PREPARATION AND CHARACTERIZATION OF TRANSDERMAL FLIMS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG KETOPROFEN

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted
By

Reg.No: 261211157

DEPARTMENT OF PHARMACEUTICS

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

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Under the guidance of

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CERTIFICATE

This is to certify that the dissertation entitled **"PREPARATION AND CHARACTERIZATION OF TRANSDERMAL FLIMS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG KETOPROFEN"** submitted by **T.Prabakaran** (Reg. No: 261211157) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutics, Edayathangudy.G.S Pillay College of Pharmacy during the academic year 2012-2014.

Place: Nagapattinam **(Prof.K.Shahul Hameed Maraicar, M.Pharm., (Ph.D).,**

Date:

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Place: Nagapattinam (**Prof.Dr.D.Babu Ananth, M.Pharm., Ph.D.,)**

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INDEX

LIST OF ABBREVIATIONS

λmax	Absorption maxima
conc.	Concentration
^{o}C	Degree centigrade
ER	Extended release
Gm	Grams
hrs/ h	Hours
∞	Infinity
LBG	Locust bean gum
g/ mcg	Microgram
mg	Milligram
ml	Milliliter
min	Minutes
M	Molar
nm	Nanometer
%	Percent
Ketoprofen	KF
rpm	Rotations per minute
SEM	Scanning electron microscopy
SD	Standard deviation
SR	Sustain release
√t	Square root of time
UV	Ultra violet
w/v	Weight by volume
w/w	Weight by weight

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INTRODUCTION

Topical drug delivery is well-known for the treatment of local skin disorders, but the use of the skin as a route for systemic drug delivery is a more recent development. Very few Transdermal products have been approved to date, largely because of the complexities involved in achieving a consistent delivery rate. There are many variables that influence the absorption of drugs across the skin and into the general circulation, including the biological properties of the skin, chemical properties of the drug, and the interactions between skin and drug delivery system. Systematic studies have led to complications of permeability data for a range of drugs through skin, both stratum corneum and dermis. These studies reflect the large variability and slowness of the process for most drugs. Consequently, only a few drug candidates are currently available for transdermal drug delivery. There are efforts to improve the process which involve conditioning the skin[1].

In recent years transdermal route now ranks with oral treatment as the most successful innovative research area in drug delivery, with around 40% of the drug delivery candidate product under clinical evaluation related to transdermal or dermal system. The transdermal drug delivery systems (TDDS) have been designed as an alternative route for systemic drug delivery. The systemic drug administration though skin holds several advantages such as maintenance constant drug level in blood, decrease of side effects, and improvement of bio availability by circumvention of hepatic first pass metabolism and increase patient compliance. Now a day's skin considered as a safe port for drug administration, to provide continuous drug release into systemic circulation. Recently, bio polymers used in the fabrication of transdermal films has received much attention due to their excellent biocompatibility and bio degradation[2]. One of the most promising techniques for enhancement for transdermal permeation of drugs is transdermal patches. Sodium alginate (SA) is a natural polymer is very promising and has been widely exploited in pharmaceutical industry, because of its tailor-made to suit the demands of applications[3]. Locust bean gum (LBG) is a hydrophilic polymer, had been limited for use in thickening, suspending, and

emulsifying water based systems. It is gaining appreciation for the fabrication of pharmaceuticals with uniform drug release characteristics[4]. Drug release property of matrices is preceded by polymer hydration and the rate of drug release from polymer carrier can be tailor-made by selecting a suitable polymer-blends composition and drug concentration[5]. The effect of hydrophilic plasticizers such as glycerin on physicochemical properties on SA/LBG film was evaluated. Ketoprofen (KF) belongs to the group of substituted 2-phenylproprionic acids which has analgesic, anti-inflammatory and antipyretic effects. KF exerts the majority of its analgesic actions through inhibition of the synthesis of prostaglandins by inhibiting the enzyme cyclooxygenase (COX)[6]. KF had the best topical penetration ability when compared to ketorlac, indomethacin and other studies have found that topical KF was effective for the treatment of well localized soft tissue injury, joint pain, in reducing muscle soreness after repetitive muscle contraction. The importance of KF in the therapeutic field has stimulated the development of topical dosage forms to improve its percutaneous absorption through the application site. Moreover topical dosage forms could provide relatively consistent drug levels for prolonged periods and avoid gastric irritation, as well as the other typical side effects of oral NSAID administration[7].

Penetration depends on ability of drug to penetrate the stratum corneum, enter the systemic circulation and to achieve the therapeutic effect. There has been increased interest during recent years in the use of chemical enhancer that could modify drug permeation through skin. Many of the chemical enhancers may be harmful, especially in chronic applications, many of them were irritant in nature. It is desirable to develop topical delivery systems that do not require the use of chemical enhancers to facilitate drug permeation through skin. In the present study we made an attempt by using menthol as a penetration enhancer. Because menthol is considered to have good permeation enhancing agent by acting as a lipid disrupting agent that increases the fluidity of stratum corneum lipid by increasing the formation of capillary channels[8]. Ttransdermal films with varied ratios nonirritating and pharmaceutically acceptable biopolymers SA and LBG combination containing the drug KF with permeation

enhancer (menthol). The prepared films were compared with the marketed conventional gel. Furthermore, films was evaluated for anti-inflammatory activity on carrageenan induced rat paw edema model. The purpose was to provide the delivery of drug at a controlled rate across intact skin to achieve a therapeutically effective drug Transdermal Drug Delivery Systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drugs, through the skin, at controlled rate to the systemic circulation. Transdermal Drug Delivery Systems are designed to support the passage of drug substances from the surface of the skin through its various layers and into the systemic circulation. In response to this new idea several transdermal drug delivery systems have recently been developed, aiming to achieve the objective of systemic medication through topical application to the intact skin surface

1.1. **Advantages of Transdermal Drug Delivery System**[9]

- ✓ Avoid GI absorption problems
- ✓ Avoid first-pass effect, thus minimizing drug input
- ✓ Oral administration to be avoided or is contraindicated
- ✓ Drugs with narrow therapeutic index can be delivered
- ✓ Better control of drug levels for potent drug is possible
- ✓ Increased patience compliance
- ✓ Self medication is possible
- ✓ Drugs with short t1/2 values are utilized.

1.2 **Disadvantages of Transdermal drug delivery system**:

- ✓ Neither practical nor affordable for drugs with large doses
- ✓ When drug or formulation is skin sensitizing or irritating

- ✓ Extensive metabolism in skin
- ✓ Large molecular size preventing diffusion through skin

1.3 **Problems associated with transdermal drug delivery system** [10]:

- ✓ Limited drug permeability of the skin
- ✓ Pharmacokinetic and pharmacodynamic restrictions
- ✓ Usable for low drug doses only
- ✓ Possibility of irritation and hypersensitivity reactions
- ✓ Adhesion problems
- ✓ Skin permeability variation
- ✓ High cost of treatment

1.4 Transdermal drug absorption markedly alters drug kinetics and depends on a several parameters including the following-

- ✓ Medicament application site
- ✓ Thickness and integrity of the stratum cornea epidermis.
- ✓ Size of the molecule that is to be administered.
- ✓ Permeability of the membrane for the transdermal drug delivery.
- ✓ Hydration state of skin.
- ✓ pH of the drug.
- ✓ Drug metabolism by skin flora.
- ✓ Lipid solubility.
- ✓ Drug depot in skin.
- ✓ Blood flow alteration in the skin by additives and body temperature

The toxic effect of the drug and problem in limiting drug uptake are major considerable potential for transdermal delivery systems, especially in children because

skin thickness and blood flow in the skin usually vary with age. The increased blood supply in the skin along with thinner skin has significant effects on the pharmacokinetics of transdermal delivery for children. In some situations this may be an advantageous, while in others systemic toxicity may occur. This was observed after using scopolamine patches that are used to prevent motion sickness, a eutectic mixture of local anesthetics (EMLA) cream used to minimize the pain, corticosteroid cream applied for its local effect on skin maladies. Episodes of systemic toxic effects, including some fatalities in children have been documented with each of these, often secondary to accidental absorption through mucous membranes[5].

1.5 **Basic components of Transdermal drug delivery systems**[11]

The basic components of Transdermal devices include

A. Baking membrane

B. Polymer matrix or matrices that regulate the release of the drug

C. Drug substance

D. Penetration enhancer

E. Plasticizer

F. Adhesive

G. Release liner

FIG NO:1 Components of Transdermal Drug Delivery System

A. Baking membrane

The backing layer must be impermeable to the drugs and enhancers, if used, and as result, it is usually impermeable to water vapor i.e. occlusive.

B. Polymer matrix

Polymers are the backbone of a Transdermal drug delivery system. The polymers play a major role in TDDS of drugs. The release of drug to the skin is controlled by drug free film known as rate controlling membrane.

- Molecular weight, glass transition temperature and chemical functionality of the polymer should allow proper diffusion and release of specific drug.
- It should be chemically compatible with drug and other components of the system, such as penetration enhancers.
- It should be stable, non-reactive with the drug, easy manufactured and fabricated into the desired product.

- The polymer and its degradation products must be non-toxic.
- They also should provide consistent, effective delivery of a drug throughout the products intended shelf-life or delivery period.
- The polymers should be inexpensive.

The polymers used in TDDS include,

(i) Natural Polymers:

Cellulose derivatives, gelatin, shellac, waxes, proteins, gums, natural rubber starch etc.

(ii) Synthetic Elastomers:

Polybutadiene, polysiloxane, silicone rubber, acrylonitrile, butyl rubber, styrenebutadiene rubber, and neoprene etc.

(iii) Synthetic Polymers:

Polyvinyl alcohol, Polyvinyl chloride, Polyethylene, Polypropylene, Polyacrylate, Polymide, Polyurea, Polyvinylpyrrolidone, Polymethylmethacrylate, etc.

C. Drug substance

The transdermal route of administration cannot be employed for a large number of drugs. Candidate feasibility can be deduced rationally at an early stage in development provided certain criteria are examined. The following are some of the desirable properties of drug suitable for transdermal delivery.

The properties of drug suitable for TDDS formulation are[12]:

The drug should be potent. The daily systemic dose should be ≤ 20 mg.

- ✓ The drug should have less molecular weight i.e., < 500 - 1000 Daltons.
- ✓ Lipophilicity- the log kp of the drug should be in the range 1-3

- ✓ The drug should have low melting point < 200° C
- ✓ Hydrogen bonding groups should be < 2
- ✓ The drug should not induce a skin irritation or allergic response.
- ✓ The drug should not stimulate an immune reaction in the skin.
- ✓ The half-life (t1/2) of the drug should be short.
- ✓ The drugs which degrade in GIT or inactivated by first pass effect.

D. Penetration enhancer [13]

Agents, which reversibly alter the barrier properties of the stratum corneum. They aid in the systemic delivery of drugs by allowing the drug to penetrate more rapidly to viable tissues are been called "penetration enhancers".

(i) The properties of an ideal penetration enhancer are:

- It should be pharmacologically inert.
- It should be non-toxic, non irritating and non allergenic.
- Following removal of the enhancer, the stratum corneum should immediately and fully recover its normal barrier property.
- The barrier should decrease in one direction only and efflux of endogenous material should not occur.
- Inexpensive and cosmetically acceptable.

(ii) Classification of penetration enhancers

a. Terpenes (essential oils)

E.g. Menthol, 1, 8 cineole, carvone, limonene and nerodilol etc.

b. Pyrrolidones

c. Azone (1-dodecylazacycloheptan-2-one)

d. Fatty acids and esters

e. Sulfoxides and similar compounds

f. Alcohols, glycols and glycerides

g. Miscellaneous enhancers

i. Cyclodextrin complexes

ii. Lipid synthesis inhibitors

iii. Enzymes

E. Plasticizers[14]

An important method of varying membrane properties is to add a plasticizer to reduce the stiffness of the polymer backbone and thereby increase drug diffusion rate from the device. Plasticizers are used to modify the characteristics of many polymers. The plasticizers increase polymer chain flexibility, so that matrices with higher plasticizer loading normally give a higher release rate than those with lower plasticizer content. In the selection of plasticizer as with the polymer itself, care must be taken to select a material that is acceptable for use in medical applications and it is clearly advantageous to choose one that has already been in pharmaceutical device. Examples are butyl benzyl phthalate, trioctyl phosphate, dioctyl phthalate, glycerol, polyethylene glycol (PEG) and polypropylene glycol.

F. Release liner

During storage the films is covered by a protective liner that is removed and discarded before the application of the film to the skin. Since the liner is in intimate

contact with TDDS, the liner should be chemically inert. It is therefore regarded as a part of the primary packaging material rather than a apart of the dosage form delivering the active principle. However, because the liner is in intimate contact with the delivery system, it should comply with specific requirements regarding the chemical inertness and permeation to the drug, penetration enhancer, and water. In case cross linking is induced between the adhesive and the release liner, the force required to remove the liner will be unacceptably high.

1.5 TYPES OF TRANSDERMAL FILMS[15]

Several technologies have been successfully developed to provide rate control over the release and skin permeation of drugs. These technologies can be classified into four basic approaches.

A. Polymer membrane permeation-controlled TDD systems

1n this system the drug reservoir is sandwiched between a drug impermeable backing laminate and a rate controlling polymer membrane. The drug molecules are permitted to release only the rate controlling polymeric membrane. The rate of drug release from these TDD systems can be tailored can by varying the composition of the drug reservoir formulation and the permeability coefficient and thickness of the rate controlling membrane. Several TDD system have been successfully developed from this technology e.g , Transderm-nitro

B. Polymer matrix diffusion-controlled TDD systems.

In this approach the drug reservoir is formed by homogeneously dispersing the drug solids in a hydrophilic or lipophilic polymer matrix and the medicated polymer formed is then molded into medicated disks with a defined surface area and controlled thickness. This drug reservoir containing polymer disk is than mounted in to an occlusive base plate in a compartment fabricated from a drug impermeable plastic backing. Instead of coating the adhesive polymer directly on the surface of the

medicated disk, in this system the adhesive polymer is applied along the circumference of the films to form a strip of adhesive rim surrounding the medicated disks. This type of TDDS is exemplified by the development of the,. Nitro-Dur system

Alternatively the polymer matrix dispersing type TDD system can be fabricated by directly dispersing the drug in a pressure sensitive adhesive polymer, e.g., polyacrylate, and then coating the drug dispersed adhesive polymer by solvent casting or hot melt on to a flat sheet of a drug impermeable backing laminate to form a single layer of drug reservoir . this yields a thinner and/or smaller TDD films. e.g Nito-glycerin-releasing TDDS, Manitran , Nitro Dur-II

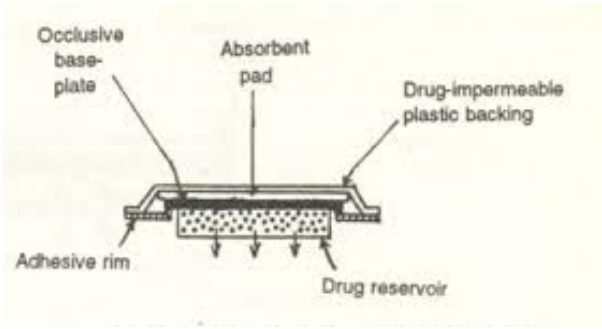


FIG NO: 2 Cross sectional view of Polymer matrix diffusion-controlled TDD systems.

