	0.258
4	0.315
5	0.396
6	0.459
7	0.535
8	0.613
9	0.671
10	0.743
11	0.813
12	0.880

Appendix 14: Drug content values after Permeation through the rat abdominal skin for formulation F2 (nm)

Time (h)	Absorbance
1	0.064
2	0.119
3	0.172
4	0.217
5	0.285
6	0.337
7	0.390
8	0.435
9	0.504
10	0.556
11	0.602
12	0.668

Appendix 15: Drug content values after Permeation through the rat abdominal skin for formulation F3 (nm)

Time (h)	Absorbance
1	0.054
2	0.080
3	0.108
4	0.137
5	0.166
6	0.198
7	0.225
8	0.282
9	0.331
10	0.372
11	0.408
12	0.430

Appendix 16: Drug content values after Permeation through the rat abdominal skin for formulation F4 (nm)

Time (h)	Absorbance
1	0.094
2	0.122
3	0.147
4	0.175
5	0.206
6	0.246
7	0.283
8	0.321
9	0.359
10	0.391
11	0.429
12	0.437

Appendix 17: Drug content values after Permeation through the rat abdominal skin for formulation F5 (nm)

Time (h)	Absorbance (nm)
1	0.09
2	0.115
3	0.145
4	0.169
5	0.198
6	0.225
7	0.246
8	0.274
9	0.302
10	0.327
11	0.351
12	0.380

Appendix 18: Drug content values after Permeation through the rat abdominal skin for formulation F1 (nm)

Time (h)	Absorbance (nm)
1	0.099
2	0.159
3	0.278
4	0.393
5	0.518
6	0.607
7	0.732
8	0.865
9	0.953
10	1.080
11	1.114
12	1.117

Appendix 19: Cumulative Amount of Drug Permeated in (mg/ 1.53 cm^2) at Various Time Intervals from the various formulations.

Time (hrs)	F1 (mg/1.53 cm^2)	F2 (mg/1.53 cm^2)	F3 (mg/1.53 cm^2)	F4 (mg/1.53 cm^2)	F5 (mg/1.53 cm^2)	F7 (mg/1.53 cm^2)
0	0.00	0.00	0.00	0.00	0.00	0.00
1	0.340	0.183	0.152	0.277	0.265	0.293
2	0.563	0.356	0.230	0.365	0.344	0.482
3	0.793	0.523	0.320	0.443	0.438	0.856
4	0.977	0.664	0.412	0.532	0.514	1.217
5	1.227	0.878	0.510	0.629	0.605	1.611
6	1.425	1.042	0.604	0.755	0.689	1.891
7	1.665	1.208	0.689	0.872	0.755	2.284
8	1.916	1.350	0.869	0.992	0.844	2.702
9	2.092	1.566	1.023	1.110	0.932	2.978
10	2.318	1.731	1.152	1.211	1.010	3.378
11	2.520	1.875	1.265	1.331	1.080	3.485
12	2.749	2.080	1.334	1.356	1.17	3.495

Appendix 20: Cumulative percentage of drug permeated (%) at different time intervals from the various formulated films

Time (hrs)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F7 (%)
0	0	0	0	0	0	0
1	5.64	3.04	2.69	4.46	4.23	4.79
2	9.34	5.92	4.07	5.88	5.53	7.87
3	13.15	8.69	5.67	7.14	7.04	13.98
4	16.21	11.04	7.3	8.57	8.26	19.88
5	20.35	14.59	8.86	10.13	9.72	26.32
6	23.65	17.32	10.7	12.17	11.06	30.89
7	27.63	20.08	12.2	14.05	12.13	37.32
8	31.79	22.44	15.4	15.98	13.56	44.15
9	34.72	25.93	18.14	17.89	14.97	48.66
10	38.46	28.78	20.43	19.52	16.22	55.19
11	41.80	31.16	22.4	21.45	17.35	56.90
12	45.61	34.57	23.6	21.86	18.79	57.10

Appendix 21: Steady State Flux (J) (µg/1.53 cm^2/hr) at different time intervals for the formulated films

Time (hrs)	F1	F2	F3	F4	F5	F7
0	0	0	0	0	0	0
1	66.15	51.56	29.57	53.89	51.56	72.95
2	54.77	36.77	22.37	35.51	33.46	59.05
3	51.43	33.92	20.75	28.73	28.40	55.51
4	47.5	32.29	20.03	25.88	25.00	59.19
5	47.74	34.16	19.84	24.47	23.54	62.68
6	46.21	33.88	19.58	24.48	22.34	61.32
7	46.28	33.57	19.14	24.24	20.98	63.48
8	46.59	32.83	21.13	24.13	20.53	65.71
9	45.22	33.85	22.11	23.99	20.15	64.38
10	45.09	33.67	22.41	23.56	19.65	65.72
11	44.57	33.16	22.37	23.54	19.10	61.64
12	44.56	33.72	21.63	21.98	18.96	56.66