C. Drug reservoir gradient controlled TDD system

To overcome the non-zero order drug release profiles, polymer matrix drug dispersion type TDD system can be modified to have the drug loading level varied in an incremental manner, forming a gradient of drug reservoir along with the diffusional path across the multi-laminate adhesive layer. E.g Deponit

D. Micro-reservoir dissolution-controlled TDD system

This type of drug delivery system can be considered as a hybrid of the reservoir –and matrix dispersion –type drug delivery system. In this approach the drug reservoir

is formed by first suspending the drug solids in an aqueous solution, of water- miscible drug solublizer, e.g., polyethylene glycol, and then homogenously dispersing the drug suspension, with controlled aqueous solubility in a lipophilic polymer, by high-shear mechanical force, to form thousand of unleachable microscopic drug reservoir. This thermodynamically unstable dispersion is quickly stabilized by immediately cross-linking the polymer chains in situ, which produces a medicated polymer disk with a constant surface area and a fixed thickness. A TDD system is then produced by mounting the medicated disk at the center of an adhesive pad. e.g., Nitro Disc

1.6 THE SKIN SITE FOR TRANSDERMAL DRUG ADMINISTRATION

Anatomy and Physiology of Skin

The skin is a large organ responsible for maintaining homeostasis through temperature, regulation, protection of underlying tissues, retardation of water loss, housing sensory receptors, synthesizing certain chemicals, and excreting wastes. The skin consists of an outer epidermis and a dermis, connected to underlying tissue by the subcutaneous layer (hypodermis). The epidermis is made up of stratified squamous epithelium and lacks blood vessels. It contains melanocytes and is well nourished with dermal blood vessels. The epidermis is important because it protects against water loss, mechanical injury, chemicals, and microorganisms. The dermis binds the epidermis to underlying tissues. The dermis consists of connective tissue with collagen and elastic fibers within a gel-like ground substance. Dermal blood vessels carry nutrients to upper layers of skin and help to regulate temperature. The dermis also contains nerve fibers, sensory fibers, hair follicles, sebaceous glands, and sweat glands. The subcutaneous layer (hypodermis) is composed of loose connective tissue and insulating adipose tissue. It binds the skin to underlying organs and contains the blood vessels that supply the skin. The accessory organs of the skin include hair follicles, sebaceous glands, nails and sweat glands.

The skin of an average body covers a surface area of approximately 2 sq. meters and receives about one third of the blood circulating through the body. The permeability barrier in the skin consists of three distinct layers in series. Epidermis, Dermis, Subcutaneous tissue

Epidermis

The outermost layer of the skin is composed of stratified squamous epithelial cells Microscopically epidermis shows two main parts one is stratum corneum and the other is stratum germinativum.

The cells in the stratum corneum are physiologically inactive keratin filled cells that are roughly pentagonal plates of 0.5 μm thick and 30-40 μm across. Filling the intercellular space between these cells are bi layer structural lipids. The structures of these lipids have proved to be important to the moisture retaining ability of stratum corneum. The stratum corneum is responsible for the barrier function of skin. It also behaves as the primary to percutaneous absorption.

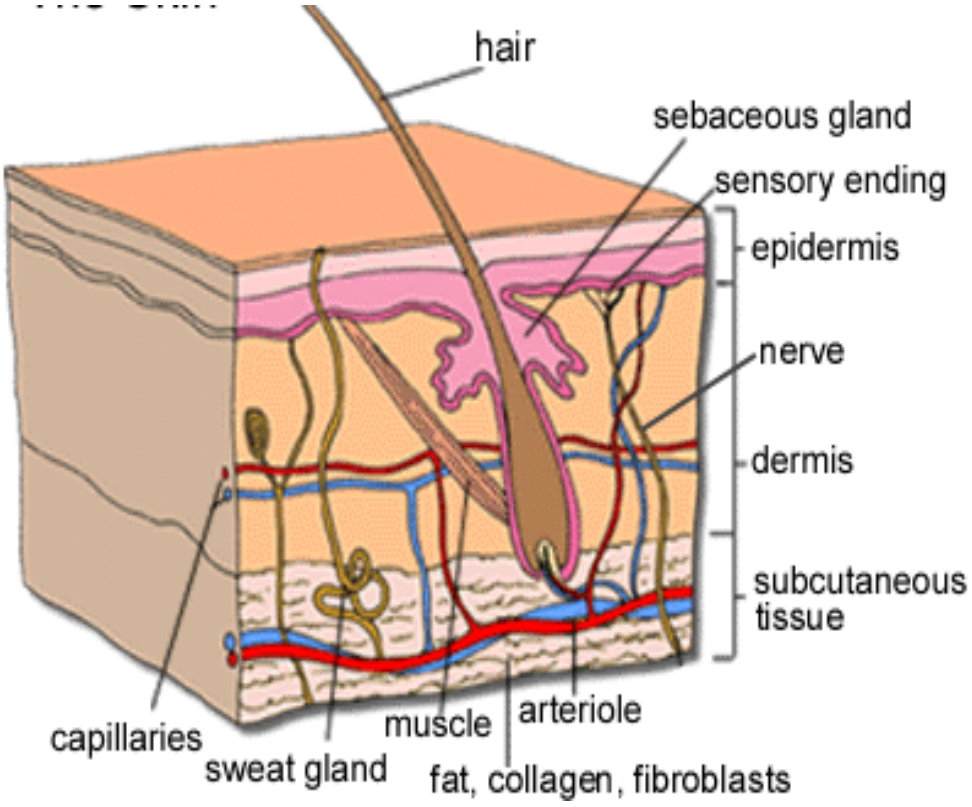


FIG NO: 3 SKIN

Dermis

It is made up of a network of robust collagen fibers of fairly uniform thickness with regularly spaced cross striations. This network responsible for the elastic properties of the skin. Beneath the epidermis, the fibrous tissue opens out and merges with the fat containing subcutaneous tissue. The upper portion of the dermis is formed into the ridges projecting into the epidermis, which contains blood vessels and nerve ending. Only nerve fibers reach into the germinativam zone of the epidermis.

Subcutaneous fat tissue

This is a sheet of fat containing areolar tissue, known as the superficial fascia attaching the dermis to underling structures. This composite structure is pierced at various places by two types of potential diffusion shunts. These skin appendages however, actually occupy only 0.1% of the total human skin surface. This route of percutaneous absorption however, provides a very limited contribution to the overall kinetic profile of transdermal permeation. Therefore, the transdermal permeation of most neutral molecules at a steady state can, thus, be considered as a process of passive diffusion through the intact stratum corneum in the inter follicular region. Thus the skin serves as the point of administration for systemically active drugs.

The drug applied topically is distributed following absorption, first into the systemic circulation and then transported to target tissues.

1.7. Factors Affecting Transdermal Permeability[16]

Physicochemical properties of the peneterants

a) Partition coefficient

b) pH conditions

c) Penetrant concentration

Physicochemical properties of drug delivery systems

a) Release characteristics

b) Composition of drug delivery system

Physiological and pathological conditions of skin

a) Reservoir effect of horny layer

b) Lipid film

c) Skin hydration

d) Skin temperature

e) Regional variation

f) Pathological injuries to skin

g) Cutaneous drug metabolism

B. Kinetics of Transdermal permeation

Transdermal permeation of a drug involves the following steps

1) Sorption by stratum corneum

2) Penetration of drug through viable epidermis

3) Uptake of drug by the capillary network in the dermal papillary layer

DISEASES THAT CAN BE TREATED BY TRANSDERMAL DRUG DELIVERY SYSTEMS

- Skin Disorders
- Fungus infections of Nails
- Hair Loss
- Cardiovascular Disorders
- Hypertension
- Anticoagulants
- Chronic Obstructive Pulmonary Disease

- Neurological and Psychiatric Disorders
- Parkinson's Disease
- Depression
- Alzheimer's Disease
- Musculoskeletal Disorders
- Osteoporosis
- Osteoarthritis
- Hormonal Disorders
- Transdermal Insulin Delivery in Diabetes
- Erectile Dysfunction
- Menopause
- Cancer
- Anticancer Drugs and Vaccines
- Pain
- NSAIDs
- Smoking Cessation
- Transdermal Nicotine Replacement
- Vaccination
- HIV/AIDS
- Influenza Vaccine

- Contraception
- Female/Male Contraception

VARIOUS METHODS FOR PREPARATION TDDS:

a. Asymmetric TPX membrane method[17]:

A prototype patch can be fabricated for this a heat sealable polyester film (type 1009, 3m) with a concave of 1cm diameter will be used as the backing membrane. Drug sample is dispensed into the concave membrane, covered by a TPX {poly(4-methyl-1-pentene)}asymmetric membrane, and sealed by an adhesive. [(**Asymmetric TPX membrane preparation):** These are fabricated by using the dry/wet inversion process. TPX is dissolved in a mixture of solvent (cyclohexane) and nonsolvent additives at 60°c to form a polymer solution. The polymer solution is kept at 40°C for 24 hrs and cast on a glass plate to a pre-determined thickness with a gardner knife. After that the casting film is evaporated at 50°C for 30 sec, then the glass plate is to be immersed immediately in coagulation bath [maintained the temperature at 25°C]. After 10 minutes of immersion, the membrane can be removed, air dry in a circulation oven at 50°C for 12 hrs].

b. Circular teflon mould method[18]:

Solutions containing polymers in various ratios are used in an organic solvent. Calculated amount of drug is dissolved in half the quantity of same organic solvent. Enhancers in different concentrations are dissolved in the other half of the organic solvent and then added. Di-N-butylphthalate is added as a plasticizer into drug polymer solution. The total contents are to be stirred for 12 hrs and then poured into a circular teflon mould. The moulds are to be placed on a leveled surface and covered with inverted funnel to control solvent vaporization in a laminar flow hood model with an air speed of 0.5 m/s. The solvent is allowed to evaporate for 24 hrs. The dried films are to be stored for another 24 hrs at 25±0.5°C in a desiccators

containing silica gel before evaluation to eliminate aging effects. The type films are to be evaluated within one week of their preparation.

c. Mercury substrate method[19]:

In this method drug is dissolved in polymer solution along with plasticizer. The above solution is to be stirred for 10- 15 minutes to produce a homogenous dispersion and poured in to a leveled mercury surface, covered with inverted funnel to control solvent evaporation.

d. By using "IPM membranes" method[20]:

In this method drug is dispersed in a mixture of water and propylene glycol containing carbomer 940 polymer and stirred for 12 hrs in magnetic stirrer. The dispersion is to be neutralized and made viscous by the addition of triethanolamine. Buffer pH 7.4 can be used in order to obtain solution gel, if the drug solubility in aqueous solution is very poor. The formed gel will be incorporated in the IPM membrane.

e. By using "EVAC membranes" method[21]:

In order to prepare the target transdermal therapeutic system, 1% carbopol reservoir gel, polyethelene (PE), ethylene vinyl acetate copolymer (EVAC) membranes can be used as rate control membranes. If the drug is not soluble in water, propylene glycol is used for the preparation of gel. Drug is dissolved in propylene glycol, carbopol resin will be added to the above solution and neutralized by using 5% w/w sodium hydroxide solution. The drug (in gel form) is placed on a sheet of backing layer covering the specified area. A rate controlling membrane will be placed over the gel and the edges will be sealed by heat to obtain a leak proof device.

f. Aluminium backed adhesive film method[22]:

Transdermal drug delivery system may produce unstable matrices if the loading dose is greater than 10 mg. Aluminium backed adhesive film method is a suitable one. For preparation of same, chloroform is choice of solvent, because most of the drugs as well as adhesive are soluble in chloroform. The drug is dissolved in chloroform and adhesive material will be added to the drug solution and dissolved. A custammade aluminium former is lined with aluminium foil and the ends blanked off with tightly fitting cork blocks.

g. Preparation of TDDS by using Proliposomes[23,24]:

The proliposomes are prepared by carrier method using film deposition technique. From the earlier reference drug and lecithin in the ratio of 0.1:2.0 can be used as an optimized one. The proliposomes are prepared by taking 5mg of mannitol powder in a 100 ml round bottom flask which is kept at 60-70°c temperature and the flask is rotated at 80-90 rpm and dried the mannitol at vacuum for 30 minutes. After drying, the temperature of the water bath is adjusted to 20-30°C. Drug and lecithin are dissolved in a suitable organic solvent mixture, a 0.5ml aliquot of the organic solution is introduced into the round bottomed flask at 37°C, after complete drying second aliquots (0.5ml) of the solution is to be added. After the last loading, the flask containing proliposomes are connected in a lyophilizer and subsequently drug loaded mannitol powders (proliposomes) are placed in a desiccator over night and then sieved through 100 mesh. The collected powder is transferred into a glass bottle and stored at the freeze temperature until characterization.

h. By using free film method[25]:

Free film of cellulose acetate is prepared by casting on mercury surface. A polymer solution 2% w/w is to be prepared by using chloroform. Plasticizers are to be incorporated at a concentration of 40% w/w of polymer weight. Five ml of polymer solution was poured in a glass ring which is placed over the mercury

surface in a glass petri dish. The rate of evaporation of the solvent is controlled by placing an inverted funnel over the petri dish. The film formation is noted by observing the mercury surface after complete evaporation of the solvent. The dry film will be separated out and stored between the sheets of wax paper in a desiccators until use. Free films of different thickness can be prepared by changing the volume of the polymer solution.

AIM

The main aim of the present study is to Formulate and Evaluate the Transdermal Drug Delivery System of Ketoprofen.

OBJECTIVE

The main objective of the study is to effectively increase the Transdermal drug permeation and diffusion of the drug, Ketoprofen using suitable polymer, plasticizer, and permeation enhancer to avoid the skin irritation.

The broad objectives of the present study is to,

1. To select suitable polymers for Ketoprofen Transdermal films.

2. To formulate Ketoprofen Transdermal films to ensure the control release of the skin.

3. To evaluate physicochemical properties for the prepared formulation.

4. To carry out in vitro diffusion studies.

5. To carry out permeability of Ketoprofen Transdermal films through albino rat skin.

A.R.Mullaicharam. et. al[27] have developed Flurbiprofen trandermal patches were formulated by using Carboxy methyl cellulose and hydroxyl propyl methyl cellulose of different concentrations and evaluate for in vitro drug release behaviour. The study shows that hydroxy propyl methyl cellulose release was more when compared to carboxy methyl cellulose.

Das. et.al[28] have investigated the effects of polymeric composition, drug content and plasticizers on the permeation of Trazodone hydrochloride across the mouse epidermis in the development of TDDS and demonstrated the potential of the fabricated pseudolatex transdermal films for sustained release of trazodone hydrochloride. The concentration of triethylcitrate in the films markedly affected the skin permeation properties of Trazodone hydrochloride.

R.V. Kulkarni. et.al[29] have taken up to prepare and evaluate Eudergit RS100 films as rate controlling membrane for transdermal use and to study the various plasticizers effect on the permeability and mechanical properties. The investigation reveals that films plasticized with PEG showed higher permeability to verapamil hydrochloride. The permeability of the drug was decreased as the concentration of dibutyl phthalate was increased. Whereas increase in the concentration of Polyethylene glycol enhanced the permeability of the films.

V.V. Dhavse. et.al[30] have prepared ointment, creams, and gel bases containing 3%w/w of Ketoprofen and evaluate for pH, drug content, rheological properties and in vitro drug release rate through cellophane membrane .The order of the in vitro release was found to be Carbapol 940gel base >polyethylene glycol ointment base >modified vanishing cream base >Canadian formulary cream base >modified hydrophilic base > beller's ointment base >oleaginous ointment base.

Saxena. M. et.al[31] have formulated the transdermal patches of metoclopramide hydrochloride by using polyvinylalcohol and polyvinylpyrrolidone for the treatment of nausea and vomiting. The formulation showed burst release of the drug in initial hours and there after drug was released slowly up to 12 h. The drug release mechanism from the patches was found to be diffusion dominated.

Dey. B. K. et. al [32], have carried out to study the effect of different proportion of ethyl cellulose and polyvinyl pyrrolidone, a hydrophobic and hydrophilic polymer on the permeation profile of the drug across the rat abdomen for the development of a reproducible transdermal therapeutic system of propranolol hydrochloride. The study was demonstrated the potential of the fabricated matrix films for prolonged release of propranolol hydrochloride.

K.Kawathekar. et.al[33, have] studied Mannich bases amides of Ketoprofen been prepared by using different secondary amines. Then evaluated for analgesic, anti- inflammatory and ulcerogenic activities and quantitative structure activity relationship.

Pophalikarr. R.N. et.al[34], have studied Mutual prodrug of ketoprofen with glucosamine was synthesized by reacting its acid chloride with d-glucosamine, in order to reduce its ulcerogenic potential and improve upon its anti arthritic activity. The study of prodrug was found to posses excellent antiarthitic activity without any ulcerogenic tendency as compared to ketoprofen when screened for ulcerogenic activity by cold stress method of rainsford.

Gattani. S.G. et. al [35]have prepared transdermal films of Ondansetron by using different hydrophilic and Lipophilic polymer combination such as poly vinyl alcohol: poly vinyl pyrrolidone, Ethyl cellulose: PVP later the study was extended to investigate the plasticizers effect such as dibutyl phthalate and propylene glycol .The result shows that release rate followed first order kinetics, and the permeation enhancement was good.

Gattani. S.G. et.al[36] have prepared Lovastatin transdermal films by casting method on mercury substrate. Eight formulations were prepared by using two different polymeric combination, Ethyl cellulose with PVP and Eudragit RL100 with Eudragit E RS 100. All the formulation carried 10%w/w of lovastatin and 30%w/w of dibutyl phthalate in chloroform solvent .The study was carried on the basis of in vitro permeation studies.

Shankar V, et al[37] explored the different suitable methods for Transdermal applications as the rate controlling membrane. They evaluated various physicochemical characters of dry free films. They studied permeability characteristics of free film by using different concentration of plasticizer. From this study they concluded that faster release was observed from ethyl cellulose patches containing glycerol as plasticizer.

Manvi FV, etal[38] studied the physicochemical properties of Transdermal film formulated using different polymeric combination plasticized with polyethylene glycol 400. They studied the effect of permeation enhancers on skin permeation kinetics by Keshary-chein diffusion cell. The results of the study show the feasibility of formulating rate controlled Transdermal therapeutic system of the drug for effective control and prophylaxis of allergic asthma.

Jamakandi VG, et al [39] reviewed in detail on various drugs have been invented in the treatment of CVS diseases in subjected to TDDS clinical risks and benefits. The study concludes that, the advanced state of research and plethora of patent applications field for Transdermal systems clearly indicate the renewed interest of pharmaceutical industry in the Transdermal field.

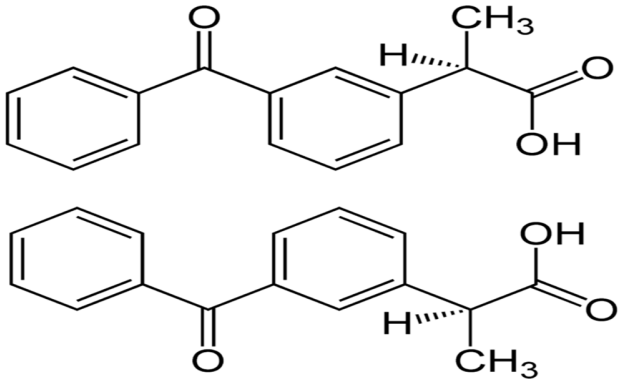
Sadashivaiah R, et. al[40] have prepared a matrix-type Transdermal drug delivery systems of haloperidol lactate using different ratios of ethyl cellulose: polyvinyl pyrrolidone by solvent-evaporation technique and 4% hyaluronidase enzyme as permeation enhancer. They have carried out different physicochemical parameters and in vitro permeation studies. As per the permeation studies they have illustrated that 4% hyaluronidase enzyme was a good enhancer. It is concluded the haloperidol lactate can be formulated into Transdermal polymeric patches to prolong its release characteristics and it is best for a sustained-release one-a-day formulation.

Xiaoping Zhan et al [41]were investigated the effect of monomers ratios, membrane thickness and drug concentration on the membrane permeation rates. The membranes were characterized by FTIR, DSC, and SEM. It was found that the copolymer membranes could control the drug release in the Transdermal drug delivery system

DRUG PROFILE

3.2.1 KETOPROFEN [42]

Structural formula



Molecular formula

$C_{16}H_{14}O_3$

Molecular weight

254.29

IUPAC Name

2-(3-benzoylphenyl)-propionic acid

Category

- Anti-inflammatory Agents
- Cyclooxygenase Inhibitors
- Analgesics
- Analgesics, Non-Narcotic
- Antipyretics
- Nonsteroidal Anti-inflammatory Agents (NSAIAs)

Description

Nonsteroidal anti-inflammatory, analgesic drug White or off-white, odorless, nonhygroscopic, fine to granular powder.

Solubility

Freely soluble in ethyl alcohol, chloroform, acetone, and ether and soluble in phosphate buffer pH 7.4, but practically insoluble in water.

Melting point

93 – 950C.

Mechanism of action

Ketoprofen is one of the most powerful inhibitors of cyclooxygenase at concentrations well within the range of therapeutic plasma concentrations (EC50 2 μg/l). It produces reversible COX inhibition by competing with the substrate, arachidonic acid, for the active site of the enzyme. This inhibition results in a reduction in the tissue production of prostaglandins such as PGE2 and PGF2α. In addition to its effects on cyclooxygenase, ketoprofen inhibits the lipoxygenase pathway of the arachidonic acid cascade. Ketoprofen is also a powerful inhibitor of bradykinin, an important chemical mediator of pain and inflammation

Pharmacokinetics

Ketoprofen is readily absorbed from the gastrointestinal tract, peak plasma concentration occur about 0.5 to 2h after a dose, 99% bound to plasma proteins and substantial concentration of drug are found in the synovial fluid. T-max-60 to 90min. The plasma elimination half life is 1.5 to 4h. It is metabolized mainly by conjugation with glucoronic acid and is excreted mainly in the urine. Food does not affect the bioavailability of Ketoprofen but the rate of absorption is slowed. Metabolism involves hydroxylation and glucuronide conjugation and elimination by first order.

Uses

1. In musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis and rheumatoid arthritis.

2. In peri-articular disorders such as bursitis and tendonitis.

3. It is also used in dysmenorrhoea, postoperative pain.

4. In painful and inflammatory conditions such as acute gout or soft tissue disorders and to reduce fever.

Adverse effects

1. Pain at injection site when administered intramuscularly and occasionally tissue damage.

2. Hypersensitive reactions like life-threatening asthma, urticaria, and angioderma.

3. It causes gastrointestinal side effects like dyspepsia, nausea, abdominal pain, diarrhea, vomiting and constipation.

Dose

100-200 mg in 2 to 4 divided dose in the treatment of rheumatic disorders.

25- 50 mg every 6-8 h in the treatment of Dysmenorrhoea

Absorption

Ketoprofen is rapidly and well-absorbed orally, with peak plasma levels occurring within 0.5 to 2 hours.

Protein Binding

99% bound, primarily to albumin

Metabolism

Rapidly and extensively metabolized in the liver, primarily via conjugation to glucuronic acid.

Route of elimination

In a 24 hour period, approximately 80% of an administered dose of ketoprofen is excreted in the urine, primarily as the glucuronide metabolite.

Half life

Conventional capsules: 1.1-4 hours

Extended release capsules: 5.4 hours due to delayed absorption (intrinsic clearance is same as conventional capsules)

Clearance

- Oral-dose cl=6.9 +/- 0.8 L/h [Ketoprofen Immediate-release capsules (4 × 50 mg)]
- Oral-dose cl=6.8 +/- 1.8 L/h [Ketoprofen Extended-release capsules (1 × 200 mg)]
- 0.08 L/kg/h
- 0.7 L/kg/h [alcoholic cirrhosis patients]

Toxicity

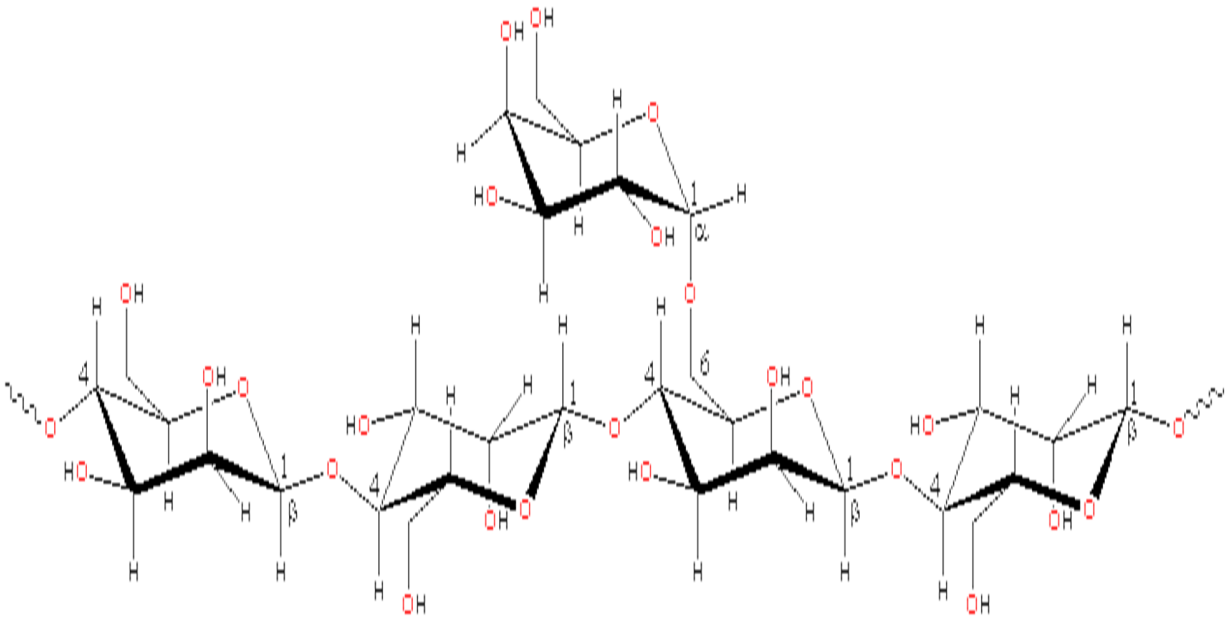
LD_{50}=62.4 mg/kg (rat, oral).

Symptoms of overdose include drowsiness, vomiting and abdominal pain.

Side effects are usually mild and mainly involved the GI tract. Most common adverse GI effect is dyspepsia (11% of patients). May cause nausea, diarrhea, abdominal pain, constipation and flatulence in greater than 3% of patients.

3.3 POLYMERS PROFILE

3.3.1. LOCUST BEAN GUM [43]



Locust bean gum is a galactomannan vegetable gum extracted from the seeds of the carob tree. It forms a food reserve for the seeds and helps to retain water under arid conditions. It is used as a thickener and gelling agent in food technology. It is also called Carob Gum or Carubin. Locust bean gum is similar to guar gum consisting of a (1→4)-linked β-D- mannopyranose backbone with branchedpoints from their 6-positions linked to α-D-galactose (i.e. 1→6-linked α-D-galactopyranose). There are about 3.5 (2.8- 4.9) mannose residues for every galactose residue.

Molecular structure

Locust bean gum is polydisperse, consisting of non-ionic molecules made up of about 2000 residues. Lower galactose substitution also decreases the stiffness (i.e. increases the flexibility) but increases the extensibility of the isolated chains. The galactose residues prevent strong chain interactions but there may be up to 10-11 unsubstituted mannose residues in a row and junction zones may form between such clear areas when they consist of greater than about six residues. These nano-crystalline

links dissociate in hot water. If the galactose residues were perfectly randomized or blocked, it is likely that each molecule would have more than four such areas capable of acting as junction zones, thus allowing gel formation.

Functionality

Locust bean gum is less water soluble and has lower viscosity than guar gum as it has fewer galactose branch points. It needs heating to dissolve but is soluble in hot water. Locust bean gum differs from guar gum in that it does form thermally-irreversible weak gels by association of the galactose deficient regions. Being non-ionic, locust bean gum is not affected by ionic strength or pH but will degrade at pH extremes at higher temperatures.

Description: White to yellowish white, nearly odorless powder.

Solubility: Soluble in hot water and insoluble in ethanol.

3.3.2. GLYCEROL[44]

Nonproprietary names:

BP: Glycerol

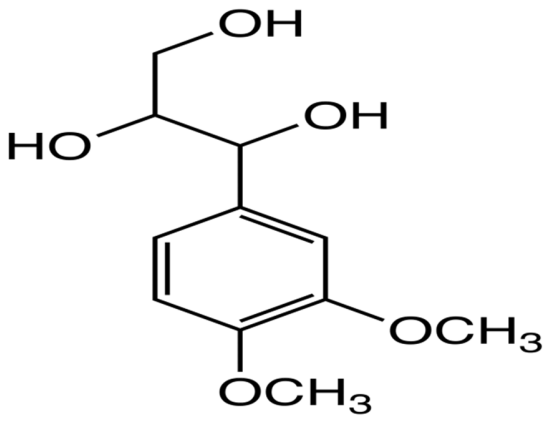
Chemical names and CAS registry number:

Propane-1,2,3- triol

Empirical formula & molecular weight:

C3H8O3 MWt: 92.09

Structure:



Functional category:

Anti microbial preservative, emollient, humectants, plasticizer, solvent, sweetening agent, tonicity agent.

Applications in pharmaceutical formulation and technology:

In topical: Humectant, emollient.

In parentrals: Solvent

IN oral solutions: Solvent, sweetening agent, antimicrobial preservative, viscosity increasing agent Plasticizer and in film coatings.