Appendix 22: Permeation Coefficient (P) (1.53 cm/ hr) for the various formulated films

Sample	Permeation Coefficient (P) (1.53 cm/ hr)
F1	0.008
F2	0.006
F3	0.004
F4	0.004
F5	0.004
F7	0.010

Appendix 23: Anti-inflammatory studies showing the paw thickness (mm) of the control group

Group A	0 min	1 hr	2 hr	3 hr	4 hr	5hr	6 hr
1	0.31	5.67	6.93	6.83	6.43	5.93	4.90
2	0.55	5.64	6.40	6.28	5.86	5.34	4.30
3	0.29	6.03	6.62	6.49	6.08	5.57	4.52
4	0.31	4.08	6.37	6.26	5.83	5.30	4.26
5	0.31	5.25	6.41	6.31	5.90	5.39	4.38
M±SEM	0.35±0.049	5.33±0.34	6.55±0.16	6.43±0.11	6.02±0.11	5.51±0.16	4.47±0.16

Appendix 24: Anti-inflammatory studies showing the paw thickness (mm) of formulation F1

Group B	0 min	1hr	2hr	3hr	4hr	5hr	6hr
1	0.55	5.46	6.21	5.87	5.12	4.20	3.11
2	0.25	4.25	4.70	4.28	3.38	2.43	1.13
3	0.69	4.88	5.04	4.63	3.74	2.53	1.04
4	0.53	4.52	5.42	5.07	4.14	3.04	1.34
5	0.65	5.73	6.38	6.00	5.05	3.86	2.26
M±SEM	0.53±0.08	4.97±0.28	5.55±0.33	5.17±0.34	4.29±0.35	3.21±0.35	1.72±0.39

Appendix 25: Anti-inflammatory studies showing the paw thickness (mm) of formulation F7

Group C	0 min	1hr	2hr	3hr	4hr	5hr	6hr
1	0.3	3.99	4.88	3.97	2.91	0.93	0.41
2	0.75	5.06	6.02	5.42	4.20	3.23	2.01
3	0.33	3.74	4.76	4.07	3.05	2.02	0.93
4	0.32	4.01	5.56	4.63	3.34	2.16	0.68
5	0.36	4.05	5.76	4.72	3.41	2.22	0.75
M±SEM	0.41±.08477	4.17±0.23	5.39±0.24	4.56±0.26	3.38±0.22	2.11±0.37	0.96±0.28

Appendix 26: Summary of the anti-inflammatory studies of both the control and the groups administered with formulations F1 and F7

Groups	Time						
	0 min	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**	**6 hr**
Group A	0.35 ± 0.05	5.33 ± 0.34^{a}	6.55 ± 0.16^{a}	6.43 ± 0.11^{a}	6.02 ± 0.11^{a}	5.51 ± 0.16^{a}	4.47 ± 0.16^{a}
Group B	0.53 ± 0.08	4.97 ± 0.28^{ab}	5.55 ± 0.33^{b}	5.17 ± 0.34^{b}	4.29 ± 0.35^{b}	3.21 ± 0.35^{b}	1.78 ± 0.39^{b}
Group C	0.41 ± 0.09	4.17 ± 0.23^{b}	5.40 ± 0.24^{b}	4.56 ± 0.26^{b}	3.38 ± 0.22^{c}	2.11 ± 0.37^{c}	0.96 ± 0.28^{b}
F-ratio	1.625	4.368	6.555	14.175	29.366	33.079	40.936
P-value	0.237	0.038*	0.012*	0.001*	< 0.001*	< 0.001*	< 0.001*

* = Significant difference exists at $p < 0.05$
Mean values with different superscripts along the columns are significantly different.
All values are given in (mean ± SEM) for n=5

Appendix 27: Percentage inhibition of formulations F1 and F7 against the control

Formulation		Percentage Inhibition					
(F1)	-	41%	47%	48%	56%	65%	79%
(F7)	-	35%	31%	41%	55%	72%	88%

Appendix 28: Absorbance values of the formulated films after storage for a period of ninety days

Sample	Absorbance (Day 30)	Absorbance (Day 60)	Absorbance (Day 90)
F1	0.196 ± 0.001	0.194 ± 0.001	0.194 ± 0.001
F2	0.196 ± 0.002	0.196 ± 0.001	0.194 ± 0.001
F3	0.191 ± 0.001	0.189 ± 0.001	0.188 ± 0.001
F4	0.201 ± 0.001	0.201 ± 0.001	0.200 ± 0.001
F5	0.204 ± 0.002	0.201 ± 0.001	0.201 ± 0.001
F7	0.198 ± 0.002	0.198 ± 0.001	0.197 ± 0.001