In capsules: plasticizer.

Typical properties:

BP: 290^0C

Flash point: 176^0C

MP: 17.8^0C

Refractive index: =1.4746

Solubility: Slightly soluble in acetone, practically in soluble in benzene, chloroform and oils. Soluble in ethanol and water

Density: 1.2620g/cm3 at 25^0C.

Stability and storage:

Mixtures of glycerine with water, ethanol(95%) and propylene glycol are chemically stable. Should be stored in airtight container in a cool dry place.

3.3.3. MENTHOL[45]

Nonproprietary names:

BP: Racementhol

USP: Menthol

Synonynms:

Hexahydrothymol; 2-isopropyl-5methyl cyclohexanol; 4-iso-propyl-1-methyl cyclohexane-3-ol.

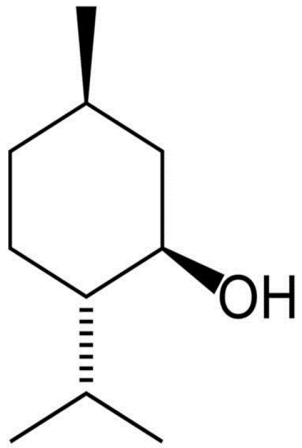
Chemical names and CAS registry number:

(±)-5-methyl-2-(1-methyl ethyl)cyclohexanol [15356-70-4]

Empirical formula & molecular weight:

C10H20O MWt: 156.27

Structure:



Functional category:

Flavoring agent and therapeutic agent

Applications in pharmaceutical formulation and technology:

Flavoring agent, cooling agent, skin penetrating enhancer and also used in perfumery, tobacco products, chewing gum and as a therapeutic agent.

Typical properties:

BP: 2120C

Flash point: 910C

MP: 340C

Refractive index: =1.4615

Solubility: Very soluble in ethanol(95%), Chloroform, ether, fatty oils and liquid paraffin. Soluble in acetone and benzene, Very slightly soluble in glycerine, Practically in soluble in water. Specific gravity: 0.904 at 150C.
Specific rotation(α)D20: -2 to +20(10%w/v alcoholic solution.

Stability and storage:

A formulation containing menthol 1% w/w in aqueous cream have been reported to be stable for up to 18 months when stored at room temperature. It should be stored in closed container at temperature not more than 250C.

3.3.5. Sodium alginate

It act as a stabilizing agent, suspending agent, disintegrant and viscosity increasing agent, good adhesive agent and better in situ gel forming agent. Sodium alginate is odorless, tasteless. Pale yellow, brown coloured powder. Alaklinity pH 7.2.Insoluble in ethanol, ether and other organic solvents. It is hygroscopic material, stable at cool room temperature

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 List of chemicals and reagents

Ketoprofen was a gift sample from Yarrow Chem Products, Wadala, Mumbai, India.

Table 1. List of chemicals and reagents

Materials	**Source**
Locust bean gum	Sisco research laboratories, Mumbai.
Sodium alginate	SD Fine chemical Ltd, Mumbai.
Glycerin	SD Fine chemical Ltd, Mumbai.
Menthol	SD Fine chemical Ltd, Mumbai.
Chloroform	SD Fine chemical Ltd, Mumbai.
Glycerin	SD Fine chemical Ltd, Mumbai.
Disodium hydrogen phosphate	SD Fine chemical Ltd, Mumbai.
Potassium dihydrogen phosphate	SD Fine chemical Ltd, Mumbai.
Sodium hydroxide	SD Fine chemical Ltd, Mumbai.

And other chemicals and reagents were of analytical grade and were used as they were procured. Distilled water was used in all experiments.

4.1.2 List of equipments and instruments

Table 2. List of equipments and instruments

Equipments / Instruments	**Source/Model**
Digital balance	Essae teraoka limited –DS 852 J
Digital pH meter	Elico L1120, Ahmedabad, Microtoniks, model M -19
Magnetic stirrer	Remi equipments, Mumbai
UV visible spectrophotometer	Systronics 2201, Ahmedabad.
Screw gauge	Micro Co., Mumbai
Hot air oven	Techno scientific products, Bangalore.
Tensile strength testing machine	Hounse field universal testing machine,U.K.
Franz diffusion cell	Orchid scientific, Nashik.
SEM	JOEL JSM-T330A Scanning Microscope
FTIR Spectrophotometer	Perkin Elmer, Germany.
Plethysmograph	MKM, Chennai.

4.2 METHODS

4.2.1 PREFORMULATION STUDIES

Preformulation may be defined as the stage of formulation development during which the physical pharmacist characterizes the physicochemical properties of the drug substance which are considered important in the formulation of a stable, effective and safe dosage form. During this evaluation, possible interactions with various inert ingredients intended for use in the final dosage form is also considered. In the present work, Preformulation studies such as solubility, determination of partition- coefficient, development of calibration curve, compatibility studies, and differential scanning calorimetry were carried out.

A. Determination of Melting Point

Melting point of drug sample was performed by using Thieles tube method. A fine powder of Ketoprofen was filled in a capillary tube, previously sealed at one end and the capillary tube was tied to the bottom of the thermometer. The thermometer and capillary tube were immersed in to the liquid paraffin taken in the tube. Bottom of the tube was heated gently by means of burner. When the sample starts to melt the reading was recorded.

B. Solubility studies[48]

The solubility was done by adding the solute in small incremental amounts to the fixed volume of solvents, after each addition, the system was vigorously shaken and examined visually for the undissolved solute particles. When some amount of the solute remains undissolved, the total solubility amount added upto the point served as a good and rapid estimate of solubility.

C. Determination of partition co-efficient [49]

The partition co-efficient study was performed using n-octanol as oil phase and phosphate buffer pH 7.4 as aqueous phase. The two phases were mixed in an equal

quantity and were saturated with each other on a mechanical water bath shaker at 32^0C for 24h. The saturated phases were separated by centrifugation at 2000 rpm on a Remi Centrifuge. Standard plots of drug were prepared from both the phosphate buffer and octanol. Equal volumes (10ml each) of the two phases were taken in triplicate in conical flask and to each 100mg of weighed amount of drug were added. The flasks were shaken at 32^0C for 6h to achieve a complete partitioning at 100rpm. The two phases were separated by centrifugation at 100rpm for 5min and they were then analyzed for respective drug contents. The partition co-efficient of drug Ko/w was calculated using the following formula.

$$Ko/w = \frac{\text{Concentration in Octanol}}{\text{Concentration in phosphate buffer pH 7.4}}$$

D. Development of Calibration Curve for Ketoprofen (□max) [50]

A stock solution of Ketoprofen was prepared by dissolving 100mg of drug in 100ml of phosphate buffer of pH 7.4. From this 10ml was taken and diluted to 100ml. From this 5, 10, 15, 20, 25 μγ/μλ dilutions were prepared using phosphate buffer of pH 7.4. The λ max of the drug was determined using UV-visible spectrophotometer. The absorption maximum of 260nm was selected and at this wavelength, the absorbance of all the other solutions was measured against a blank. The concentration ranges and absorbance data were reported in Table 4 Calibration graph was plotted using the data and presented in the Figure 5.1

E. FTIR study [51]

FT-IR spectroscopy study was carried to assess the compatibility between Ketoprofen and polymer locust bean gum, sodium alginate. The pure drug and drug with excipients were separately scanned. The pellets were prepared on potassium

bromide press. Both the spectra were compared for confirmation of peaks. The ketoprofen spectra and spectra data were shown in the Figure 5.2 and Table 5.

F. Differential Scanning Calorimetry (DSC) [52]

The dynamic DSC studies were carried out on pure drug and drug loaded films, the obtained thermo grams are presented in Figure 5.3. The data obtained from the DSC scans for the drug Ketoprofen and Ketoprofen loaded films are given in terms of onset of melt (To), melting points (Tm) and completion of melt (Tc). The amount of energy consumed for melting or the area under the endothermic peak of DSC curve of the pure drug and drug loaded films also presented in figure 5.3.

4.2.2 FORMULATION AND EVALUATION OF TRANSDERMAL FILMS

In the present study, drug loaded matrix type transdermal films of Ketoprofen were prepared by molding and solvent casting method. A mould of 5cm length and 5cm width with a total area of 25cm^2 was fabricated.

A. Preparation of drug-loaded transdermal films

The Transdermal films were prepared by using solvent casting technique. The bottom of the mold was wrapped with aluminum foil which was used as backing membrane. Drug containing films were prepared by solution casting method. In brief, the required amounts of a mixture of LBG (Table 1) were weighed and prepared polymeric solution using quantity sufficient water, kept aside for 2h after stirring. Accurately weighed KF (2.5 mg/mm2) and menthol (3% w/w) was dissolved in ethanol (6mL) by stirring for 10 min. The above mixture mixed with different concentrations of glycerin (1–5% w/w) and prepared polymeric solutions for 30 min. Finally mixed soft mass was poured on to cleaned specially designed glass molds with the plastic transparent sheet and kept in a vacuum drier until to get the dried membrane. The cast polymer films with different formulations were then peeled off covered with aluminum foils and stored in a desicator until further study.

Table 3. Formulation chart of Ketoprofen transdermal films

Formulation code	Ketoprofen (%)	Locust bean gum (%)	Sodium alginate (%)	Glycerin (%)	Menthol (%)
F1	2.5	4.0	92.0	0.5	1.0
F2	2.5	25.5	70.0	1.0	2.0
F3	2.5	40.5	52.0	2.0	3.0
F4	2.5	30.5	60.0	3.0	4.0
F5	2.5	10.5	80.0	3.5	4.5
F6	2.5	1.5	87.0	4.0	5.0

B. Evaluation of transdermal films [53]

The prepared films were evaluated for its uniformity of weight, uniformity of film thickness, tensile strength, percentage elongation, folding endurance, percentage moisture absorption, percentage moisture loss, drug content, scanning electron microscopy, drug diffusion study, primary skin irritation test and anti-inflammatory activity.

a. Uniformity of weight [54]

Weight uniformity was done by weighing three different films of the individual batch and the average weight was calculated. Care was taken that the individual weight should not deviate significantly from the average weight of the three. The tests were performed on films, which were dried at 400C for 3h prior to testing. The data of uniformity of weight is shown in Table 7.

b. Uniformity of film thickness [55]

The thickness of the films was measured at different points using screw gauge. The average of three readings of each film at different area were measured and presented in the Table 8.

c. Tensile strength [56]

Tensile strength of the films was determined using Hounse field universal testing machine. It consisted of two loaded grips, the upper one was movable and the lower one was fixed. The test films of specific size (5x1cm2) was fixed between these load grips and force was gradually applied till the films broke. The tensile strength of the films was taken directly from the dial reading in Newtons. The data is shown in the Table 9.

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}}$$

d. Percentage elongation of the films [57]

Percentage elongation of films is the ratio of increased length to its original length. It gives information of how much a specimen can elongate before it breaks. It is carried out by Hounse field universal testing machine. The percentage elongation at break point is measured on scale and the data of the percentage elongation is presented in the Table 9.

$$\%\text{Elongation} = \frac{\text{Maximum length recorded at break} - \text{Original length}}{\text{Original length}} \times 100$$

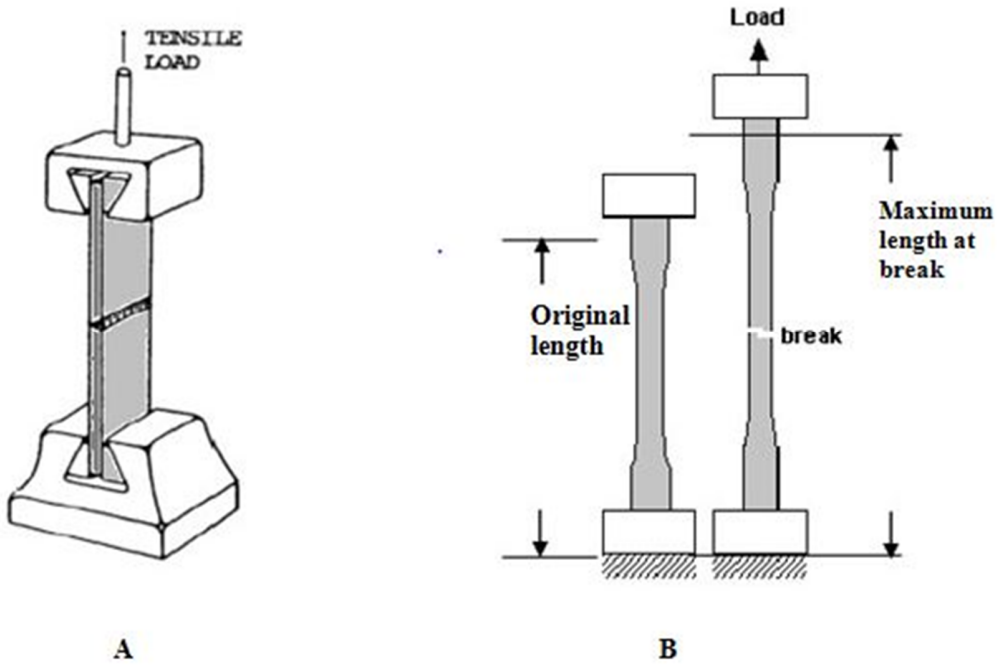


FIG 4 (A) Transdermal films between upper and lower jaw of tensile strength machine (B) Original and maximum length of films at break

e. Folding endurance [58]

The folding endurance was measured manually for the prepared films. A strip of film 2x2cm was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance.Table10.

f. Percentage moisture absorption [59]

The moisture absorption studies of various films were studied at 80% relative humidity (RH). Films of 1cm^2 of all the batches were selected. The films were weighed accurately and placed in a desiccator containing 100ml of fused aluminum chloride,

which maintains 80% of RH. After 3days, the films were taken out and weighed. The percentage moisture absorption was calculated using the formula.Table11

$$\% \text{ moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

g. Percentage moisture Loss [60]

Films of $1cm^2$ of all the batches were selected. The films were weighed accurately and placed in a desiccator containing anhydrous calcium chloride. After 3days, the films were taken out and weighed. The percentage moisture loss was calculated using the formula Table 11.

$$\%\text{moisture loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

h. Drug content [61]

A formulated film having 1cm2 area was cut into small pieces and weighed separately. The films were transferred into a 100ml volumetric flask and 100ml of phosphate buffer solution (pH 7.4) was added. The medium was stirred with magnetic stirrer for 12h. The contents were filtered using Whatmann filter paper. The filtrate was analyzed at 260nm spectrophotometrically for drug content against the reference solution containing only placebo films. Table 12.

i. Scanning electron microscopy

SEM studies are carried using JOEL JSM-T330A Scanning Electron Microscope. The external morphology of the formulation F3 was analyzed using a

scanning electron microscope to determine the drug distribution in the film and the SEM photos presented in Figure 5.4.

J. stability of the transdermal films and prepared kf gel[62]

Formulation F3(2.5cm^2)and conventional gel were subjected for stability studies at 250C/60%RH, 300C/65%RH,400C/75%RH for 90 days and the above formulations were evaluated for drug content periodically Table.13.

K. In vitro Drug Diffusion Study [63]

Drug diffusion studies were carried out in an open glass diffusion tube. A specimen dimension of films (2.5 cm^2) was fixed to the hydrated cellophane membrane at one end of the open glass tube and placed in the receptor compartment containing buffer solution. The assembly was placed on a magnetic stirrer and stirred at 100 rpm. The temperature of the system was maintained at 37°C ± 1°C. A known amount of receptor medium (buffer) was withdrawn at regular intervals of time and sink condition was maintained by replacing equal volume of fresh saline. The drug concentration samples were measured spectrophotometrically at 260nm against blank. Table 14-20

l. In vitro skin permeation studies

In vitro skin permeation studies were performed on a Franz diffusion cell with an effective diffusion area of 2.5 cm2 and 16 ml of receiver chamber capacity using rat abdominal skin. The animal study protocol was reviewed and approved by the Animal Ethics Committee at the Department of Pharmaceutics, MMU College of Pharmacy, Bangalore, Male albino rats weighing 128-130 g were used to excise full thickness skin. Rats were anaesthetized by ether and then hair of abdominal skin was removed by using electric clipper. Special care was taken while removing hairs, not to destroy the stratum corneum. The cleaned skin was washed with distilled water and stored in the deep freezer at –21oC until further use. The skin was brought to room temperature and

mounted between the donor and receiver compartment of the Franz diffusion cell, where the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. Initially the donor compartment was empty and the receiver chamber was filled with ethanolic phosphate-buffered saline (PBS) pH 7.4 (30:70% v/v). The receiver fluid was stirred with a magnetic rotor at a speed of 300 rpm, to maintain the hydro dynamics of receiver fluid and the temperature maintained at 320C ± 10C. All the ethanolic PBS was replaced every 30 minutes to stabilize the skin. It was found that the receiver fluid showed negligible absorbance after 5 hours and beyond, indicating complete stabilization of the skin. After complete stabilization of the skin, 2.5 cm2 of the optimized film was placed. In to each donor compartment and sealed with paraffin film to provide occlusive conditions. Samples (0.5mL) were withdrawn at regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8 hours), filtered through a0.45-membrane filter. The volume of release media was maintained by adding equal volume of fresh media after every sampling, Concentration of the KF in the sample was measured by m.

Anti-inflammatory activity [64]

The Anti inflammatory test was carried out on male albino rats weighing 128 to130 g. The animals were kept under standard laboratory conditions, with temperature of 250C ± 10C and relative humidity of 60% ± 5%. The animals were housed in cages, 5 per cage, with free access to a standard laboratory diet. The anti-inflammatory activity of KF from film formulation F3 was evaluated by the carrageenan induced hind paw edema method in albino rats and compared with conventional gel. The transdermal film was applied to the shaved abdominal skin of male rats. Just before administration of transdermal film, 1% carrageenan saline solution (0.1 ml) was injected into each hind paw of rats. The thickness of paw edema induced by carrageenan was measured by using a standard screw gauge during 8 h after application of KF transdermal film.

Permeation data analysis

Results are given mean ± standard deviation (S.D).The cumulative amount of drug permeated through the skin (mg/cm2) was plotted as a function of time (t) for each formulation. Drug flux (permeation rate) at steady state (Jss) was calculated by dividing

the slope of the linear portion of the graph by the area of the diffusion cell. The permeability coefficient (Kp) was calculated by dividing Jss by the initial concentration of the drug in the donor cell (Co)

$$Kp = Jss / Co \ldots\ldots 1$$

Enhancement ratio (Er) was calculated by dividing the flux of the respective formulation by the flux of the control formulation:

$$Er = Jss \text{ of formulation} / Jss \text{ of control} \ldots\ldots 2$$

The results were analyzed statistically using Student's t test and significance was determined at 95% confident limit ($P < 0.05$).

5. RESULTS

5.1 PREFORMULATION STUDIES

The drug and the compatiability of the other excipients used in the formulation. The results of the various Preformulation characterizations are given below.

A. Determination of Melting Point

Melting point of Ketoprofen was found to be 93.5^{o}C.

B. Solubility studies[51]

Ketoprofen is freely soluble in phosphate buffer pH 7.4, ethyl alcohol, methyl alcohol, chloroform, acetone, dichloro methane, and ether insoluble in water.

C. Determination of partition co-efficient

The partition co-efficient studies were performed in triplicate. The value of partition co-efficient (P) value was experimentally found to be 0.840. The results obtained also indicate that the drug possesses sufficient lipophilicity, which fulfill the requirements of formulating the selected drug into a transdermal film. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin.

D. Development of Calibration Curve for Ketoprofen (λmax)

The absorption maximum (λmax) is determined as 260nm. The concentration ranges and data are reported in Table 4. Calibration curve of Ketoprofen was plotted using this data and shown in the Figure5.1.

Table 4. Calibration curve for Ketoprofen in pH 7.4 phosphate buffer

Sl. No.	Concentration (in µg/ml)	Absorbance Peak Area± S.D Mean*	R^2 Value
1.	0	0	0.9998
2.	5	0.1540±0.0016	
3.	10	0.3097±0.0022	
4.	15	0.4504±0.0021	
5.	20	0.6113±.0103	
6.	25	0.7561±0.0038	

*Standard deviation, n = 3

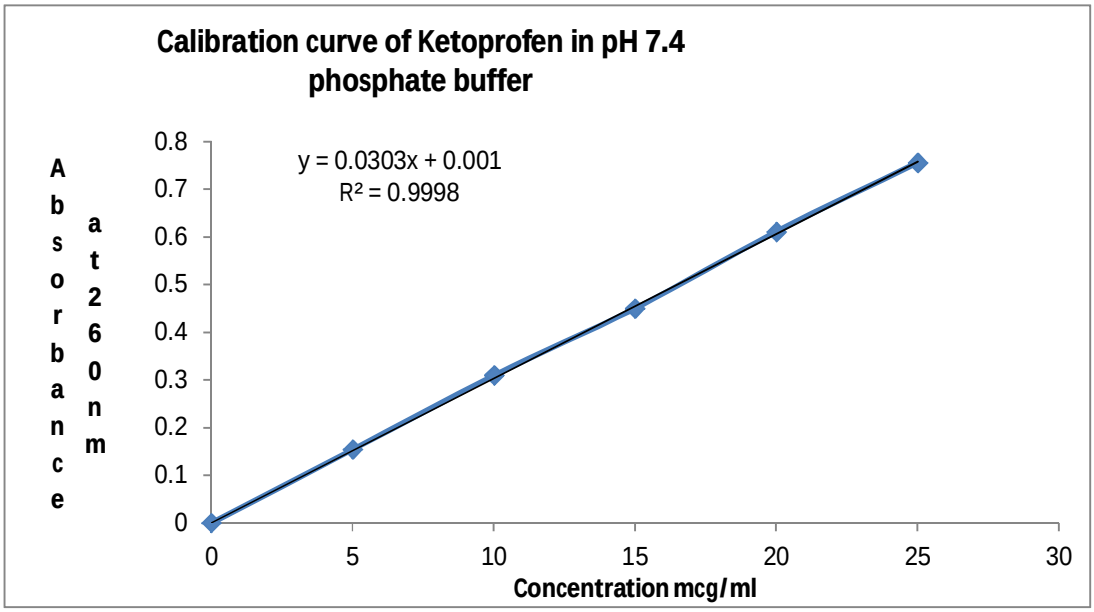


Figure5 Calibration curve of Ketoprofen in pH 7.4 phosphate buffer

E. FT-IR study

FT-IR study was employed to ascertain the compatibility of the drug ketoprofen with locust bean gum and sodium alginate. Both the spectra were compared for confirmation of common peaks. Specific peaks of pure drug and formulation showed no significant variation in height, intensity and positions of peaks. This proved that drug and excipients were compatible. There is no interaction between drug and polymer. The FT-IR spectra and data of ketoprofen drug and ketoprofen films are shown in the figure 5.2 and table 5.

Figure 6 FTIR spectra of ketoprofen and Ketoprofen films

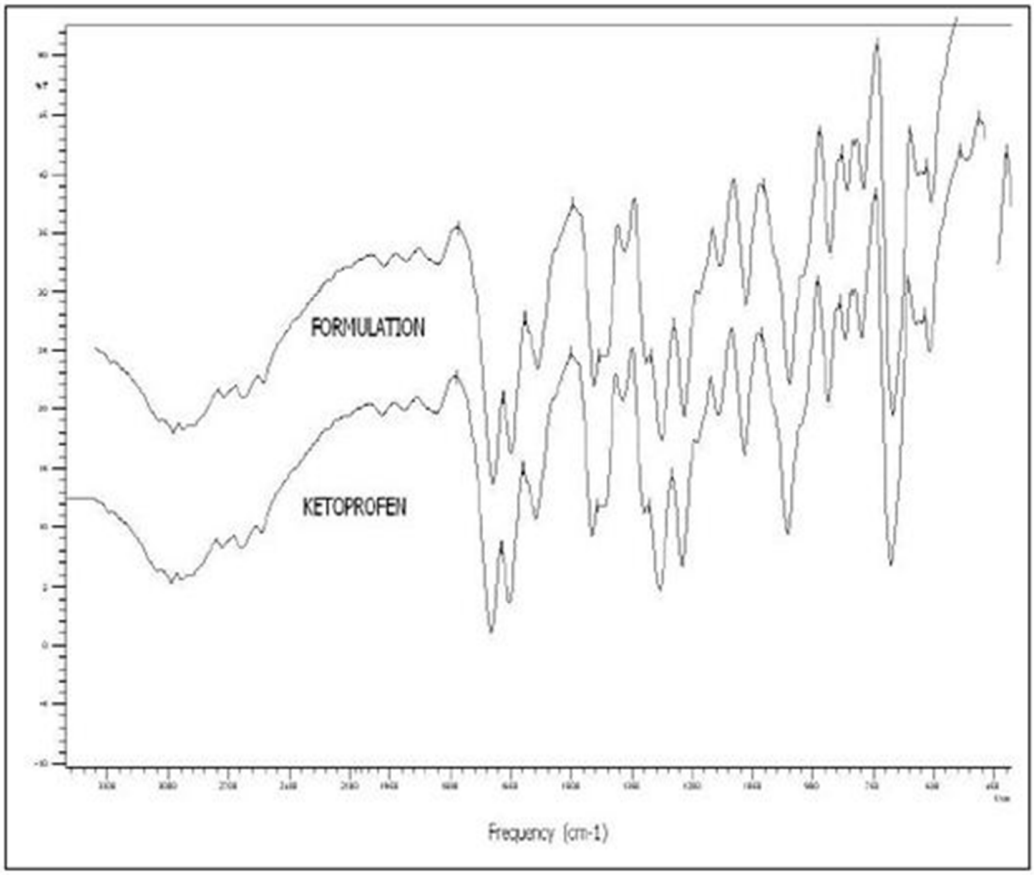


Table 5. FTIR spectra data of Ketoprofen and Ketoprofen films

Assignment	Band position pure drug (cm-1)	Band position of formulation (cm-1)
C - H stretching of CH3 group(asymmetric) masked by O-H stretching	2974-2932	2979-2936
C - H stretching of CH3 group(symmetric) masked by O-H stretching	2882	2878
C = O stretching of acid	1696	1696
C = O stretching of ketone	1654	1649
C = C stretching of aromatic ring	1593	1597
C - H deformation of CH3 group (asymmetrical)	1441	1444
C - H deformation of CH3 group (symmetrical)	1372	1369
C - H deformation of aromatic ring	867-691	829 – 634

From the FTIR studies, the characteristic absorption bands for important functional group of pure drug, empty films and drug-loaded films were identified and presented in Figure 5.2. IR spectra at 2979,2936cm-1 (C-H stretching of CH3 group (asymmetric) masked by O-H stretching), 2878cm-1 (C-H stretching of CH3 group (symmetric) masked by O-H stretching), 1696cm-1(C=O stretching of acid),1597cm-1 C=C stretching of aromatic ring, 1444cm-1 (C-H deformation of CH3 group(asymmetrical)), 1369cm-1 (C-H deformation of CH3 group- symmetrical) and 829 - 634cm-1 C-H deformation of aromatic ring. FTIR spectra showed that the

characteristics bands of ketoprofen were not altered after successful drug loading without any change in their position, indicating no chemical interactions between the drug and used polymers. Compared the IR spectra and interpretation of this region in our spectra agrees with their conclusions[52].

F. Differential Scanning Calorimetry (DSC)

To understand the compatible state of the drug, DSC studies were carried out on pure drug and drug loaded films, the thermo grams data obtained are shown in Figure 5.3. Ketoprofen exhibits a sharp endothermic peak at 93.25^{0}C. It was observed that presence of the endothermic peak at 93.36^{0}C in the drug loaded films indicated, that the drug is distributed in the films without any degradation and compatible with LBG/SA. Compared the DSC data and interpretation of this region in our data agrees with their conclusions as shown in Table 6.

Figure7 DSC thermogram of Ketoprofen and Ketoprofen films

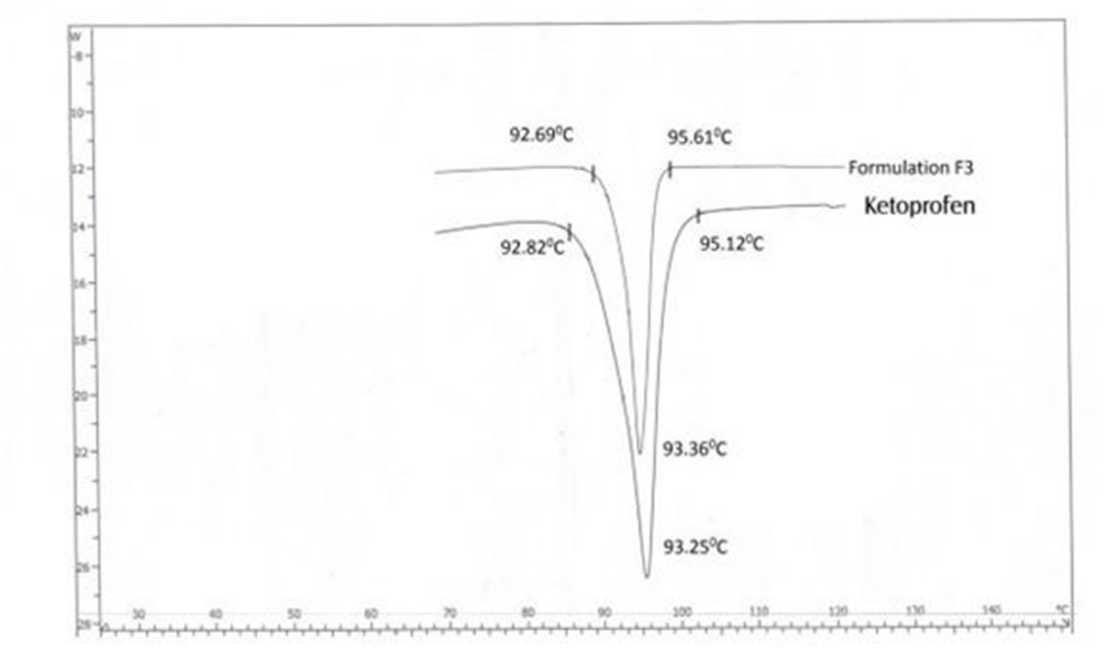


Table 6. DSC thermogram data of Ketoprofen and Ketoprofen films

Formulation	To (^{0}C)	Tm (^{0}C)	Tc (^{0}C)
Ketoprofen drug	92.82	93.25	95.12
Ketoprofen film (F3)	92.69	95.36	95.61

5.2 Evaluation of Transdermal films

Seven film formulations of films were prepared using solution casting method and dried. Films consist of glycerin as a plasticizer and menthol as permeation enhancer. Drug loaded films were light yellow opaque in colour. All surface of the film was smooth, with elegant appearance, good Physical properties. Flatness of the films was observed better when the amount of SA > 50% in the formulated films, might be SA having α- L-glucoronic acid, which is interact with LBG produces good flatness to the films. Thus these formulations can maintain a smooth and uniform surface when applied on skin.[53]

The drug loaded films were formulated using drug, polymer (LBG/SA), Glycerin used as plasticizer and different concentrations of menthol used as permeation enhancer. The prepared films were evaluated for its uniformity of weight, uniformity of film thickness, tensile strength, percentage elongation, folding endurance, percentage moisture absorption, percentage moisture loss, drug content, scanning electron microscope, in vitro drug diffusion study, primary skin irritation test and anti-inflammatory activity.

Mechanical properties

Thickness of the prepared films was in the range of 0.13 to 0.17 mm is shown in Table 8. Thickness, tensile strength and % elongation of the films increasing by increased ratio of LBG and plasticizer in the films. Added glycerin alters the physical and mechanical properties by enhancing the mobility of polymers chains of SA, LBG by hydrogen bonding[54]. However it was found that 2% of glycerin gives the best plasticizer effect for KF loaded film.

Moisture uptake

Low moisture uptake was found in films with less percent of plasticizer, after stored in the above conditions. Films with low percent of plasticizer showed a lower capacity to absorb water compared to those with plasticizer. As the ratio of plasticizer and RH increases, moisture uptake was increased. This effect was more pronounced on films containing more amount of plasticizer and more amount of plasticizer showed an significant increases in moisture up take at increased RH[55]

a. Uniformity of weight

Three different films of the individual batch are weighed and the average weight was calculated. The dried films were weighed on digital balance. The films exhibited uniform weight. The data of the individual weights are shown in the Table 7.

Table 7. Uniformity of weight of Ketoprofen films (2.5 mg/cm^2)

Sl.No.	Formulation code	Weight of the patch in mg			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	F1	164	166	169	166.33±2.08
2.	F2	167	171	170	169.67± 1.53
3.	F3	184	187	182	184.67± 3.51
4.	F4	190	193	195	192.67± 1.15
5.	F5	199	196	195	196.33± 1.53
6.	F6	171	173	174	170.33 ±0.23
7.	F7	172	176	175	177.53 ±1.63

*Standard deviation, n=3

b. Uniformity of film thickness

The thickness of the films was measured at different points using screw gauge. The average of three readings were measured and presented in Table 8.

Table 8. Uniformity of thickness of Ketoprofen film (2.5 mg/cm^2)

Sl.No.	Formulation code	Thickness of the patch in mm			Mean±S.D*
		Trial I	Trial II	Trial III	
1.	F1	0.11	0.13	.16	0.13±0.02
2.	F2	0.12	0.15	0.15	0.13± 0.01
3.	F3	0.13	0.15	0.16	0.14± 0.01
4.	F4	0.15	0.13	0.15	0.14± 0.01
5.	F5	0.16	0.15	0.16	0.15± 0.01
6.	F6	0.17	0.14	0.18	0.16± 0.01
7.	F7	0.18	0.14	0.17	0.17± 0.01

*Standard deviation, n=3

c. Tensile strength

Tensile strength of the films was determined using Hounse field universal testing machine. The tensile strength of the films was taken directly from the dial reading in Newton's. The films have shown reasonable tensile strength and moderate percentage elongation. The tensile strength increased whereas percentage elongation decreases. The data is shown in the Table 9.

d. Percentage elongation

Percentage elongation of films is gives information of how much a specimen can elongate before it breaks. It was carried out by Hounse field universal testing machine. The percentage elongation at break point is measured on scale and the data of the percentage elongation is presented in the Table 9.

Table 9. Tensile strength and % elongation of Ketoprofen film(2.5 mg/cm^2)

Sl. No.	Formulation code	Tensile strength(N/mm^2)	Percentage elongation
1.	F1	2.43± 0.01	21.30± 0.63
2.	F2	2.53± 0.04	21.56± 0.23
3.	F3	2.67± 0.02	22.45± 0.31
4.	F4	2.86± 0.02	24.4±1 0.14
5.	F5	3.08± 0. 15	26.38± 0.28
6.	F6	3.20± 0.12	29.36± 0.13
7.	F7	3.27± 0.03	34.14± 0.21

e. Folding endurance

The folding endurance was measured manually for the prepared films. A strip of film 2x2 cm was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance. The data were presented in the table 10.

Table 10. Folding endurance of Ketoprofen drug loaded film (2.5 mg/cm^2)

Sl. No.	Formulation code	Folding Endurance			Mean ± S.D*
		Trial I	Trial II	Trial III	
1.	F1	246	236	235	237.33± 5.86
2.	F2	246	254	246	248.67± 7.02
3.	F3	264	265	272	268.67± 4.16
4.	F4	266	276	262	267.33± 5.03
5.	F5	273	271	271	268.33± 7.37
6.	F6	276	283	278	276.50± 6.37
7.	F7	277	273	271	274.01± 6.87

*Standard deviation, n=3

f. Percentage moisture absorption

The moisture absorption studies carried out at 80% relative humidity. All the films showed least percentage moisture absorption. The data of the same is presented in the Table 11

g. Percentage moisture Loss

The moisture loss studies carried in a desiccator containing anhydrous calcium chloride. All the films showed least percentage moisture loss. The data of the same is given in the Table 11

Table 11. Data of percentage moisture absorption and moisture loss (2.5mg/cm^2)

Sl.No.	Formulation code	% Moisture Absorption Mean ± S.D*	% Moisture Loss Mean ± S.D
1.	F1	1.61± 0.34	1.23± 0. 15
2.	F2	1.60± 0.42	1.06± 0.10
3.	F3	1.63± 0.64	0.98± 0.16
4.	F4	1.38± 0.41	0.91± 0.10
5.	F5	1.87± 0.38	0.82± 0.40
6.	F6	1.92± 0.26	0.87± 0.26
7.	F7	1.95± 0.29	0.85± 0.37

*Standard deviation, n=3

h. Drug content

The drug content was analyzed by spectrophotometrically at 260nm and the data is given in the Table 12. The formulations exhibited uniform drug content and minimum batch variability.

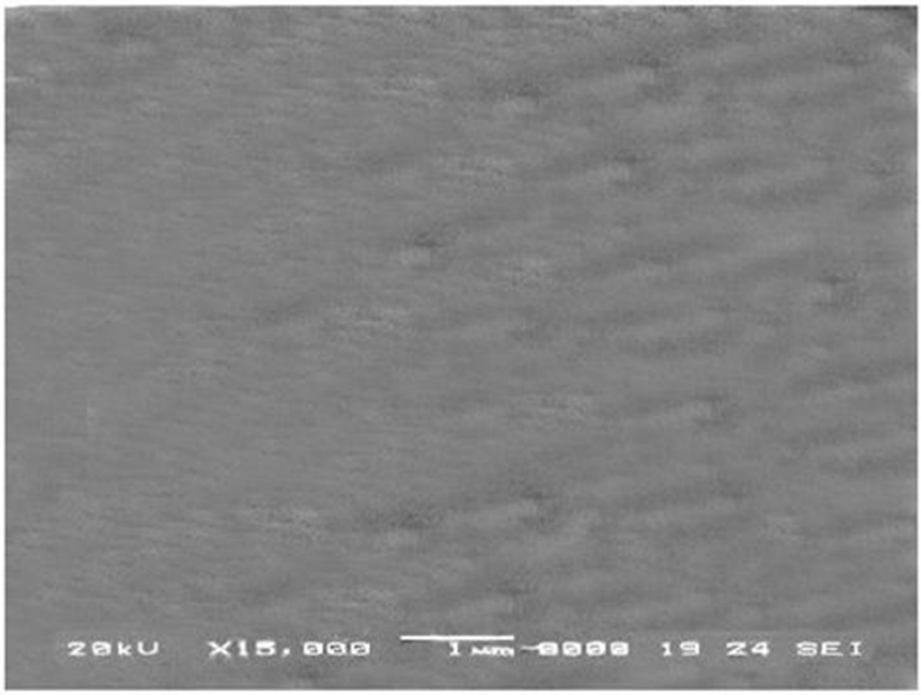
Table 12. Drug content uniformity and percentage of drug content (2.5 mg/cm^2)

Sl.No.	Formulation code	Drug content in mg	% of drug content
1.	F1	2.37	95.53
2.	F2	2.40	96.16
3.	F3	2.48	98.52
4.	F4	2.42	96.62
5.	F5	2.41	95.78
6.	F6	2.38	94.14
7.	F7	2.40	95.12

i. Scanning Electron Microscopy (SEM)

The surface morphology of this film was observed using SEM as shown in F.5.4 which indicates that the formulated film has smooth uniform surface.

Figure 5.4: Scanning electron microscopy of Ketoprofen films



j. Stability Studies

Stability studies of KF loaded transdermal films were carried out to determine the amount of drug content as presented films and also determine the physical stability of the film given in table.13.

Table 13. Stability studies of F3 formulation.

Sampling Intervals in days	**Drug content 25^0C/60% HR**	**Drug Content 30^0C/65% HR**	**Drug content 40^0C/75% HR**
15	98.46	98.28	98.18
45	98.34	98.22	98.10
60	98.21	98.12	98.10

k. Diffusion Study

The diffusion carried out for all the formulations to study for their in vitro drug diffusion. The kinetics of drug diffusion profiles was found out by using plotting different graphical models. All the profiles were presented in the respective table 14 to 20.

Table 14. In vitro Diffusion profile of Ketoprofen TDDS Formulation F1 (2.5 mg/cm^2)

Times (hrs)	√T	LogT	%Cumulative Drug released	Log %Cum Drug released	%Cum Drug Retained	Log %Cum drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	13.74	1.138	86.30	1.936
2	1.125	0.085	16.95	1.230	83.10	1.918
3	1.634	0.214	22.34	1.346	77.65	1.892
4	1.900	0.280	28.84	1.460	72.12	1.57
5	2.149	0.372	35.66	1.560	65.12	1.810
6	2.800	0.423	43.02	1.64	57.14	1.760
7	3.400	0.486	48.90	1.702	52.04	1.710
8	3.366	0.518	55.86	1.782	45.28	1.648

Table 15. In vitro diffusion profile of Ketoprofen TDDS Formulation F2(2.5 mg/cm^2)

Time(hrs)	√T	Log T	% Cumulative Drug Released	Log %Cum Drug Released	% Cumulative Drug Retained	Log %Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.34	1.156	85.66	1.938
2	1.214	0.084	20.22	1.305	79.78	1.901
3	1.632	0.212	24.48	1.388	75.72	1.878
4	1.900	0.278	32.34	1.509	67.66	1.830
6	2.149	0.370	37.82	1.577	62.18	1.773
8	2.728	0. 434	40.62	1.669	59.38	1.770
10	3.062	0.485	51.20	1.708	49.80	1.679
12	3.364	0.518	58.24	1.720	47.76	1.620

Table 16. In vitro diffusion profile of Ketoprofen TDDS Formulation F3 (2.5 mg/cm^2)

Time(hrs)	√T	Log T	% Cumulative Drug Released	Log %Cum Drug Released	% Cumulative Drug Retained	Log %Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	15.22	1.182	84.78	1.928
2	1.214	0.084	22.34	1.349	77.66	1.890
3	1.632	0.212	30.14	1.479	69.86	1.844
4	1.900	0.278	40.82	1.611	59.18	1.772
6	2.149	0.370	47.94	1.681	52.06	1.717
8	2.728	0. 434	53.12	1.725	46.88	1.671
10	3.062	0.485	63.72	1.804	36.28	1.560
12	3.364	0.518	70.28	1.847	29.72	1.473

Table 17. In vitro diffusion profile of Ketoprofen TDDS Formulation F4 (2.5 mg/cm^2)

Time(hrs)	√T	Log T	% Cumulative Drug Released	Log %Cum Drug Released	% Cumulative Drug Retained	Log %Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.28	1.155	85.72	1.933
2	1.214	0.084	20.86	1.319	79.14	1.898
3	1.632	0.212	28.74	1.458	71.26	1.853
4	1.900	0.278	38.48	1.585	61.52	1.789
6	2.149	0.370	40.94	1.612	59.06	1.771
8	2.728	0. 434	41.8	1.621	58.20	1.764
10	3.062	0.485	42.70	1.630	57.20	1.758
12	3.364	0.518	50.66	1.704	49.34	1.693

Table 18. In vitro diffusion profile of Ketoprofen TDDS Formulation F5 (2.5 mg/cm^2)

Time(hrs)	√T	Log T	% Cumulative Drug Released	Log %Cum Drug Released	% Cumulative Drug Retained	Log %Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	14.86	1.172	85.14	1.930
2	1.214	0.084	21.72	1.336	78.28	1.894
3	1.632	0.212	29.84	1.478	70.16	1.846
4	1.900	0.278	40.12	1.603	59.88	1.777
6	2.149	0.370	42.16	1.624	57.16	1.752
8	2.728	0. 434	50.84	1.703	49.16	1.691
10	3.062	0.485	52.86	1.723	47.14	1.673
12	3.364	0.518	56.88	1.754	43.12	1.634

Table 19. In vitro diffusion profile of Ketoprofen TDDS Formulation F6 (2.5 mg/cm^2)

Time(hrs)	√T	Log T	% Cumulative Drug Released	Log %Cum Drug Released	% Cumulative Drug Retained	Log %Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	11.34	1.054	88.66	1.940
2	1.214	0.084	14.15	1.150	85.58	1.930
3	1.632	0.212	19.86	1.297	80.14	1.903
4	1.900	0.278	26.32	1.420	73.38	1.867
6	2.149	0.370	33.12	1.520	66.88	1.925
8	2.728	0. 434	40.82	1.610	59.18	1.772
10	3.062	0.485	46.54	1.667	53.47	1.728
12	3.364	0.518	53.12	1.725	46.88	1.670

Table 20. In vitro diffusion profile of Ketoprofen TDDS Formulation F7 (2.5 mg/cm2)

Time(hrs)	√T	Log T	% Cumulative Drug Released	Log %Cum Drug Released	% Cumulative Drug Retained	Log %Cum Drug Retained
0	0	0	0	0	0	0
1	1.000	0.000	10.12	1.005	89.88	1.953
2	1.214	0.084	13.12	1.117	86.88	1.936
3	1.632	0.212	18.18	1.259	81.82	1.912
4	1.900	0.278	25.19	1.401	74.81	1.873
6	2.149	0.370	32.46	1.511	67.54	1.829
8	2.728	0. 434	39.12	1.592	60.88	1.784
10	3.062	0.485	45.35	1.656	54.65	1.737
12	3.364	0.518	32.10	1.716	47.90	1.860

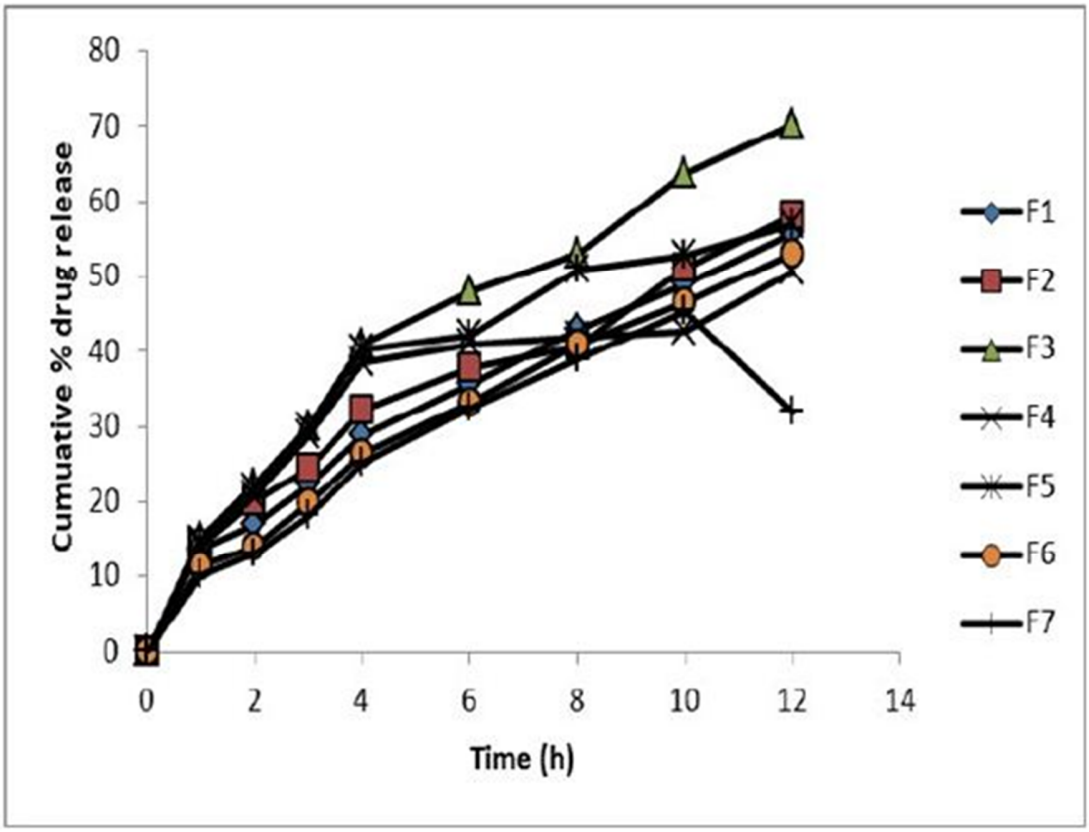


Figure 5.5: Comparative in vitro diffusion profiles of Ketoprofen TDDS according to Zero order kinetics

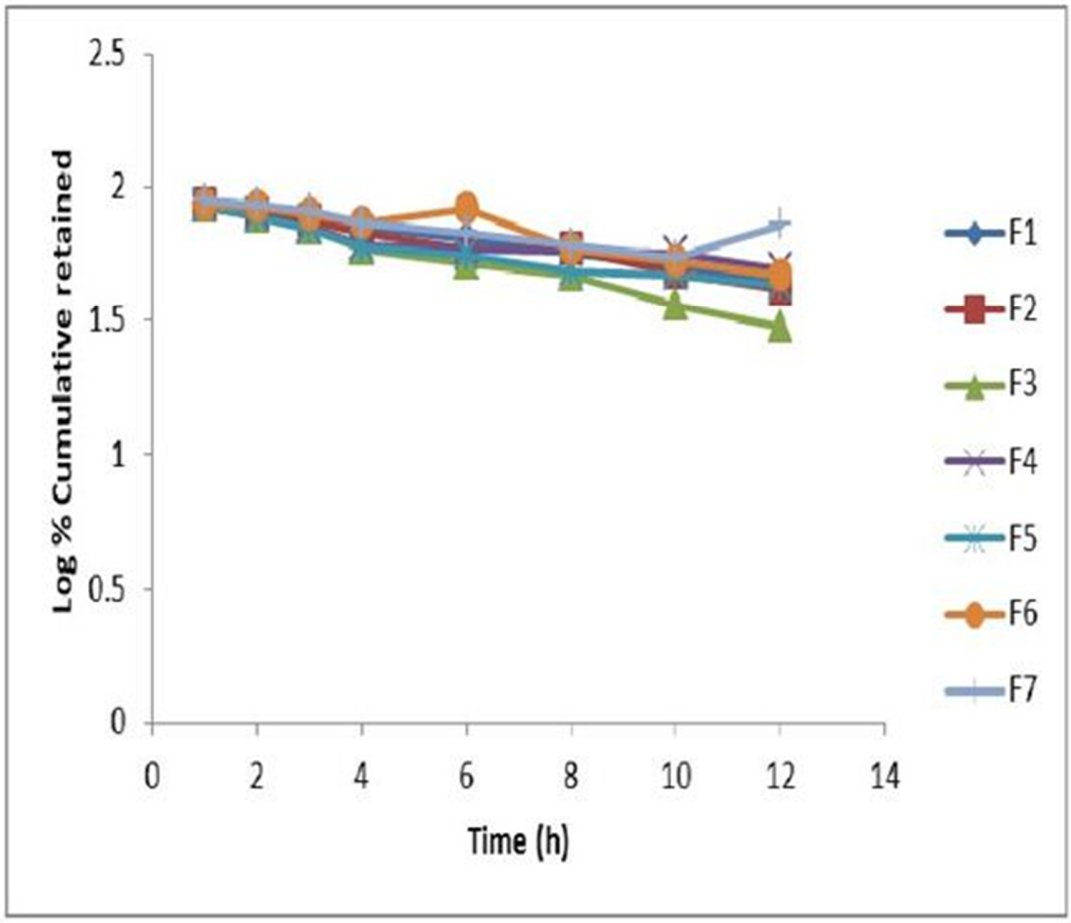


Figure 5.6: Comparative in vitro diffusion profiles of Ketoprofen TDDS according to First order kinetics

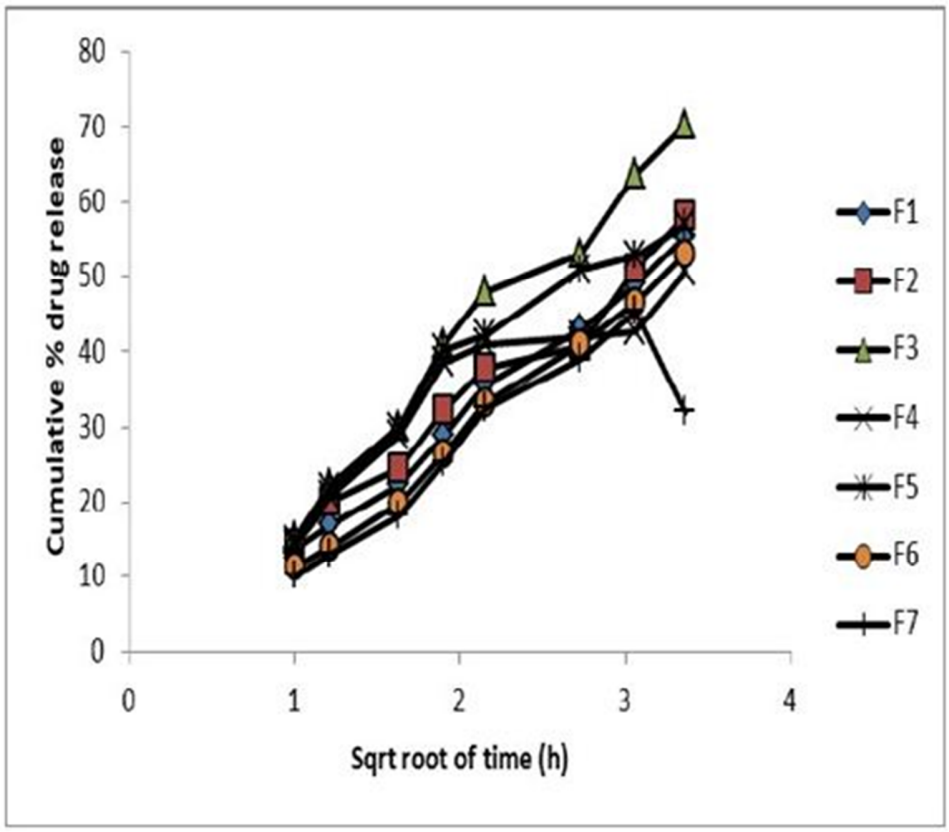


Figure 5.7: Comparative in vitro release profiles of Ketoprofen TDDS according to Higuchi matrix

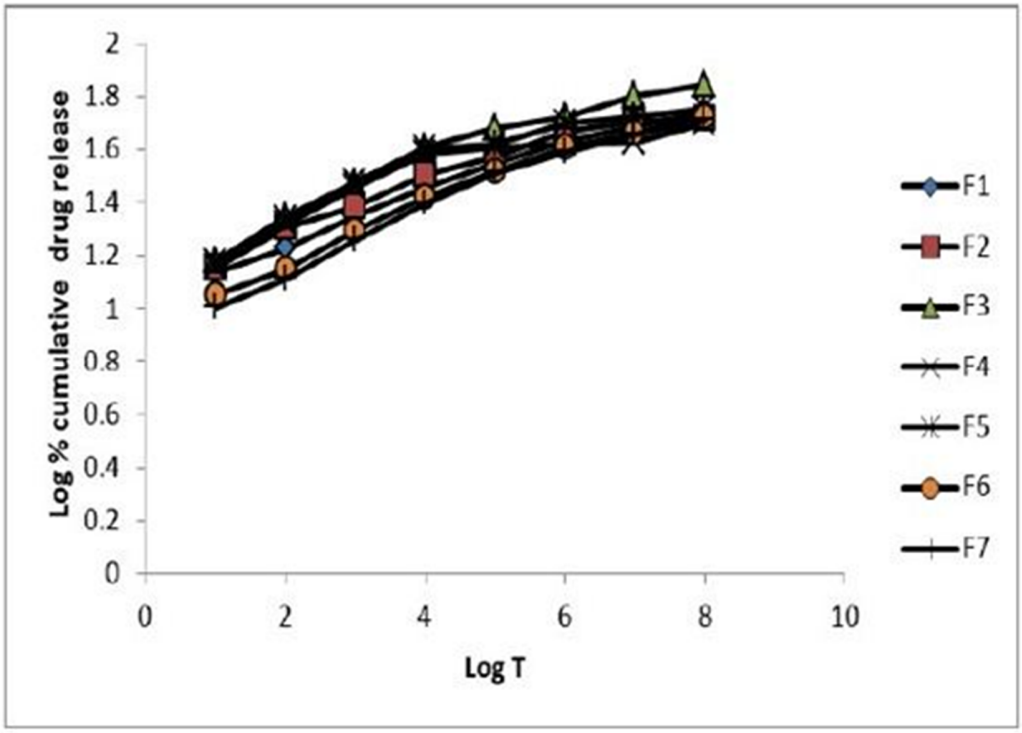


Figure 5.8: Comparative in vitro diffusion profiles of Ketoprofen TDDS according to Peppas kinetics

Table 21. Results of Model Fitting of Ketoprofen TDDS

Formulation	Zero order	First order	Higuchi Matrix	Peppas Plot	'n' values
F1	0.9590	0.9976	0.9897	0.9969	0.6111
F2	0.9427	0.9935	0.9924	0.9944	0.6013
F3	0.9492	0.9911	0.9871	0.9912	0.6360
F4	0.9447	0.9674	0.9876	0.9899	0.6278
F5	0.9417	0.9654	0.9878	0.9896	0.6211
F6	0.9413	0.9632	0.9853	0.9886	0.6201
F7	0.9401	0.9618	0.9832	0.9879	0.6203

l. The comparative skin permeation parameters of different formulations obtained were shown in Table no.22.

Table 22: *In vitro* skin Permeability parameters of different formulations

Formulation	**Menthol (%)**	**Jss (mg/cm2/h)**	**± Permeability coefficient (Kp)**	**Er**
Gel Control	-	0.029±0.034	0.120±0.086	1.0±0.12
F1	1.0	0.160±0.013	0.151±0.067	5.3±0.14
F2	2.0	0.231±0.024	0.210±0.054	7.7±0.16
F3	3.0	0.250±0.013	0.219±0.32	8.3±0.21
F4	3.5	0.226±0.022	0.210±0.053	7.5±0.35
F5	4.0	0.208±0.053	0.190±0.066	6.9±0.10
F6	4.5	0.196±0.042	0.180±0.021	6.5±0.09
F7	5.0	0.170±0.011	0.161±0.010	5.6±0.19

m. Anti-inflammatory activity

The anti-inflammatory efficacy of Ketoprofen films in mice paw oedema induced by the Carrageenan was tested and the results were compared with that of the conventional gel without menthol. The paw oedema volume was measured for 12h period, after the Carrageenan injection. The inhibition of oedema was observed and the comparative reduction in paw oedema volume, according to the mode of administration was recorded with respect to the control group. A significant inhibition of inflammation was found with conventional gel formulation (without menthol) presented in figure 9.

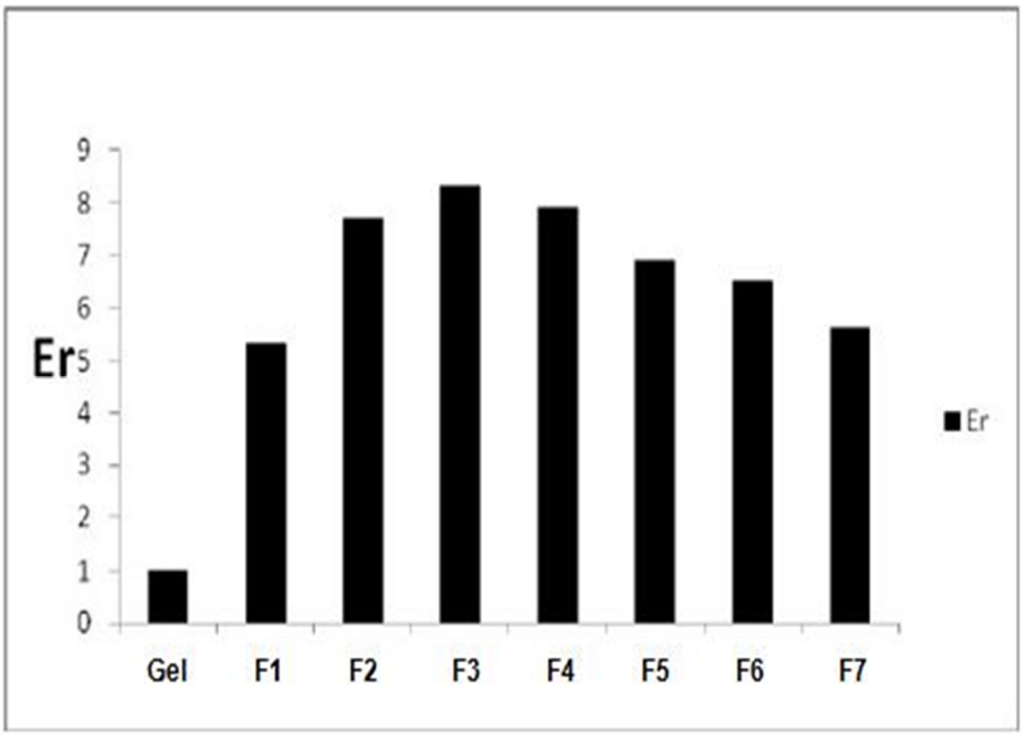


Figure 5.9: Comparative inhibition of inflammation from formulations and gel.

6. DISCUSSION

6.1 PREFORMULATION STUDIES

Preformulation studies are necessary to understand the physicochemical properties of the drug and the compatibility of the other excipients used in the formulation. The results of the various

Preformulation characterizations are discussed below.

A. Determination of melting point

Melting point of Ketoprofen was found to be 93.50C

B. Solubility studies

Ketoprofen is freely soluble in soluble in phosphate buffer pH 7.4, ethyl alcohol, methyl alcohol, chloroform, acetone, dichloro methane, and ether insoluble in water.

C. Determination of partition co-efficient

The value of partition co-efficient obtained indicates that the drug possesses sufficient lipophilicity, which fulfill the requirements of formulating the selected drug into a transdermal film. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin.

D. Development of calibration curve for Ketoprofen (λmax)

The absorption maximum (λmax) was obtained as 260nm. This implies the purity of the sample drug ketoprofen.

E. FT-IR study

FT-IR study was employed to ascertain the compatibility of the Ketoprofen with the LBG and SA. Both the spectra were compared for confirmation of common peaks. Specific peaks of ketoprofen and ketoprofen films showed no significant variation in height, intensity and positions of peaks. This confirms that there is no chemical interaction between drug and polymers.

F. Differential Scanning Calorimetry (DSC)

DSC studies were carried out on pure drug and drug loaded films. It was understood that the drug and the excipients used were compatible. Also indicated, that the drug is distributed uniformly in the films without any degradation.

6.2 EVALUATION OF TRANSDERMAL FILMS

A. Determination of film forming character

The films prepared using 40.5 % of LBG, 52.0% SA, 2 % Plasticizer and 3.0% W/W of menthol forms smooth, flexible, transparent and having sufficient mechanical strength.

B. Evaluation of drug loaded films

The drug loaded films were formulated using polymer, glycerin used as plasticizer, and menthol as permeation enhancer. The prepared films were evaluated for its physicochemical and mechanical properties and the same were discussed individually.

a. Uniformity of weight

Three different dried films of the individual batch were weighed using digital balance and the average weight was calculated. The films exhibited uniform weight and there was no deviation in the weight of any formulation.

b. Uniformity of film thickness

The thickness of the films was measured at different points using screw gauge. The films showed uniformity in their thickness.

c. Tensile strength

Tensile strength of the films was determined using Hounse field universal testing machine. The tensile strength increased where as percentage elongation decreases with increasing the concentration of Glycerin. The tensile strength results obtained in formulations indicate the risk of film cracking. The films have shown reasonable tensile strength and no sign of cracking in the films observed, which may be attributed to addition of plasticizer.

d. Percentage elongation

The films have shown moderate percentage elongation and exhibited satisfactory elongation. The tensile strength increased whereas percentage elongation decreases with increasing the concentration of LBG and glycerin.

e. Folding endurance

The folding endurance was determined manually by repeatedly folding the film at same place until it broke. The formulated film F3 exhibited optimal folding endurance without any batch variation.

f. Percentage moisture absorption

The moisture absorption studies carried out at 80 % relative humidity. All the films showed least percentage moisture absorption. This shows that the film protects the materials from microbial contamination and bulkiness of the films. Low moisture absorption was found in films with less percent of plasticizer.

j. Moisture loss

Moisture loss was carried out in desiccators containing anhydrous calcium chloride .The films should containing least percentage of moisture loss

h. Drug content

The drug content was analyzed by spectrophotometrically at 260nm. All the formulations determine fairly uniform drug content ranging from 2.37to2.48 mg/cm2. The drug content analysis of the formulations have showed that the process employed to prepare films in this study was capable of giving films with uniform drug content and minimum batch variability.

i. Scanning Electron Microscopy (SEM)

The surface morphology of this film was observed using scanning electron microscopy and which indicates that the formulated film has smooth surface with elegant appearance and good physical appearance. It shows that the drug is uniformly distributed throughout the film. Flatness of the films was observed better when the amount of LBG was more than 52 .5% in films. The viscosity of the films was found in the range of 392.13 to 403.45 Cp and increased concentrations of SA more viscosity.

j. Stability studies

There was no significant change in the physical property and drug content during the study period.

k. Diffusion Study

The matrix diffusion controlled transdermal drug delivery system of ketoprofen was studied for their in vitro drug diffusion to observe the kinetics of drug diffusion from the formulations. The in vitro drug release kinetics of the prepared ketoprofen films was found out by using plotting different graphical models. Diffusion studies were carried out in an open glass diffusion tube, using hydrated cellophane as a diffusion membrane.

Diffusion studies for all thefilms were carried out for 8 h in normal saline. From the diffusion studies, it was observed that, there was no significant diffusion of drug from KF films at gastric pH. At the end of 8th h, drug diffuses from formulation F3 (70.28) was maximum than F1 (55.74%), F2 (58.24%), F4 (50.66%), F5(56.88%), F6 (53.12%), F7 (52.10%) and conventional gel (88.3%) shown in Fig5.5 From the figure, it was clear that maximum amount of KF was diffuses from the formulation (F3). From

the above results, it can be concluded that drug diffusion from the films was controlled due to increased amounts of LBG showed higher swellability of the film and leached plasticizer from the film could reduce tortuosity of aqueous pore channels of the films, respectively[56]. In order to understand mechanism of drug release, in vitro release data were treated to kinetic models and linearity was observed with respect to Higuchi equation. The correlation coefficient obtained from Higuchi plot was found to be in the range of 0.9832 to 0.9924. This indicates that mechanism of drug release was diffusion type. As indicated by higher R2 values, the drug release from all formulations follows first order release and Higuchi model. Since it was confirmed as Higuchi model, the diffusion mechanism was swelling and diffusion controlled. The Peppas model is widely used to confirm whether the release mechanism is fickian diffusion, non-fickian diffusion or zero order. 'n' value could be used to characterize different release mechanisms.

Peppas-korsmeyer equation is given as:-

$$\% R = K t^n$$

$$\text{Or } \log \% R = \log K + n \log t$$

Where, R = drug release, n = slope,k = constant, t = time.

'n'	Mechanism
0.5	Fickian Diffusion (Higuchi matrix)
$0.5 < n < 1$	Non Fickian Diffusion
1	Case II Transport (Zero order)

The 'n' values for all formulations were found to be more than 0.50. Hence, this indicates that the diffusion approximates Fickian diffusion mechanism.

L. In vitro skin permeation studies

In vitro skin permeation studies were performed to compare the release of drug from 7 different film formulations (F3- F7) and conventional gel, all having the same quantity of (2.5% w/w)KF. As expected the flux of KF from films was found significantly higher ($P < 0.05$) than the flux of KF from conventional gel presented in Table.22. In vitro skin permeation was highest and lowest in formulation F3 and F7 respectively, The formulations F4 showed an intermediate skin permeation profile.

Increasing the concentration (3 to 5% w/w) of penetration enhancer showed a significant difference P < 0.05) in the flux of KF. The highest flux and enhancement ratio for KF from the film (F3) containing menthol was found to be 0.250 ± 0.13 mg/cm2/h & 8.3 mg/cm2/h respectively. The skin permeation profile of film F3 was significantly different (P <0.05), when compared with that of F4. Thus, menthol is expected to be a moderate skin permeation enhancer. In contrast, menthol enhanced the skin permeation of the drug by increasing both the skin concentration and the diffusion rate in skin because menthol contains functional group of hydrogen bonding. KF is lipophillic drug and menthol is a lipophillic terpene found to be more effective because menthol found to enhance the penetration of drug by both lipid and pore pathway [57, 58]. Increase in the concentration of penetration enhancer from 1% wt/wt to 3% even after increasing the penetration enhancer from 4% w/wt to 5% and plasticizer from 2.5% to 4% w/w in formulation F4 and F7 showed decreased enhancement ratio. Because increased ratio of LBG/SA in the films showed higher swellability of the film, plasticizer leaches from the film could reduce tortuosity of aqueous pore channels of the films. So that delivery of drug at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer duration of time from transdermal films. When enhancement ratio <1.0 indicates that enhancer
has no permeation enhancing activity.

m. Anti inflammatory studies

Based on higher drug permeation, formulation F3 was selected for the in vivo anti inflammatory effects and compare with conventional gel. A significant inhibition (p < 0.05) of inflammation was found with the film formulation F3 containing 3 % w/w menthol in comparison to the conventional gel without menthol. The percent inhibition value after 24h was found to be more F3 as compared to gel formulation without penetration enhancer and the difference between formulation F3 and conventional gel percent inhibition was significant (p < 0.05). The enhanced anti inflammatory effects of formulation F3 could be due to the enhanced permeation of KF though the skin. The anti inflammatory studies was performed to confirm the safety of optimized formulation F3. Literature survey reported that a value between 0 to 9 indicates that the applied formulation is generally not an irritant to human skin. The enhancement ratio was found

to formulation F3 8.3. From this it was concluded that optimized formulation F3 was safe to be used for transdermal drug delivery. Based on higher drug permeation and lower Viscosity formulation F3 was selected for the study of anti inflammatory effect. The anti inflammatory was evaluated by reported carragenean induced hind paw oedema method on rats and the inhibition of Inflammation was compared with conventional gel without penetration enhancer. The conventional gel shows less inhibition and significant inhibition ($P<0.5$), of inflammation was found with other formulation in comparison showed in figure 9. The in vivo results found to be correlation with in vitro permeation results. The enhanced anti inflammatory effects could be due to the enhanced permeation through the skin.

7. SUMMARY

The principle of transdermal drug delivery systems is to deliver the drug across epidermis to achieve systemic effect over a prolonged period of time. Because of these attributes, transdermal drug delivery systems offer many advantages such as reduced side effects, improved patient compliance, elimination of first-pass metabolism and sustained drug delivery. Ketoprofen is one of the important NSAID that have been widely used in the treatment of rheumatoid arthritis and related conditions. In the present study was aimed to formulate and evaluate the Transdermal drug delivery system of Ketoprofen. Ketoprofen selected as the drug candidate for the development of transdermal films, because of its low molecular weight, lipophilicity, considerable first-pass metabolism in liver, shorter plasma half life and excellent permeability through skin. Locust been gum and sodium alginate was used as polymers for the fabrication of matrix film because of its excellent film forming property. Glycerin was used as plasticizer to maintain the flexibility of the film and study the effect of drug diffusion property. Menthol was used as permeation enhancer to study the permeability effect. The method of casting the film on the fabricated mould was found to be satisfactory. The films were prepared with polymer alone were found to be brittle. 5 % w/w plasticizer glycerin was used to prevent embrittlement andwhich gave thin, flexible, smooth and transparent films. Physicochemical evaluation carried out for all the formulations. Solubility study was conducted to determine the sink condition property of the media phosphate buffer (pH 7.4) in diffusion studies. Phosphate buffer dissolves sufficient amount of drug and shows necessary character to maintain sink condition. The drug determines the sharp melting point at 93.5 C. The partition co-efficient values indicate that the drug possesses sufficient lipophilicity and fulfills the requirements of formulating the selected drug. FT-IR spectroscopy was used to study the drug-excipients interactions and it shows no physical and chemical interaction between drug and polymer. The DSC studies were carried out to understand the drug and the excipients used were compatible. It was indicated, that the drug is distributed in the films without any degradation. The SEM study indicates that the formulated film has smooth uniform surface and shows that the drug is uniformly distributed throughout the film. The drug loaded film exhibits satisfactory results of uniformity of weight,

uniformity of thickness, tensile strength and percentage of elongation. The films were exhibited optimal folding endurance without any batch variation. It was observed that films were having low average of % moisture absorption and moisture loss is one of the physicochemical characteristics in the formulation of transdermal films. The films shows small amount of moisture absorption. This result favours the stability and compatibility of the formulation in a high humid environment. The formulations determine fairly uniform drug content ranging from 95.5 to 98.5% with minimum of batch variability. The diffusion study of the formulation was essential for ensuring a sustained release and reproducibility of the rate and the duration of drug release. Drug diffusion study was conducted using Franz diffusion cell and synthetic release membrane. All the formulations were shown satisfactory cumulative percentage of drug release. According to the cumulative percentage of drug release profiles it was understood that the cumulative percentage drug release increased with increase in the plasticizer concentration. It can be stated that there is a significant variation in the cumulative percentage of drug release between F1 to F7. But there was no significant variation of cumulative percentage of drug release between F3 to F5. Hence the formulation F3 was found to be ideal formulation for satisfactory drug diffuse. The diffusion values revealed that the diffusion kinetic model follows first order, Higuchi, and Zero order. Based on the R2 value, it was confirmed as Higuchi model, the release mechanism was swelling and diffusion controlled. According to Peppas diffusion exponent of release profiles the ‘n’ values lies from 0.6 to 0.63, which indicates non-fickian transport diffusion. A primary skin irritation test studies was conducted on Albino rabbit skin and there was no signs of erythema or edema. It indicates that the transdermal films are safe for use. The efficacy of the anti-inflammatory activity was carried out by Carrageenan induced inflammation edema in mice. The formulation F3 showed a comparative reduction in the edema volume with respect to the oral solution of Ketoprofen. It revealed that the formulation F3 can able to produce maximum percutaneous penetration in a sustained release manner.

8. CONCLUSION

On the basis of good mechanical properties, better compatibility and stability of drug with polymer, highest drug permeation, we selected film formulation F3 (3% Menthol) for use in In vivo studies. The in vivo studies revealed a significant increase in anti inflammatory effects as compared with conventional gel without menthol. From in vitro and in vivo data it can be concluded that the developed film formulation F3 have great potential for transdermal drug delivery. Developed film formulation F3 has the best effective combination of polymer to achieve therapeutic plasma concentration. But additional experiments should be carried out before the film formulations are used on humans.

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